Recent Progress in the Chemistry of the Pectic Materials and Plant Gums.

THE TILDEN LECTURE, DELIVERED BEFORE THE CHEMICAL SOCIETY AT THE UNIVERSITY, BIRMINGHAM, ON FEBRUARY 19TH, 1940.*

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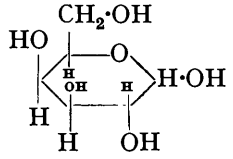
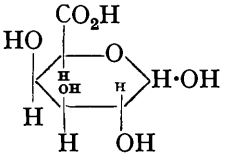
IT has long been considered probable that the pentose sugars arise in Nature from the corresponding hexoses by a process which involves oxidation to a uronic acid, followed by decarboxylation. It is known, for instance, that *d*-glucose and *d*-xylose are closely associated in the cellulosic constituents of plants and it is clear that *d*-xylose would be formed by the decarboxylation of *d*-glycuronic acid.



(I.)



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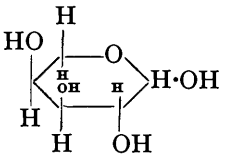


(III.)

An even more significant example is provided by the inter-relationship of galactose and arabinose, since the *l*- and not the *d*-form of arabinose would be produced by the decarboxylation of *d*-galacturonic acid (II), and it is *l*-arabinose (III) which is associated in nature with *d*-galactose (I). Relationships of this type may give an indication of the kind of mechanism involved in the formation of pentoses, but nothing is known concerning the circumstances in which this transformation takes place and no answer can as yet be returned to the question whether the xylose residues in xylan arise by oxidation and decarboxylation of the glucose residue of cellulose or whether xylan is built up by condensation of preformed xylose molecules.

Similar problems abound in connection with natural substances which contain arabinose and galactose residues as integral parts of the molecule. In no instance is there direct evidence concerning the mode of origin of the pentose and this lecture is concerned mainly with a discussion of the problem in the light of results obtained by an indirect method of approach. That the obvious explanation must be regarded with caution is well illustrated by the case of xylan already cited. Here the position is complicated and the simple hypothesis of direct derivation from cellulose has been rendered uncertain by recent work which has established the presence of *l*-arabofuranose as terminal end groups in the xylan molecule (Haworth, Hirst, and Oliver, J., 1934, 1917).

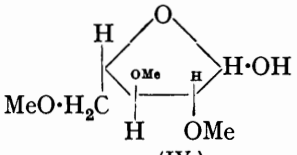
In order to gain further information concerning the inter-relationship of pentoses and hexoses, workers in the Bristol University laboratories are examining the structure of hexose and pentose residues associated with one another in certain polysaccharides of plant origin. The groups of natural products which afford special opportunities for this type of investigation are the pectic substances, the plant gums, and the plant mucilages. Members of each of these groups afford favourable conditions for comparing the ring structure and mode of linkage of the arabinose, galactose, and galacturonic acid residues present in the molecule, and, as will appear in what follows, the somewhat unexpected conclusion has been reached that in none of the substances investigated is it possible for the arabinose to arise intramolecularly from the associated galacturonic acid or from the associated galactose residues. Furthermore, the galactan which is associated with the pectin complex cannot, by oxidation at C_6, give rise to the galacturonic acid residues present in pectic acid, unless by way of hydrolysis and subsequent re-synthesis. Furthermore, it is clear that the araban which accompanies pectic acid in the pectin complex cannot be formed directly by decarboxylation of the pectic acid chains. It seems, therefore, that the natural processes involve hydrolysis of one polysaccharide, transformation of the resulting hexose into the corresponding uronic acid or pentose, and lastly, synthesis of another polysaccharide from the uronic acid or pentose so formed. Some further details of the evidence upon which these conclusions are based will now be given.

Pectic Substances.—It has long been known that " pectin " consists essentially of a mixture of polysaccharides, including an araban, a galactan, and a polygalacturonide (pectic acid). These three are present in most (or perhaps all) pectic substances, but their relative proportions vary widely with the

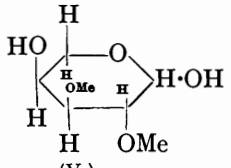
* The preparation of this lecture for publication was unavoidably delayed and the account now presented has been compiled in collaboration with Dr. J. K. N. Jones, to whom I wish to express my best thanks. The opportunity has been taken to render the survey more complete by the inclusion of references to some recent work, notably that of Dr. F. Smith on gum arabic, the full details of which became available after the date on which the lecture was delivered. E. L. H.

origin of the pectin. Advantage has been taken of this in a preliminary survey of the subject, in that different pectic substances can be utilised as convenient sources for the various components. For instance, a convenient source of araban was found in the peanut, from which, after removal of the oil, protein, and other materials, a crude pentosan was isolated. After further purification, this was found to contain neither galactose nor galacturonic acid residues, and on hydrolysis with 0·01N-acid it gave rise to *l*-arabinose only. This ease of hydrolysis indicated that the polysaccharide was built up of arabofuranose molecules. The araban, after methylation by treatment with thallous hydroxide and methyl iodide, was transformed into the fully methylated derivative, which has a high negative rotation indicating α-linkages.

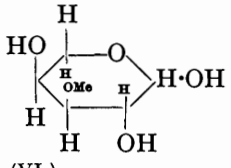
It was then shown that arabans from peanut, apple, and citrus pectins had very similar properties and after methylation they gave similar methyl derivatives which yielded on hydrolysis equimolecular proportions of 2 : 3 : 5-trimethyl *l*-arabinose (IV), 2 : 3-dimethyl *l*-arabinose (V) and a monomethyl *l*-arabinose which is probably the 3-derivative (VI). The dimethyl and monomethyl arabinoses are isolated as pyranose sugars, but in the unhydrolysed araban the corresponding residues are present in the furanose form. The absence of a methyl group at C_5 permits ring change after hydrolysis. These observations demonstrate the presence of branch chains in the araban molecule and one of the possible structural formulæ is typified in (VII). At present a decision cannot be made between (VII) and its obvious variants, but the general type of structure is apparent (Hirst and Jones, J., 1938, **496**; 1939, **452**, **454**; Beaven, Hirst, and Jones, J., 1939, 1865).



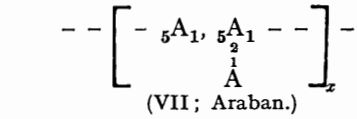
(IV.)



(V.)



(VI.)

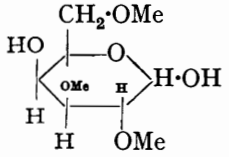


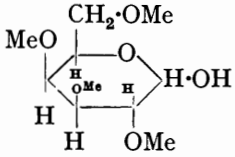
(VII; Araban.)

A = α-arabofuranose residue united to other residues through the numbered C-atoms.

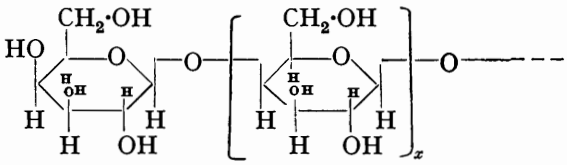
The isolation in the pure state of the galactan portion of a pectin has been achieved, up to the present, in one case only, namely, from the seeds of *lupinus albus*, which are a rich source of the polysaccharide (Schulze and Steiger, *Ber.*, 1887, **20**, 290). We are indebted to Professor Skene, of the University of Bristol, for drawing our attention to the possibilities afforded by this material, in which the galactan occurs, together with smaller quantities of araban and pectic acid. The araban was first removed by hydrolysis with 0·01N-acid, and the pectic acid was precipitated as its insoluble calcium salt. The residual polysaccharide showed a low positive rotation and was hydrolysed, giving *d*-galactose, at a rate which indicated a pyranose structure. On methylation, this polysaccharide gave a trimethyl galactan, hydrolysis of which yielded 2 : 3 : 6-trimethyl *d*-galactose (VIII), accompanied by a small quantity of 2 : 3 : 4 : 6-tetramethyl *d*-galactose (IX). The polysaccharide (X) consisted, therefore, of a chain of β-*d*-galactose units linked through the 1- and 4-positions and it could not, on oxidation and decarboxylation, give rise to an araban with furanose ring structure [compare (VII)] without intermediate hydrolysis followed by re-synthesis (Hirst, Jones, and Walder, unpublished results).

(VIII.)





(IX.)



(X; Galactan.)

A small quantity of crude araban was isolated from the seeds of *lupinus albus* by extraction with 70% alcohol. It had a high negative rotation and after methylation and hydrolysis gave a mixture of sugars,

among which 2 : 3 : 5-trimethyl (IV) and 2 : 3-dimethyl *l*-arabinose (V) could be detected. It was evident that this araban was very similar to, if not identical with, the araban isolated from peanut, apple, and citrus pectins.

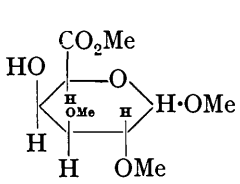
The pectic acid component of pectin was isolated in a nearly pure state from white lupin seeds (which provided a source from which all the three main components were isolated) and from apple, citrus, peanut and hawthorn pectins, by precipitation as the copper and calcium salts, followed by purification of the free acid (Beaven, Hirst, Jones, and Walder, unpublished results). It is difficult to remove the last traces of water, ash, and associated araban and galactan, but the purest samples isolated had equivalent weights close to 176, high positive rotations ($[\alpha]_D$ + 170° to + 230°) and after hydrolysis no substance other than *d*-galacturonic acid could be detected. Owing mainly to difficulties connected with the viscosity of alkaline solutions of the pectic acid, methylation proceeded unsatisfactorily, and for preliminary structural work a degraded pectic acid ester was prepared by boiling the pectic acid for some time with methyl-alcoholic hydrogen chloride. This treatment served to remove ash, adsorbed galactan and araban, caused partial hydrolysis of the polysaccharide, and esterification of the carboxyl group (Morrell, Baur, and Link, *J. Biol. Chem.*, 1934, **105**, 1).

TABLE I.

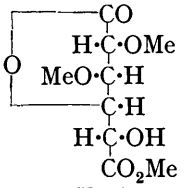
Source.	Equiv. wt.	Specific rotation.	Methoxyl, %.	Barium salt. Ba, %.	Barium salt. OMe, %.
Strawberry	200	—	17·4	25·2	1·8
Raspberry	187	+197°	15·7	25·1	0·8
Apple	195	+229	17·4	24·6	1·5
Citrus	215	+212	13·8	26·8	1·3
Lupinus albus	210	+170	17·9	—	—
Required for a methyl poly-galacturonide methyl ester containing eight units :				27·7	1·6

By this means degraded polyesters were prepared from citrus, apple, raspberry, strawberry, and *lupinus albus* pectic acids (see Table I) (Beaven, Hirst, Jones, and Walder, unpublished results). Methylation of these with thallous hydroxide and methyl iodide gave a trimethyl derivative, which underwent hydrolysis only with extreme difficulty. Hydrolysis in a sealed tube with methyl-alcoholic hydrogen chloride at an elevated temperature gave the methyl ester of 2 : 3-dimethyl methyl-*d*-galacturonoside (XI), the structure of which was proved by oxidation with bromine water, followed by esterification, giving the 1 : 4-lactone of 2 : 3-dimethyl mucic ester (XII), which with periodic acid gave *l*-dimethoxysuccinic acid (XIII), after oxidation of the aldehyde with bromine water. This result, taken in conjunction with the extraordinarily high stability of pectic acid to acidic reagents and its high positive rotation (over + 200°), indicates that in the pectic acid molecule there is present a series of pyranose α-galacturonic acid residues mutually linked through positions 1 and 4 (XIV) (Beaven and Jones, *Chem. and Ind.*, 1939, **58**, 363).

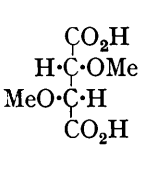
Simultaneously with this work the direct methylation of pectolic acid derived from citrus pectin was carried out by Smith at the University of Birmingham (*Chem. and Ind.*, 1939, **58**, 363; J., 1940, 1106, 1506). On hydrolysis of the methylated derivative, only 2 : 3-dimethyl galacturonic acid was obtained, which was proved by oxidation to be the 1 : 4-lactone of 2 : 3-dimethyl mucic ester (XII), identical with material obtained by synthesis. Although the dimethyl galacturonic acid was isolated after the methanolysis as the furanose form of the methylgalacturonoside, Smith's views concerning the pyranose ring of the uronic acid residues in pectin are in agreement with those indicated above, the transformation to the furanose ring form taking place after hydrolysis of the pectic acid (Luckett and Smith, J., 1940, 1506).



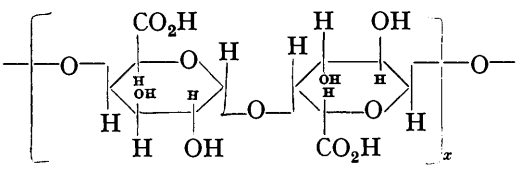
(XI.)



(XII.)



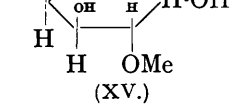
(XIII.)

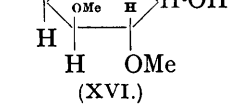


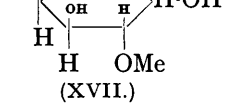
(XIV; Pectic acid.)

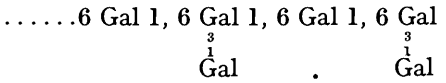
If (XIV) represents the type of structure present in pectic acid, it is clear that the latter cannot be derived from the accompanying galactan (X) by oxidation of the primary alcoholic grouping at C_6 to a carboxyl group, since in (X) the repeating units are β-*d*-galactopyranose residues whereas in pectic acid the repeating units are present in the α-form. So far as present knowledge goes, it would be necessary for hydrolysis and re-synthesis to take place if the galactan were to be the forerunner of pectic acid in the plant.

Further evidence in favour of the hypothesis that hydrolysis, followed by re-synthesis, occurs during the transformation of certain hexosans into the corresponding pentosans, has been obtained from a study of the so-called ε-galactan or galacto-araban of larchwood (Schorger and Smith, *Ind. Eng. Chem.*, 1916, **8**, 494). This has been shown to be a mixture of araban and galactan and there is evidence that the araban consists of arabofuranose units whereas the galactan portion is composed of galactopyranose units. This galactan, on methylation, followed by hydrolysis, gives rise to equimolecular proportions of tetramethyl *d*-galactopyranose, 2 : 4-dimethyl *d*-galactopyranose (XV), and a trimethyl *d*-galactopyranose, the constitution of which has not yet been established with certainty [it may be 2 : 3 : 4- (XVI) or 2 : 4 : 6-trimethyl galactose (XVII)]. It follows, therefore, that the galactan is of the branch chain type (XVIII is one of the possible formulæ) and is very different from the galactan present in pectins. Furthermore, it is impossible for the galactan to give the araban by oxidation and decarboxylation without intermediate hydrolysis, since (*d*-galacto)pyranose units are present in one and (*l*-arabo)-furanose units in the other (Campbell, Hirst, and Jones, *Nature*, 1941, **147**, 25). At present, therefore, little definite can be said regarding the inter-relationship of galactans, polygalacturonides and arabans, apart from the fact that *d*-galactose, which gives rise to *l*-arabinose, is almost invariably associated in natural products with *l*-arabinose and is probably its precursor.


(XV.)

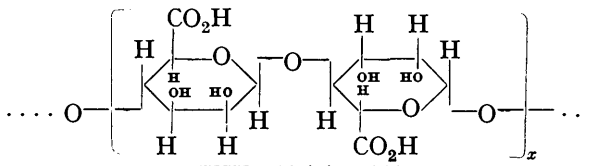

(XVI.)

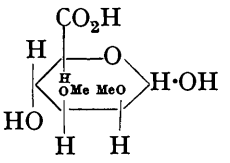

(XVII.)

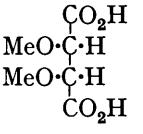

(XVIII; ε-Galactan.)

Gal = Galactopyranose residue, linked as shown. It is assumed that the trimethyl galactose residue is the 2 : 3 : 4-derivative.

Another instance of a polyuronide is found in the alginic acid (XIX) obtainable from various types of seaweed. This consists solely of β-*d*-mannuronic acid residues in the pyranose form, linked through positions 1 and 4, since on methylation, followed by hydrolysis, it gives 2 : 3-dimethyl *d*-mannuronic acid (XX), which can be oxidised to inactive dimethoxysuccinic acid (XXI) with periodic acid and bromine water (Hirst, Jones, and Jones, J., 1939, 1880).


(XIX; Alginic acid.)


(XX.)


(XXI.)

Plant Gums.—A study of the plant gums which have been obtained from many species of *Rosaceæ* and *Leguminosæ* throws further light on the problems under discussion, since these substances form a series of structurally complex polysaccharides in which hexose residues, uronic acid residues, pentose residues, and methyl pentose residues occur combined with one another in the most diverse fashions within the same molecule. An opportunity is thus provided for comparative studies of the ring form and the mode of linkage of the various sugars. A review of present knowledge in this field reveals that arabinose is present in the furanose form and is attached as a side chain to the main chain of the molecule. On the other hand, xylose has been encountered so far only in the pyranose form. Galactose, which is usually in the main chain of the polysaccharide, also favours the pyranose form and is found usually in a 1 : 3- or 1 : 6-linkage rather than in the 1 : 4-linkage so characteristic of the glucose polysaccharides,

starch, cellulose, lichenin and glycogen. The uronic acid so far met with in the plant gums has been glycuronic acid, which is attached in the pyranose form to *d*-galactose or *d*-mannose. These generalisations have been arrived at from the study of several different gums, which, despite their diversity of type and structure, have certain points of resemblance. For instance, they all appear to possess a repeating unit comprised of an aldobionic acid residue united to two *d*-galactose residues, together with side chains of various types.

In determining the constitution of the gums it is essential that a homogeneous material be used. In the case of almond, cherry, damson, egg plum, lemon and orange gums the most satisfactory procedure is to collect the nodules of gum from one tree if possible, and examine selected nodules for homogeneity. Once it has been established that each type of tree produces a characteristic gum, its collection is much simplified. After purification of the gum, its equivalent weight, rotation, uronic anhydride and pentosan content are determined and from these figures the percentage content of pentosan present in the gum may be calculated. " Auto-hydrolysis " of the purified, ash-free gum results in removal of all the pentofuranose molecules from the polysaccharides, together with other sugars which may be attached to them,

TABLE II.

Approximate Composition of Plant Gums (*mols. per repeating unit*).

Gum.	*d*-Glycuronic acid or methoxy-deriv.	*d*-Galactose.	*d*-Mannose.	*l*-Arabinose.	*d*-Xylose.	*l*-Rhamnose.
Arabic	1	3	nil	2	nil	1
Damson	1	2	1	3	*ca.* 3%	nil
Cherry	1	2	1	6	*ca.* 3%	nil
Egg plum	2	6	nil	7	1	nil
Purple plum	1	3	nil	3(?)	1(?)	nil
Almond tree	1	3	nil	4	2	nil
Lemon	+ve	+ve	nil	+ve	nil	nil
Orange	+ve	+ve	nil	+ve	nil	nil
Grape fruit	+ve	+ve	nil	+ve	nil	nil
Cholla	+ve	+ve	nil	+ve	nil	+ve
Mesquite	*d*-Galacturonic	+ve	nil	+ve	nil	—

For references see text, except for cholla gum (Sands and Klaas, *J. Amer. Chem. Soc.*, 1929, **51**, 3441) and mesquite gum (Anderson and Sands, *Ind. Eng. Chem.*, 1925, **17**, 1257; *J. Amer. Chem. Soc.*, 1926, **48**, 3172).

and leaves the skeleton of the gum, which may still contain some pentose molecules united in the pyranose form. Further hydrolysis of this degraded polysaccharide results in the formation of an aldobionic acid, which usually consists of *d*-glycuronic acid united to another hexose, together with *d*-galactose and small quantities of other sugars. By this means some idea of the constituents of the gum, together with their order of linkage, is obtained. Further evidence on the mode and position of linkage is obtained from a study of the products of hydrolysis of the methylated aldobionic acid, degraded gum and original gum.

The plant gum which has been most extensively studied is gum arabic. This has been the subject of a series of detailed investigations by Dr. F. Smith of Birmingham University, and notwithstanding the difficult and complicated nature of the problem, so much progress has been made that the main features of the structure of the repeating unit have been elucidated and the possibilities remaining in structural detail have been very considerably narrowed down.

The nature of the constituent sugars of gum arabic had already been investigated, but some doubt remained as to the presence of *l*-rhamnose (Norman, *Biochem. J.*, 1929, **23**, 524). However, by autohydrolysis of the acidic polysaccharide, Smith demonstrated the presence of *l*-arabinose, *l*-rhamnose, and *d*-galactose. A portion of the galactose was isolated after partial hydrolysis of the gum in the form of a disaccharide, 3-*d*-galactosido-*l*-arabinose, the constitution of which was proved by the usual methods (Smith, J., 1939, 744). The constitution of the aldobionic acid which resulted from further hydrolysis of the degraded polysaccharide had already been determined as 6-*d*-glycuronosido-*d*-galactose (Challinor, Haworth, and Hirst, J., 1931, 258; Hotchkiss and Goebel, *J. Amer. Chem. Soc.*, 1936, **58**, 858). Prolonged autohydrolysis of the degraded gum gave small quantities of a disaccharide, 3-*d*-galactosido-*d*-galactose, the constitution of which was proved by hydrolysis of the completely methylated material and identification of the resulting sugars (Smith, J., 1940, 79). The isolation of these products is in itself sufficient demonstration of the complexity of the gum arabic molecule. Degraded arabic acid (obtained by autohydrolysis of the gum and consisting of nine residues of *d*-galactose and three residues of *d*-glycuronic acid) was then methylated. From an examination of the sugars resulting from hydrolysis of the methyl derivative (Smith, J., 1939, 1724), the presence of four end groups, one of *d*-galactopyranose and three of *d*-glycuronopyranose, was demonstrated. The hydrolysis products included also 2 : 3 : 4-trimethyl *d*-galactose (XV) (5 molecular proportions) and 2 : 4-dimethyl *d*-galactose (XIV) (3 molecular

proportions). The isolation of these proved that all the galactose molecules were in the pyranose form, all the linkages in the molecule were either 1 : 3 or 1 : 6, and the 1 : 4-linkage characteristic of starch

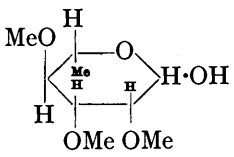
Gal 1, 3 Gal 1, 3 Gal 1, 3 Gal 1, 3 Gal 1, 3 Gal.....
(6 1 Gal, 6 1 Gly)

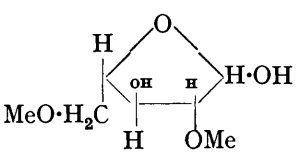
Degraded gum arabic.

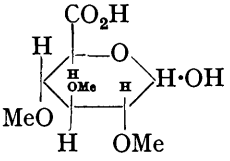
(Gal = *d*-galactopyranose; Gly = *d*-glycuronic acid, pyranose form)

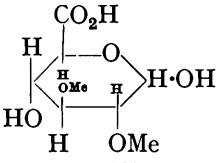
and cellulose was entirely absent. From these facts it was concluded that degraded gum arabic must have one of three formulæ. The possibilities for the structure of degraded gum arabic were further limited to two, by the isolation under carefully controlled conditions of hydrolysis of a hexamethyl 6-β-glucuronosido-*d*-galactose from methylated degraded arabic acid (Jackson and Smith, J., 1940, 74). One of these which illustrates the general type of structure is shown above.

From a study of the products of hydrolysis of methylated arabic acid, Smith was able to draw further conclusions as to the positions of the arabinose, rhamnose, and galactose units. Hydrolysis gave 2 : 3 : 4-trimethyl *l*-rhamnopyranose (XXII), 2 : 3 : 5-trimethyl (IV) and 2 : 5-dimethyl *l*-arabofuranose (XXIII), 2 : 3 : 4 : 6-tetramethyl (IX) and 2 : 4-dimethyl *d*-galactopyranose (XV) and 2 : 3 : 4-trimethyl (XXIV) and 2 : 3-dimethyl *d*-glucuronic acid (XXV).


(XXII.)


(XXIII.)


(XXIV.)


(XXV.)

" The identification of these products demonstrates the branched chain structure of arabic acid and also shows that those labile sugar residues, namely, *l*-arabinose, *l*-rhamnose, and 3-galactopyranosido-*l*-arabinose, which are liberated during the autohydrolysis of arabic acid, are joined to the nucleus of degraded arabic acid in the form of *l*-arabofuranose, *l*-rhamnopyranose, and 3-galactosido-*l*-arabofuranose " (Smith, J., 1940, 1035). In addition to the 1 : 3- and 1 : 6-linkages present in arabic acid, the 1 : 4-linkage also is present, since 2 : 3-dimethyl methyl-*d*-glucuronoside is one of the products isolated. Many formulæ, varying, however, only in detail, are ascribable to the undegraded gum arabic (one of which is shown below) on the above evidence, and still further work is required for the allocation of a definite detailed structure.

A 3, 1 Gal (6 1) A 3, 1 Gal (6 1)

Gal 1, 3 Gal 1, 3 Gal 1, 3 Gal 1, 3 Gal 1, 3 Gal.....
(6 1)

R 1, 3 Gal (6 1) Gly (4 1) A

Gal = *d*-Galactopyranose
Gly = *d*-Glycuronic acid
R = *l*-Rhamnopyranose
A = *l*-Arabofuranose

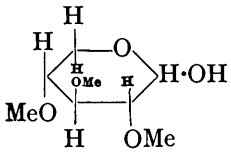
Gum arabic.

Structural investigations on damson gum have been carried out in the Bristol University laboratories. Samples of the gum were collected from trees in many different parts of the country and were found to be essentially the same in physical and chemical properties. The ash-free polysaccharide (A) had an equivalent weight of 1100, and estimation of pentosan and uronic anhydride indicated the presence of some 37·6% of araban and 16·4% of uronic anhydride. Autohydrolysis of the polysaccharide gave reducing sugars and a degraded polysaccharide. The sugars were shown to consist almost entirely of *l*-arabinose contaminated with a small quantity of *d*-galactose and the whole of the *l*-arabinose was removed from the polysaccharide during autohydrolysis. The degraded polysaccharide (B) obtained by autohydrolysis gave values for uronic acid and pentosan which were in agreement with those required by a polysaccharide containing one uronic acid residue, combined with three hexose residues, together with a pentosan present in much smaller stoicheiometric ratio. Controlled hydrolysis of this degraded polysaccharide with N-sulphuric acid gave two molecular proportions of *d*-galactose admixed with a little

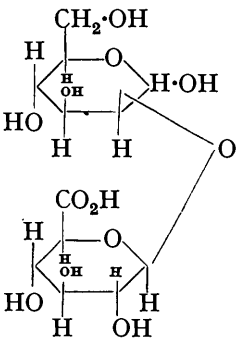
d-xylose and one molecular proportion of an aldobionic acid (2-*d*-glycuronosido-*d*-mannose). The aldobionic acid on further hydrolysis gave equimolecular proportions of *d*-mannose and *d*-glycuronic acid; the gum, therefore, consists of repeating units which contain the following sugars: *l*-arabinose (3 mols.), *d*-galactose (2 mols.), *d*-mannose (1 mol.), *d*-glycuronic acid (1 mol.). In addition, *d*-xylose occurs to the extent of about 3% (Hirst and Jones, J., 1938, 1174).

Further evidence on the structure of the gum was obtained from a study of the products of hydrolysis of the methylated gum (A) and of the methylated degraded gum (B). Methylation was carried out by heating the thallium derivative with methyl iodide—the product was essentially homogeneous and in each case was hydrolysed with methyl-alcoholic hydrogen chloride. The hydrolysed products from methylated damson gum (A) consisted of a complex mixture of methylated sugars, in which the following sugars were identified in the approximate molecular proportions indicated: 2 : 3 : 5-trimethyl *l*-arabinose (IV) (8 parts), 2 : 3-dimethyl *l*-arabinose (V) (4 parts), 2 : 4 : 6-trimethyl *d*-galactose (XVII) (3 or 2 parts), 2 : 4-dimethyl *d*-galactose (XV) (3 or 4 parts), 2 : 3 : 4-trimethyl *d*-glycuronic acid (XXIV) (2 parts), 2 : 3-dimethyl *d*-glycuronic acid (XXV) (2 parts), and a partially methylated *d*-mannose (4 parts). 2-Methyl galactose (1 part) and 4-methyl *d*-galactose (1 part) were also identified, but these may have been isolated as a result of incomplete methylation of the polysaccharide. A methylated derivative of *d*-xylose was also present, but was not identified (Hirst and Jones, unpublished results).

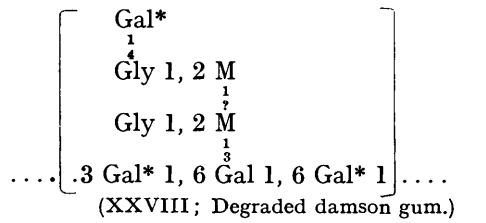
Methylated degraded gum (B) on hydrolysis gave the following sugar derivatives in approximately the molecular proportions indicated (Hirst and Jones, J., 1939, 1482): 2 : 3 : 4-trimethyl *d*-xylose (XXVI) (3—4%), 2 : 3 : 4 : 6-tetramethyl *d*-galactose (IX) (1 part), 2 : 3 : 4-trimethyl *d*-galactose (1 part), 2 : 4 : 6-trimethyl *d*-galactose (XVII) (1 part), 2 : 4-dimethyl *d*-galactose (XV) (1 part) (previously reported erroneously to be the 4 : 6-dimethyl derivative), 2 : 3 : 4-trimethyl *d*-glycuronic acid (XXIV) (1 part), and 2 : 3-dimethyl *d*-glycuronic acid (XXV) (1 part). A portion, at least, of the mannose (2 parts) is known to be a dimethyl derivative. The fully methylated undegraded damson gum gives neither tetramethyl nor 2 : 3 : 4-trimethyl galactose on hydrolysis, from which it may be inferred that the arabinose side chains are attached to positions C_6 and C_3 in those galactose residues which give rise to the above-mentioned sugars in the methylated degraded gum. Since 2 : 3-dimethyl and 2 : 3 : 5-trimethyl arabinose are present in the hydrolysis products in the ratio 1 : 2, it follows that the side chains in question have the structures A 1, 5 A 1 . . . and A 1 . . . respectively, where A = an arabofuranose residue. From these facts and from the observation that the aldobionic acid in damson gum consists of 2-*d*-glycuronosido-*d*-mannose (XXVII) some idea can be



(XXVI.)



(XXVII.)



(XXVIII; Degraded damson gum.)

Gal = Galactopyranose
Gly = Glycuronic acid (pyranose)
M = Mannopyranose

obtained of the type of structure present in the damson gum molecule. Neglecting, for the moment, the small proportion of xylose, the rôle of which is unknown, one of many possible structures for degraded damson gum is depicted in (XXVIII). In the undegraded gum the arabinose side chains may be attached to any of the galactose residues marked with an asterisk (at least two being involved). It is obvious that at this stage of the investigation, when detailed information concerning the mannose and xylose residues is lacking, it is not possible to indicate more than an outline of the type of structure involved.

Cherry gum (C) closely resembles damson gum in its physical properties but differs from it considerably in its chemical constitution. Its equivalent weight is very much higher (1503) and it contains more pentosan (Butler and Cretcher, *J. Amer. Chem. Soc.*, 1931, **53**, 4160; Jones, J., 1939, 558). Analysis indicates that it contains the following sugars in the approximate proportions indicated: *l*-arabinose (6 parts), *d*-xylose (*ca.* 1·5%), *d*-galactose (2 parts), *d*-mannose (1 part), and *d*-glycuronic acid (1 part). Autohydrolysis of the acidic gum (C) results in the removal of practically all the pentose, together with a little *d*-xylose, leaving a degraded cherry gum (D) which very closely resembles the degraded gum from

the damson. On further hydrolysis this gives two molecular proportions of *d*-galactose together with an aldobionic acid, 1 : 2-*d*-glycuronosido-*d*-mannose, identical with the aldobionic acid present in damson gum. Both cherry gum (C) and degraded cherry gum (D) are readily methylated by thallium hydroxide and methyl iodide.

Methylated cherry gum (C) gives on hydrolysis (Jones, unpublished results) a complex mixture of sugars, among which the following have been identified: 2 : 3 : 5-trimethyl *l*-arabinose (IV), 2 : 5-dimethyl *l*-arabofuranose (XXIII), 2 : 4 : 6-trimethyl *d*-galactose (XVII), 2 : 4-dimethyl *d*-galactose (XV), 2 : 3 : 4-trimethyl *d*-glycuronic acid (XXIV), and a dimethyl *d*-glycuronic acid (2·3 ?). A derivative or derivatives of *d*-mannose and *d*-xylose remain to be identified. It is possible that degraded cherry gum (the polysaccharide obtained by autohydrolysis of the original gum) has the same general structure as is present in degraded damson gum, but these investigations were interrupted by the outbreak of war.

Our state of knowledge of other plant gums is not so advanced, but some information (Hirst and Jones, unpublished) has been obtained concerning egg plum, purple plum, almond, grape-fruit, orange and lemon tree gums (for composition, see Anderson, Russell, and Seigle, *J. Biol. Chem.*, 1936, **113**, 683).* For example, egg plum gum is an acidic polysaccharide which consists of residues of *l*-arabinose (7), *d*-xylose (1), *d*-galactose (6), and *d*-glycuronic acid (2) in approximately the proportions indicated. All the arabinose units appear to be in the furanose form, since they are eliminated under very mild conditions of hydrolysis. The *d*-xylose appears to be united in the pyranose form to a molecule of *l*-arabinose and the *d*-galactopyranose units are united to an aldobionic acid (*d*-glycuronosido-*d*-galactose) (1 : 6 ?).

Almond tree gum on autohydrolysis gives *l*-arabinose and a disaccharide which on further hydrolysis gives *d*-xylose and *l*-arabinose. The degraded gum remaining after removal of the pentose gave on further hydrolysis *d*-galactose and an aldobionic acid, 1-glycuronosido-6-*d*-galactose, identical with the aldobionic acid in gum arabic. Almond tree gum, therefore, contains the following sugar residues: *l*-arabinose (4 parts), *d*-xylose (2 parts), *d*-galactose (2 parts), aldobionic acid (1 part).

Mucilages.—Of the mucilages examined, those from slippery elm (Anderson, *J. Biol. Chem.*, 1934, **104**, 163), flax seed (*idem, ibid.*, 1933, **100**, 249), lucerne seed (May and Schulze, *Z. Biol.*, 1936, **97**, 201), *plantago lanceolata* seed (Mullan and Percival, J., 1940, 1501), *plantago psyllium* seed (Anderson and Fireman, *J. Biol. Chem.*, 1935, **109**, 437), and *plantago fastigiata* seed (Anderson, Gillette, and Seeley, *ibid.*, 1941, **140**, 569) may be referred to briefly.

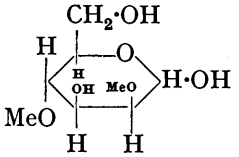
It appears that all these mucilages may contain a protein-like compound as an integral part of the molecule. The mucilages from the seeds of *plantago psyllium* and of *plantago fastigiata* have both been shown to contain *l*-arabinose, *d*-xylose, and *d*-galacturonic acid. *Plantago fastigiata* mucilage contains an aldobionic acid consisting of *d*-galacturonic acid and *l*-arabinose, but knowledge of the detailed constitution of this aldobionic acid is not yet available. On the evidence presented it is just possible that the *l*-arabinose molecules may occur in the mucilage in the pyranose form, as they seem to be more resistant to hydrolysis, but further work is needed to settle this point. The mucilage from *plantago lanceolata* resembles those from *plantago psyllium* and *plantago fastigiata* in that it contains *d*-galacturonic acid and *d*-xylose, but differs in that it also contains *d*-galactose and a methyl pentosan, but no *l*-arabinose. Its structure appears to comprise many side chains of *d*-xylose. Very little is known of the detailed structural constitution of flax seed mucilage and only the aldobionic acid obtainable from it has been critically examined (Tipson, Christman, and Levene, *J. Biol. Chem.*, 1939, **138**, 609).

On the other hand, some evidence concerning the ring structure and linkage of the component sugars of lucerne seed mucilage and of slippery elm mucilage has been obtained. Lucerne seed mucilage gives on hydrolysis two sugars only, namely, *d*-galactose (2 parts) and *d*-mannose (1 part) (compare May and Schulze, *loc. cit.*). Attempts to separate the polysaccharide into a galactan and a mannan were fruitless and a study of the products of hydrolysis of the methylated product showed that the two sugars are combined together in the same polysaccharide.

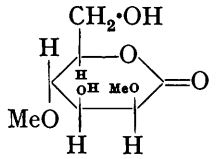
The purified lucerne seed mucilage was a cream powder soluble in water, giving viscous solutions, and it underwent slow hydrolysis with N-sulphuric acid, the presence of pyranose rings being thus indicated (compare the mannogalactan of the seeds of the fenugreek; Daoud, *Biochem. J.*, 1932, **26**, 255). The methylated product, which was free from protein, gave on hydrolysis three methylated sugar derivatives in approximately equal proportions (Hirst, Jones, and Walder, unpublished results); these were 2 : 3 : 4 : 6-tetramethyl *d*-galactose (IX), 2 : 4 : 6-trimethyl *d*-galactose (XVII), and a mannose derivative which was almost certainly 3 : 4-dimethyl *d*-mannose (XXIX), since it gave no furanose derivative with cold methyl-alcoholic hydrogen chloride and on oxidation gave a δ-lactone (XXX) which with liquid ammonia

* We are indebted to Dr. Misener of Toronto for the gift of orange, lemon, and grapefruit gums.

gave an amide with a positive Weerman reaction. From these results, taken in conjunction with the rotation of the polysaccharide (+ 85°), one of the possible formulæ is outlined below (XXXI).



(XXIX.)



(XXX.)

Gal 1, 3 Gal 1, 6 M 1, 3 Gal 1, 6 M 1, 3 Gal 1, 6 M 1, 3 Gal.....

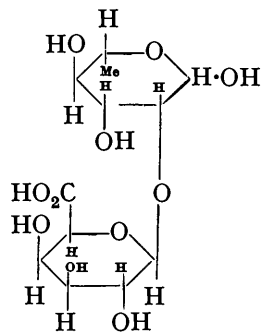
(XXXI; Lucerne seed mucilage.)

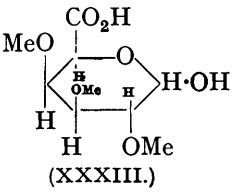
(Gal = galactopyranose; M = mannopyranose)

Slippery elm mucilage, in addition to some 10% of protein, contains *d*-galacturonic acid (1 part), *l*-rhamnose (1 part), and *d*-galactose (1 part). The ash-free mucilage, on being heated, is partly hydrolysed with the precipitation of coagulated protein; the protein-free polysaccharide underwent further hydrolysis at a rate normal for a pyranoside and gave rise to *d*-galactose and an aldobionic acid. The latter was *d*-galacturonosido-*l*-rhamnose, since on further hydrolysis it gave *d*-galacturonic acid and *l*-rhamnose (Anderson, *loc. cit.*).

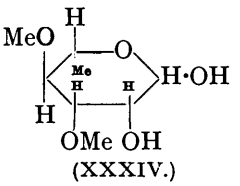
Complete methylation of the aldobionic acid, followed by hydrolysis of the products, proved its structure (Gill, Hirst, and Jones, J., 1939, 1469) (XXXII) to be identical with that of the aldobionic acid present in flax seed mucilage (Tipson, Christman. and Levene, *loc. cit.*), since it gave equimolecular proportions of 2 : 3 : 4-trimethyl *d*-galacturonic acid (XXXIII) and 3 : 4-dimethyl *l*-rhamnose (XXXIV). The linkage is evidently between the reducing group of the galacturonic acid and C_2 of the rhamnose molecule—an unusual type found hitherto only in aldobionic acids in damson and cherry gums and flax seed mucilage.

(XXXII.)





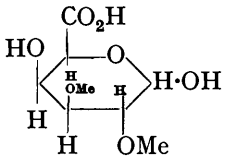
(XXXIII.)

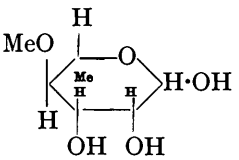


(XXXIV.)

Methylation of the mucilage with sodium hydroxide and methyl sulphate proceeded unsatisfactorily; methylation by means of thallous hydroxide and methyl iodide, however, proceeded smoothly and rapidly. The methylated polysaccharide was comparatively stable to methyl-alcoholic hydrogen chloride and prolonged boiling with this reagent was necessary for completion of hydrolysis. Fractionation of the

(XXXV.)





(XXXVI.)

Galac 1, 2 R 1, 4 Galac 1, 2 R 1, 4 Galac 1, 2 R 1, 4 Galac 1, 2, R1,.....

Gal (3, 1) Gal (3, 1) Gal

Gal (3, 1) Gal (4, 1) Gal (XXXVII; Slippery gum mucilage.)

Gal = *d*-Galactopyranose R = *l*-Rhamnopyranose Galac = *d*-Galacturonic acid (pyranose)

products gave 2 : 3 : 4-trimethyl *d*-galacturonic acid (trace) (XXXIII), 2 : 3-dimethyl *d*-galacturonic acid (4 parts) (XXXV), 2 : 3 : 4 : 6-tetramethyl *d*-galactose (2 parts) (IX), 2 : 4 : 6-trimethyl *d*-galactose (1 part) (XVII), 2 : 3 : 6-trimethyl *d*-galactose (1 part) (VIII), 3 : 4-dimethyl *l*-rhamnose (2 parts) (XXXIV), and 4-methyl *l*-rhamnose (2 parts) (XXXVI). From the evidence given, the general type of structure present in the mucilage may be inferred and one formula which illustrates typical features is (XXXVII) (Gill, Hirst, and Jones, unpublished results). There are obvious variants, but other methods of approach must be developed before it will be possible to differentiate unequivocally between them.