STRUCTURAL CHEMISTRY OF PLANT GLYCURONOGLYCANS

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ABSTRACT

This paper is essentially a brief review of structural studies on plant acidic polysaccharides (glycuronoglycans) containing glycuronic acid residues. The polysaccharides have been classified according to the contents of glycuronic acids and their basal core structure. The structures of certain glycuronoglycan groups are discussed. Further development of the structural chemistry of plant glycuronoglycans is regarded as dependent on elaboration of new precise methods of structural analysis and elucidation of the biological functions of this class of polysaccharides.

I. INTRODUCTION

Plant polysaccharides are very diverse[1–15]. At present they are classified by properties in the following groups: (1) cellulose[6–14]; (2) reserve polysaccharides[6–10]; (3) hemicelluloses[4, 6, 15]; (4) pectic substances[1, 5, 6, 9]; and (5) gums and mucilages[2, 3, 6–8, 10–13]. There are no strict boundaries among these groups, and very often the same polysaccharide can belong to several groups. The above classification has been developed historically and is mainly associated with localization of polysaccharides in plants and with their biological properties.

In recent years, the classification of plant polysaccharides by chemical properties such as neutrality, acidity and basicity is gaining increasing support[7].

The present brief review is concerned with a large group of plant glycuronoglycans containing glycuronic acid residues in their structure.

The class of glycuronoglycans can be split into the three groups according to the uronic acid content (*Table 1*), differing in structures and properties: (1) glycuronans composed of glycuronic acid residues as virtually single components of their carbohydrate chain; (2) glycanoglycuronans, whose backbones are composed of uronic acid residues as the main constituents with other sugar residues in both the inner and the outer chains, the uronic acid content generally exceeding 50 per cent; and (3) glycuronoglycans, which, even though they comprise uronic acids in widely variable amounts, generally do not exceed 50 per cent of the total contents, the molecules being based, however, on neutral monosaccharides and their derivatives.

The molecules of plant acidic polysaccharides quite often comprise acetyl, sulphate, methyl and ester groups as non-carbohydrate components.

In nature, glycuronoglycans are found as calcium, magnesium, iron salts, frequently accompanied by proteins, peptides and neutral polysaccharides; complex relationships with cellulose are also observed[6–10]. Individual glycuronoglycan molecules are often interlinked to form a complex reticular structure, whose formation essentially involves both covalent and non-covalent bonds of obscure nature.

Table 1. Glycuronoglycan classification.

I. Glycuronans	1. Guluronomannuronans	Alginic acids
II. Glycanoglycuronans	2. Glycanogalacturonans	Pectic substance Gums and mucilages
III. Glycuronoglycans	3. Sulphated glucurono-glycans	Brown seaweed mucilages
	4. Glucuronomannans	Gums and mucilages
	5. Glycuronoxylans	Hemicelluloses Gums and mucilages
	6. Glycuronogalactans	Gums Hemicelluloses

It should be noted that there is virtually no glycuronoglycan with a fully established structure at present. On the contrary, for a relatively large number of polysaccharides the structures of the backbones or basal cores have been clarified, thus allowing classification of glycuronoglycans using the structure of their cores.

An attempt to classify plant polysaccharides in this way was first made by Aspinall[12, 13] for gums and mucilages. Such a classification may apparently be used also for the entire class of plant acidic polysaccharides containing glycuronic acid residues.

Hence, the plant acidic polysaccharides, for which sufficient information has been obtained, may be divided into the following principal groups: guluronomannuronans, glycanogalacturonans, sulphated glucuronoglycans of brown seaweeds, glucuronomannans, glycuronoxylans and glycuronogalactans (*Table 1*). At the same time, it should be borne in mind that present knowledge of the detailed structures of certain polysaccharides is not yet sufficient to permit an unambiguous structural classification of all glycuronoglycans[13]. There can be no doubt that with the elucidation of the structure of still newer polysaccharides several additional groups may possibly appear. The discovery of unusual structural features in different polysaccharides would lead to the detection of similar fragments in other polysaccharides. Moreover, a classification based on basal structure revealed a new approach for examining polysaccharides with very complex structures and clarified the relationship between different groups of plant polysaccharides[13].

Beyond doubt, every plant contains polysaccharides belonging to different groups. Yet the amounts of polysaccharides occurring in a certain plant vary very widely.

Thus, to obtain hemicelluloses, hardwood and softwood are used along with stalks of numerous cereals and annuals[15]. The largest amounts of pectic

substances are contained in vegetable root-crops and fruit[5,9]. Mucilages are obtained from plant seeds and different algae[7,8]. Thus, brown seaweeds contain large amounts of alginic acids[11], whose commercial production mostly involves the use of laminarian, fucus and macrocystic seaweeds[8].

Numerous plants exude gums[7], such as the well-known cherry and arabic gums. However, the most common gum sources are tropical and subtropical plants.

The isolation[6–11] of glycuronoglycans from plant material is often preceded by removal of low molecular weight admixtures, usually by alcohol extraction. Some glycuronoglycans are readily soluble in water, which is used to extract such polysaccharides. More often, extraction is performed with aqueous salt solutions or alkaline agents.

In certain cases, highly polar organic solvents, e.g. dimethyl sulphoxide, are used. Further purification is carried out by means of dialysis and reprecipitation with ethanol.

The polysaccharide preparations thus obtained frequently contain more than one polysaccharide component. Fractionation is achieved by precipitation from an aqueous solution with different water-miscible solvents, such as alcohol, acetone and acids. In some cases, fractionation involves precipitation with special reagents, e.g. quaternary ammonium bases of the Cetavlon type[16]. Homogeneity is controlled by means of different kinds of electrophoresis[17], ultracentrifugation[18], chromatography on DEAE–cellulose[19] and gel-filtration on Bio-Gels and Sephadex[20,21]. In recent years, molecular-sieve chromatography has begun to be used also for preparative fractionation of polysaccharides.

All the usual methods for the structural study of the carbohydrate chain were used to establish the structure of glycuronoglycans[7,10,22]. However, the presence of uronic acid residues also affects to some extent the study of glycuronoglycan structures. Very close attention has been given to the essentially lower acid hydrolysis rate of glycuronoside linkages compared with the usual glycoside bonds. This circumstance is widely used in obtaining oligouronides or aldobiouronic acids. The elucidation of the structure of such fragments permits us to obtain very important evidence on the structure of the macromolecule.

Contrariwise, in the study of polysaccharides or their fragments, with carbohydrate chains consisting only of uronic acid residues, reduction of the acid polysaccharide to a corresponding neutral material is often used to facilitate further structural studies[23].

The wider use of enzymes in the study of the structure of glycuronoglycans in recent years should be noted. However, their use still remains insufficient because of difficulties connected with the availability of specific enzymes[10,24].

II. GLYCURONANS

Alginic acids
(GULURONOMANNURONANS)

The group of guluronomannuronans is represented by a large number of alginic acids. Alginic acid was originally obtained by Stanford in 1883 during

treatment of *Laminariaceae*, brown seaweeds, with aqueous sodium carbonate[7]. It formed very viscous solutions when acidified and precipitated from the extract in the form of a jelly.

Being present in seaweeds in the form of salts, alginic acid is usually linked by bivalent-cation bridges with other polysaccharides. Such complexes apparently fulfil very important biological functions in brown seaweeds and ensure their vital activity. Thus, for example, they regulate the water and salt contents in cells and effect the growth and development of the seaweed[7, 11]. The content and structure of alginic acid noticeably depend on its origin.

Nevertheless, glycuronic acids are virtually the sole components of the carbohydrate chain in alginic acids[7, 25]. Originally, it was maintained that the carbohydrate chain of alginic acid is composed of D-mannuronic acid residues. Later, however, it was proved that it also contains noticeable amounts of L-guluronic acid[25, 26] (see *Figure 1*).

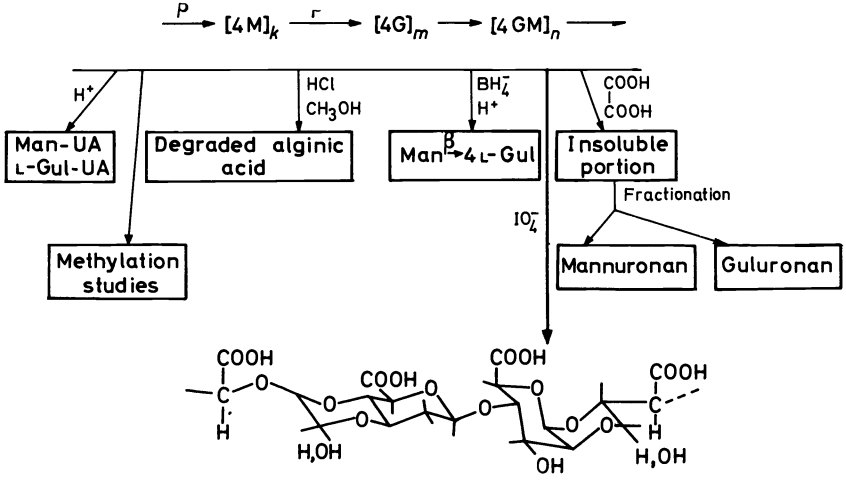


Figure 1. Alginic acid

Numerous analyses of alginic acids from different sources showed that the molar ratio between mannuronic and guluronic acids varies within a wide range from 0.5 to 3.0[7, 10, 25].

The great commercial significance of alginic acid has resulted in numerous works on its structure; such studies are at present continuing[7, 10, 11].

Partial hydrolysis of alginic acid with methanolic hydrogen chloride afforded a degraded alginic acid[7] (see *Figure 1*).

A study of this fragment showed the presence of β-1,4-glycoside linkages between the glycuronic acid residues. Moreover, the linear character of the carbohydrate chain of different alginic acids was established using methylation studies[27]. The isolation of 4-*O*-β-mannosyl-gulose (*Figure 1*) indicated that alginic acids contained fragments composed simultaneously of residues of both glycuronic acids. The hydrolysis of alginic acid with oxalic acid resulted in the formation of two fractions, which differed sharply in solubi-

lity[29]. The insoluble fragment was separated by means of an acid into two fractions, one of which represented virtually pure mannuronan, and the other guluronan, to show the presence in the alginic acid of sites consisting of residues of only one kind of uronic acid. Thus, alginic acid is essentially a block-polymer consisting of fragments of guluronan and mannuronan of different length and blocks comprising simultaneously the residues of both uronic acids[30–32] (*Figure 1*).

This structure was also confirmed in intense study of alginic acids with the aid of enzymic methods.

Although most of the enzymes called alginases were used as crude preparations, the examination of fragments that form after enzymatic digestion proved to be a substantial contribution to elucidation of the alginic acid structure. Furthermore, two specific alginases were isolated[33, 34] from *Haliotis* sp. These enzymes split β-1,4-linked mannuronic or guluronic acid residues, respectively. The successive actions of both enzymes showed the presence of such linkages in alginic acid. True, alginic acids did not completely decompose, this possibly being due to spatial difficulties or the presence of a small number of other types of linkages. The latter circumstance was noted earlier by Hirst and co-workers[27, 28], who suggested the presence of a certain number of 1,3-linkages in the macromolecule.

Periodate oxidation of alginic acid provided some interesting data (*Figure 1*). Under conditions that would exclude overoxidation, periodate consumption was only 0.45–0.55 mol per anhydro unit[35, 36]. These results contradict methylation evidence and data obtained by other structural methods, indicating the predominance of 1,4-linkages in the alginic acid molecule[7, 11, 27, 28]. The structural methods used previously were revised[37], only to confirm the existing understanding of alginic acid structure.

The problem was solved when it was suggested and shown that the low periodate consumption was due to the formation of six-member cyclic hemiacetals between neighbouring residues of monosaccharides[38, 39] (*Figure 1*).

Formation of such fragments impedes further oxidation with periodate. When sodium alginate was oxidized with periodate to its limiting consumption of 0.45 mol of the oxidant and then reduced with sodium borohydride, it again became susceptible to periodate, and further oxidation proceeded smoothly up to a second oxidation limit corresponding to a total consumption near to 1.0 mol of oxidant per anhydro unit, as expected.

This serves as convincing proof that periodate oxidation of alginic acids proceeded via initial formation of cyclic hemiacetals. This should also be taken into account when using periodate oxidation in the study of other polysaccharides containing polyuronide chains in their structure.

Thus, at present a large amount of information has been accumulated on the structure of alginic acids. However, numerous details of the precise structure of the macromolecule still remain unresolved.

III. GLYCANOGLYCURONANS

Glycanogalacturonans: pectic substances and related compounds

The next very wide group of plant acidic polysaccharides comprises

glycanogalacturonans represented by pectic substances and certain gums and mucilages, whose structures and properties resembled those of the pectin substances[1, 5–7, 9, 40].

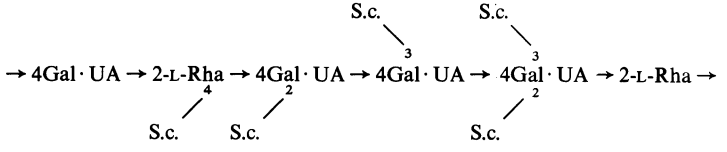
Pectic substances are contained in virtually all higher flowering plants[6, 9], *Zosteraceae* plants[41–43] and certain fresh-water algae[44, 45]. They represent a structural component of cell-walls fulfilling important biological functions[9, 45]: influence seed germination and cell growth; protect plants from withering; render plants more resistant to drought and cold; act as protectors of plants against phytopathogens. Possessing jelly-forming properties, pectins are widely used in the food industry and perfumery; they have also found application in medicine as substitutes for blood plasma and in the treatment of gastric diseases, etc.[9, 46].

The biological functions of gums and mucilages have not been sufficiently studied[7]. There are indications that gums play a certain role in the mechanical damage of vegetative tissues, while mucilages serve as the medium in which biochemical processes take place.

Gums and mucilages are very widely used as commercial products in different branches of industry and agriculture[7, 8].

Pectic substances were discovered as a particular group of compounds in 1825 by Bracconot[47], who gave them their name. The chemistry of pectic substances began in 1917[48], when it was shown that they are based on D-galacturonic acid, often esterified by methyl alcohol.

The existing nomenclature of pectic substances[49] considers them to be galacturonan derivatives. In this connection, several notions used in contemporary literature on pectic substances are distinguished. Protopectin represents an insoluble high molecular weight pectic complex, which is contained in plants, and when treated with diluted acids yields soluble pectin usually extracted from plant material. Pectin is a complex glycanogalacturonan. Pectinic acid is a galacturonan containing carboxyl groups esterified by methanol. Pectic acid is a galacturonan with free carboxyl groups.



Oligosaccharides:

1. Gal · UA → 2-L-Rha
2. Gal · UA → 2-L-Rha → 2-L-Rha
3. R → 2Gal · UA
4. R → 3Gal · UA
5. R → 4Rha → 4Gal · UA

where S.c. denotes side chains
R denotes residues of Xyl, Ara·f, Gal, GlcUA, etc.

Figure 2. Glycanogalacturonans

Many reports have been devoted to structural studies of pectic substances[9, 10, 40]. At present, one may consider it established that pectic substances represent complex glycanogalacturonans, which are often accompanied by neutral glycans, usually galactans, arabans and arabinogalactans.

The structure of pectic substances and other related glycanogalacturonans is known to remain far from being completely elucidated. There is very little information on the structure of protopectin[50].

Partial hydrolysis of glycanogalacturonans results in galacturonan[51]. The assumption concerning the linear structure of galacturonans as the backbone of pectic substances was suggested at the beginning of the 1930s[52] and further developed in the research of several authors[5] (*Figure 2*). At the same time, Hirst[1] suggested the presence of 1,4-linkages in galacturonan as well as in cellulose. The structure of galacturonan was established with the aid of all the structural methods of carbohydrate chemistry. As a result, galacturonan proved to be essentially a linear polysaccharide with α-1,4-linkages between the residues of D-galacturonic acid[5, 9, 40]. The conclusion on the α-configuration of glycosidic bonds was made on the basis of the high positive rotation of galacturonan.

The pyranose form of galacturonic acid was assumed on the basis of the very high stability of galacturonan to hydrolysis, although there is still no definite proof for the above assumption.

At the end of the 1940s, it was established[1] that the carbohydrate chain of galacturonan contains some residues of L-rhamnose. As was shown later[13], the various polysaccharides of this group contain interior chains of residues of D-galacturonic acid and L-rhamnose. The relative proportions of these two sugars in the inner chain vary within quite a wide range. As a rule, pectic substances contain a low percentage of rhamnose residues[7]. Among the gums of this group, three distinct sub-groups may be recognized: those in which the inner chains contain D-galacturonic acid residues with trace amounts of rhamnose residues; those in which the inner chains contain some blocks of D-galacturonic acid units interrupted by regions in which units of both sugars are present; and those in which substantial portions of the interior chains are composed of alternating sequences of the two sugars[13]. For instance, tragacanthic acid, *Khaya* gums and *Sterculia* gums belong to the first, second and third sub-group, respectively. It was assumed that rhamnose plays a substantial role in forming the precise structures of pectic substances and other glycanogalacturonans. Subsequently, numerous works dealing with partial acid and enzymatic hydrolysis of this group of compounds led to the isolation of an aldobiouronic acid (*Figure 2*, **1**), a trisaccharide (**2**) (see *Figure 2*), and oligosaccharides containing residues of galacturonic acid, rhamnose and other neutral monosaccharides[5]. This justified the conclusion that rhamnose residues are involved in the carbohydrate chain of galacturonan by 1,2-linkages and interlink separate galacturonan blocks to represent some branching points of glycanogalacturonans[53, 54] (*Figure 2*).

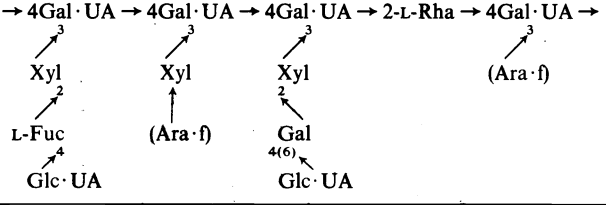
It was shown that residues of neutral sugars, mainly D-galactose and L-arabinose[5], are involved in the carbohydrate chain of glycanogalacturonans. Other monosaccharides are usually observed as minor components. The relationship of sugar residues in pectic substances and other related glycanogalacturonans noticeably differs, depending on the origin of the material[40]. At present, the results of fractionation and the determination of homogeneity[55] allow us to consider the presence of a covalent bond between neutral and acidic fragments of pectic substances and other glycanogalac-

turonans to be an authentically established fact; this is also confirmed by isolation of different aldobiouronic acids in the course of partial hydrolysis[53, 54, 56, 57] and by the results of Smith degradation studies on glycanogalacturonans[58]. In addition, these data demonstrated that side chains appeared to be joined to the backbone chain at positions C-2 and C-3 of the galacturonic acid residues, as well as to rhamnose residues. Hence, the general scheme of glycanogalacturonan structure may be presented in the way shown in *Figure 2*.

Despite intensive structural studies of glycanogalacturonans, there is not a single representative of this class of compounds today with completely established structure.

The pectic substances of apples have attracted the attention of investigators for many years[59], but information on their structure was obtained only recently[59, 60]. As a result of partial hydrolysis, two oligosaccharides, viz. Gal → Gal-UA and Xyl → Gal-UA, were obtained along with the aldobiouronic acids usually observed in such cases. The isolation of these oligosaccharides shows that the carbohydrate chain of apple pectin is branched; a covalent bond exists between the neutral and acidic components. The study of pectin by periodate oxidation showed the presence of branchings at C-2 and C-3 of the galacturonic acid residues.

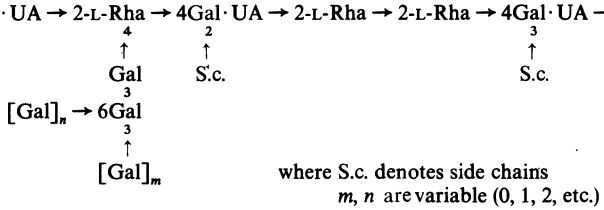
Aspinall conducted quite an exhaustive study of the pectins of alfalfa[61, 62] and lemon peel[53], tragacanth gum[63] and soyabean glycanogalacturonans[56, 64–66] (*Figure 3*). Partial hydrolysis yielded several acid and neutral oligosaccharides, which were separated and studied (*Figure 3*) to show close structural affinity of all the above-mentioned glycanogalacturonans. This was also confirmed by methylation studies.



Oligosaccharides	Tragacanth gum	Lemon peel pectin	Lucerne pectin	Soyabean polysaccharides
Xyl → 3Gal	+	+	+	+
Ara·f → 3Gal·UA	...	...	+	...
Glc·UA → 4-L-Fuc	+	+	+	+
Glc·UA → 4Gal	+	...	...	...
Glc·UA → 6Gal	+	+	+	+
L-Fuc → 2Xyl	+	...	...	+
Gal → 2Xyl	+	...	...	+

Representatives:
Tragacanth gum — Pectic substances of lucerne — Plantaglucide of *Plantago major* L.
Lemon peel pectin — Mucilage of *Tussilago farfara* L. — Acidic polysaccharides of soyabeans

Figure 3. Glycanogalacturonans



Oligosaccharides:

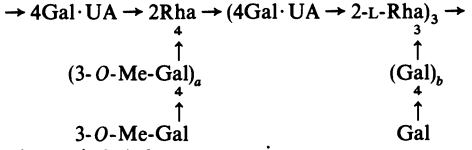
Gal → L-Rha → 4Gal·UA — Panaxan
Gal → 4-L-Rha → 4Gal·UA } *Khaya* gums
4-OMe-Glc-UA → 4Gal } Mucilage of opium poppy capsules

Figure 4. Glycanogalacturonans

Along with the usual oligosaccharides from residues of galacturonic acid and rhamnose (see *Figure 4*), oligosaccharides from residues of xylose and galacturonic acid appear to be highly interesting. They confirm the presence of branchings at C-2 and C-3 of the galacturonic acid. Also interesting is the presence of glucuronic acid residues, even though their number is very small. It should be noted that in all the cases examined, glucuronic acid is linked with fucose and galactose residues. The position of these fragments in the side chain has been clarified only in the case of tragacanth gum; however, these fragments seemed to occupy similar positions also in other glycanogalacturonans. Arabinofuranose residues are located mainly as terminals on the side chains. The frequency of branches in the relatively small number of rhamnose residues remains obscure.

The approximate structure of this group of glycanogalacturonans is shown in *Figure 3*. The pectic substances of sisal[23], coltsfoot *Tussilago farfara* L.[67] and the plantain *Plantago major* L.[68] have apparently similar structures, although there is still insufficient experimental evidence for a final conclusion.

The pectin of *Panax ginseng* C. A. Mey, which was named panaxan[69, 70], is probably very closely related to this group (*Figure 4*). However, a peculiarity about this pectin resides in the presence of a branched side chain



Oligosaccharides:
(1) (3-*O*-Me-Gal 1) → 4-L-Rha
(2) (3-*O*-Me-Gal 1) → 4(3-*O*-Me Gal)
(3) (3-*O*-Me-Gal 1) → 4(3-*O*-Me Gal 1) → 4-L-Rha

Figure 5. Slippery-elm mucilage

comprising 1,3- and 1,6-linked galactose residues. Most probably, the carbohydrate chain is attached to rhamnose residues, this being indicated by isolation of the acidic oligosaccharide shown in *Figure 4*.

The attachment of a galactan chain to rhamnose residues was shown in the case of the *Khaya* gum[13] by isolation and determination of the structure of the trisaccharide containing residues of galactose, rhamnose and galacturonic acid (*Figure 4*). An interesting feature of this glycanogalacturonan is the presence of terminal residues of 4-*O*-methyl-D-glucuronic acid connected by 1,4-linkage with galactose residues. A similar feature is a characteristic of the glycanogalacturonan mucilage from opium poppy capsules[71].

In all these cases, moreover, arabinofuranose residues are terminal.

Pectic substances have been isolated repeatedly from the bark of trees, e.g. fir[51], spruce[72], birch[73], elm[74], etc. These polysaccharides have been studied insufficiently; more detailed information has been obtained only for the glycanogalacturonan from the bark of *Ulmus fulva* (slippery-elm mucilage)[74] (*Figure 5*). The specific feature of this polysaccharide is the presence of 3-*O*-methyl-galactose residues in the side chains and the presence of a 1,3-bond between the galactose and rhamnose residues.

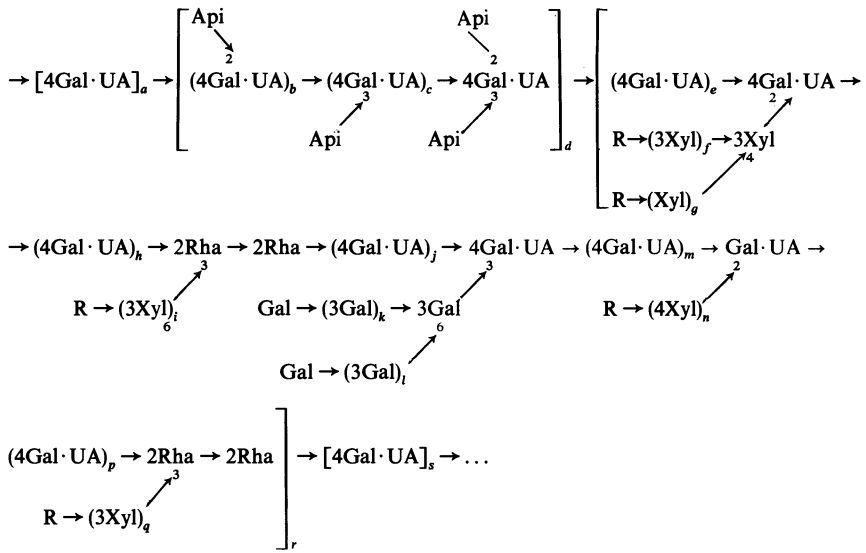
A very peculiar pectin was obtained from the cell walls of *Lemma* sp.[75, 76]. It is essentially a mixture of related apiogalacturonans, whose D-apiose content varies from 7.9 to 38.1 per cent. Apiogalacturonans with relatively high apiose content are pectinase-resistant. The study of their structure led to the conclusion that the existence of disaccharide side chains connected with galacturonan and comprising 1,3-linked apiose residues is possible[77].

A very specific pectin was isolated from *Zosteraceae* plants. It was first obtained in 1940 by Miroshnikov[41] from a White Sea *Zostera marina* L. He named it zosterine and showed that it belongs basically to the class of pectic substances. A structural study of zosterine isolated from various *Zosteraceae* plants collected in the Sea of Japan was carried out quite recently[43, 78].

The presence of a sufficiently large number of D-apiose residues is a characteristic feature of the carbohydrate chain of zosterine.

The results obtained allow us to assume the structural scheme of a zosterine molecule illustrated in *Figure 6*.

Thus, it is apparent from the above scheme that zosterine has a block structure and consists of the following main fragments: galacturonan, apiogalacturonan and heteroglycanogalacturonan, interlinked by galacturonic acid or rhamnose residues. Such a structure undoubtedly shows its polyfunctional character with respect to its biological properties. The apiogalacturonan fragment, distinguished by increased resistance to the action of pectolytic enzymes, most probably protects the plant from phytopathogens and causes the formerly noted[79] resistance against the natural processes of putrefaction and decomposition. Earlier it was shown[80] that zosterine is apt to form a high molecular weight aggregate in aqueous and salt-water solutions. In acid solutions, zosterine shows its jelly-forming properties, which are the more pronounced the more complex is the neutral portion of its molecule[81]. In this connection, galacturonan and heteroglycanogalacturonan fragments, which are readily susceptible to enzyme digestion, probably play a significant role in maintaining the aqueous and salt-water



where R are terminal residues (L-Ara·f, L-Ara·p, D-Xyl·p, 2-*O*-Me-Xyl·p)
coefficients *a*, *e–n*, *p–s* are variable (0, 1, 2...); *b*, *c* = 1, 2; *d* = 10–12; *r* = 5

Fragments:
I Galacturonan
II Rhamnogalacturonan
III Apiogalacturonan
IV Heteropolysaccharides A and B
(Apioxyloarabinorhamnogalacturonans)
V Oligosaccharides:

1. Oligogalacturonic acids
2. Xylooligosaccharides
3. Galactooligosaccharides
4. Xyl → 3Xyl → 3Rha
5. Xyl → 3Rha → 2Rha
6. Rha → 2Rha
7. Xyl → 2Gal·UA
8. Xyl → 4Xyl → 2Gal·UA
9. Gal·UA → 2Rha
   3
   Xyl
10. Ara → Xyl

Figure 6. Zosterine

régimes of *Zosteraceae* plants and in promoting their growth and development.

IV. GLYCURONOGLYCANS

1. Glycuronoglycans of brown seaweeds

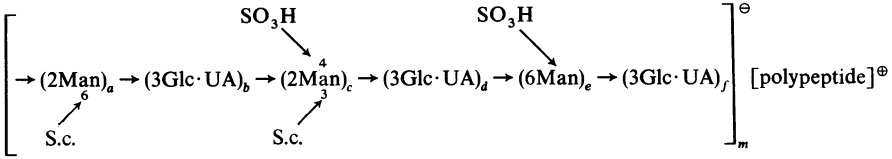
The presence in brown seaweeds of acidic polysaccharides containing glucuronic acid residues was indicated a relatively long time ago[7,25]. In the mid-1960s, Norwegian scientists carried out systematic investigations of brown seaweed polysaccharides to isolate a sulphated glucuronoglycan,

containing a firmly linked polypeptide component, from the seaweed *Ascophyllum nodosum*[82, 83]; they named the component 'ascophyllan'. Its carbohydrate chain comprises residues of D-glucuronic acid (19.2 per cent), L-fucose (25.2 per cent) and D-xylose (26 per cent). Moreover, sulphate (12.9 per cent) and peptide (*ca.* 12 per cent) were detected. Another glucuronoglycan related to ascophyllan was isolated from the same seaweed[84].

A similar polysaccharide was also obtained from the brown seaweeds *Laminaria hyperborea*[85] and *Fucus vesiculosus*[86], this being indicative of the presence of sulphated glucuronoglycans in other brown seaweeds. A systematic study[87] of brown seaweeds collected in the Sea of Japan showed that thirteen main species contain sulphated glucuronoglycans, which comprise four or five per cent of a firmly linked polypeptide component and are similar to ascophyllan[87]. A sulphated glucuronoglycan very similar in composition was recently isolated also from the brown seaweed *Sargassum linifolium*[88].

Unfortunately, only preliminary evidence on the structure of the majority of the said compounds has been obtained up to the present time. Thus, it was shown that they are based on a carbohydrate chain from glucuronic acid residues alternating with neutral monosaccharide residues[83, 84, 89], the side chains comprising mainly xylose and fucose residues linked by a β-1,3-bond[89, 90]. Moreover, the presence of 1,4-linked xylose residues and 1,2-linked fucose residues, containing sulphate groups, was also indicated[89]. It was likewise noted that this group of polysaccharides is distinguished by a high degree of branching[83, 84, 89].

Sulphated polysaccharides from *Sargassum pallidium*[91] and *Pelvetia wrightii*[92] have been examined in greater detail; they were called sargassan and pelvetian, respectively. Both polysaccharides contain four or five per cent of a firmly linked polypeptide component, and are closely related with respect to their main characteristics. Their carbohydrate chains contain residues of the same monosaccharides; D-galactose, D-mannose, D-xylose, L-fucose and D-glucuronic acid, whose content reaches 25 per cent.



where S.c. denotes side chains; polypeptide—polypeptide portion
Coefficients: *a–f* are variable (0, 1, 2, etc.); *m* is unknown value

Fragments:
I Glucuronomannan
II Glucuronoxylomannan
III Xylofucan
IV Oligosaccharides (fragments of branches):

1. Xyl → 3·L-Fuc
2. Xyl → 4Gal
3. Xyl → 6Gal → 6Man
4. L-Fuc → 2Xyl
5. L-Fuc → 2L-Fuc
6. L-Fuc → Gal
7. Xyl → 6Gal
8. L-Fuc → 2Xyl → Gal

Figure 7. Sargassan and pelvetian

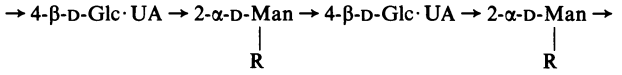
The composition of the polypeptide component involves sixteen amino acids with predominantly histidine content. In the course of ion-exchange chromatography on a cationite, a noticeable decrease of protein content was observed with preparations of sargassan and pelvetian; furthermore, this method allowed us to separate both compounds into carbohydrate and peptide portions. These results show that polypeptide is not a structural element in the carbohydrate chain. Probably sargassan and pelvetian represent carbohydrate–protein complexes, in which the polysaccharide and polypeptide are interlinked by ionic bonds, the polysaccharide component playing the role of a polyanion, and the polypeptide one that of a polycation.

It is quite possible that such complexes are involved in seaweeds as regulators of water–salt exchange; they may also take part in other specific biological functions, whose clarification will undoubtedly be interesting.

A structural study[93–99] of sargassan and pelvetian allowed us also to elucidate some structural features of their molecules, whereas their precise structure still will need examination. The structures of sargassan and pelvetian are shown schematically in *Figure 7*.

2. *Glucuronomannans*

This class of glycuronoglycans includes numerous gums[13], of which five (see *Figure 8*) have been studied in greater detail.



Oligosaccharides:
Glc·UA → 2Man (**1**)
Glc·UA → 6Gal (**2**)
Glc·UA → 2Man → 4Glc·UA → 2Man (**3**)
Xyl → 6Man (**4**); L-Ara·f → 3Man (**5**)
Gal → $[6Gal]_n$ → 6Gal (**6**)
Gal → $[6Gal]_n$ → 3L-Ara·f (**7**)
Xyl → 5L-Ara·f (**8**)
L-Ara·f → $[5L\text{-Ara}\cdot f]_n$ → 5L-Ara·f (**9**)

Representatives:
Cherry gum (*Prunus* sp.)
Gum ghatti (*Anogeissus batifolia*)
Leiocarpan A (*Anogeissus leiocarpus*)
Encephalartos longifolius gum
Virgilia oroboides gum

Figure 8. Glucuronomannans

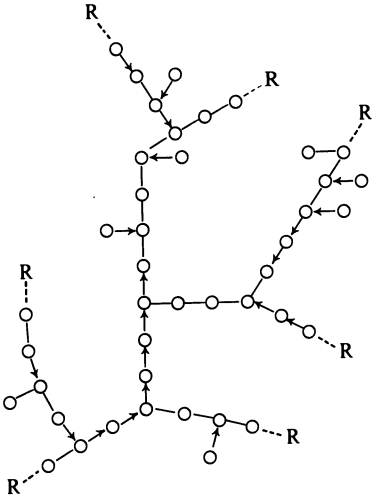
Aldobiouronic acids (**1**) and (**2**) were isolated as products of partial hydrolysis of all the polysaccharides of this type. In the case of the gum ghatti from *Anogeissus* sp., an acidic tetrasaccharide (**3**) was characterized to allow the determination of the sequence of sugar residues in the basal chains of glucuronomannan. Side chains are attached to mannose residues and, in some cases, are quite complex. The simplest are the side chains from the sole residue of D-xylose or L-arabinofuranose attached to the backbone by 1,6- and 1,3-linkages, respectively. This is evidenced by disaccharides (**4**) and (**5**).

The oligosaccharides (**6**)–(**9**) reflected the structure of more complex side chains (see *Figure 8*).

There are also still other more complex and branched side chains, whose structures remain rather obscure.

3. Glycuronogalactans

The largest single group of exuded gums belongs to the glycuronogalactan family of polysaccharides[13]. The group includes gum arabic, other gums from *Acacia* species, mesquite gum, etc. (*Figure 9*). The polysaccharides all contain: (a) a core of D-galactopyranosyl residues mutually joined by 1,3- and 1,6-linkages; (b) residues of D-glucuronic acid or its 4-*O*-methylether, or both, in the outer chains in terminal or near-terminal positions; and (c) outer chains of L-arabinofuranosyl residues and, in some cases, L-rhamnopyranosyl residues. With respect to the core structure and to certain aspects of that of the periphery, the hemicelluloses[15] from coniferous woods and hardwoods are similar to the exuded gums of this group. However, the hemicelluloses of this type are not too numerous (*Figure 9*).



○ are D-Gal·p
— β1,3-linkage
→ β1,6-linkage

R—outer chains:
L-Ara·f; L-Ara·f → 3-L-Ara·f → 3-L-Ara·f;
L-Ara·p → 3L-Ara·f → ; L-Gal → 3-L-Ara·f →;
(4-O-Me)-Glc·UA → 4Gal →; (4-O-Me)-Glc·UA → 6Gal—;
L-Rha → 4Glc →; Xyl → 3-L-Ara·f →.

Representatives:

Gums	Hemicelluloses
1. Gum arabic and others *Acacia* sp. gums	Softwoods
2. Mesquite gum (*Prosopis* sp.)	Larch (*Larix* sp.)
3. *Khaya* gum 6. Cholla gum (*Opuntia fulgida*)	Pinewood (*Pinus* sp.)
4. *Araucaria* gum 7. Jeol gum (*Odina wodier*)	Hardwoods
5. Lemon gum	Aspenwood (*Populus* sp.)

Figure 9. Glycuronogalactans

Many works[7, 13] have been devoted to the clarification of the structure of glycuronogalactans. Particular attention was given to the elucidation of the basal core structure, since, in this case, it has a very complex network structure. Recent years have also witnessed continued efforts to specify the individual elements of this structure. It is shown schematically in *Figure 9*.

Formation of degraded polysaccharides by controlled acid hydrolysis of the parent gums involves the preferential scission of arabinofuranosyl and

rhamnopyranosyl linkages. This shows that these residues are located exclusively on the outer chains. The isolation of different oligosaccharides in the course of fragmentation allowed us to establish the structure of several outer chains (*Figure 9*).

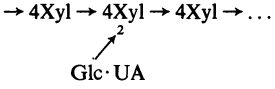
It is noteworthy that this group of plant acidic polysaccharides is distinguished by the highest complexity, and several of their structural features still remain obscure.

It is interesting to note that very recently Percival and Smestad[100] isolated from the green seaweed *Acetabularia crenulata* a glucuronogalactan, which probably belongs to the above-mentioned group, and differs in the presence of sulphate groups linked with galactose residues in the core.

4. Glycuronoxylans

A comparatively large number of gums belong to the glycuronoxylans; however, only two polysaccharides have been studied in detail[13]. Structurally, these gums are related to hemicelluloses, the majority of which belong precisely to this group of plant acidic polysaccharides[15]. After partial hydrolysis, it is characterized by the formation of one or two acidic trisaccharides (**1**) or (**2**), one of which contains glucuronic acid, and the other its 4-*O*-methyl ether (*Figure 10*).

In this connection, two types of structures, namely glucuronoxylans and 4-*O*-methyl-glucuronoxylans, may be examined.



Oligosaccharides:	Origin and sources:
Glc·UA → 2Xyl → 4Xyl (**1**)	cereals and annuals
4-O-Me-Glc·UA → 2Xyl → 4Xyl (**2**)	

Figure 10. Glucuronoxylans.

4-O-Me-Glc·UA 10–15%; OAc 3–19%; OMe—2%; $[\alpha]_D^{20}$ − 60 − 100°; DP 100–200

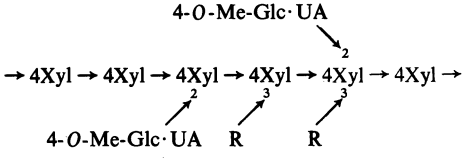
Origins and sources:

Hardwoods	Conifers	Cereals and annuals
Betula sp. (14–27%)	*Picea* sp.	Sisal (*Agave sisalana*)
Populus sp. (19–21%)		Hemp (*Cannabis* sp.)
Acer sp. (12–22%)		Sunflower (*Heliantus* sp.)
Fagus sp. (13–18%)		Cotton (*Gossipium* sp.)
Alnus sp. (14–24%)		
Malus sp.		
Prunus sp.		
Salix sp. (13–15%)		
Platanus orientalis		

Figure 11. 4-*O*-Methyl-glucuronoxylans (type I)

A relatively small group of glucuronoxylans involves the hemicelluloses of some cereals and annual grasses. Nearly all of them have quite simple structures (*Figure 10*).

4-*O*-methyl-glucuronoxylans should essentially be divided into two groups. In the first group, the polysaccharide side chains consist only of 4-*O*-methyl-D-glucuronic acid rèsidues. The schematic structure of these compounds is shown in *Figure 11*. These polysaccharides are contained in considerable amounts in hardwoods, and are virtually absent from coniferous woods[15]. Some cereals and annual plants contain quite a high percentage of such compounds. The distinctive feature of alfalfa hemicelluloses, which belong to this type of compound, is the presence in the backbone of slight amounts of 1,3-linked L-rhamnopyranose residues.



R is Xyl or LAra·f or Gal → 3L-Ara·f
4-*O*Me-Glc·UA 5–25%; $[\alpha]_D^{20}$ – 20–40°; DP 100–150

Origins and sources:		
1. Hemicelluloses		2. Gums
Softwoods (3–5%)	Cereals and weeds	
Picea sp. (4–5%)	Reed (*Phragmites* sp.)	*Sapote achras*
Pinus sp. (3–5%)	Fern (*Dryopteris* sp.)	*Watsonia* sp.
Larix sp.	*Dactylis glomerata*	
Abies sp.	Corn cob (*Zea mays*)	
	Wormwood (*Artemisia* sp.)	
	Corn (wheat, oats, etc.)	

Figure 12. 4-*O*-Methyl-glucuronoxylans (type II)

The second group of 4-*O*-methyl-glucuronoxylans is shown schematically in *Figure 12*. The side chains of these polysaccharides consist not only of single 4-*O*-methyl-D-glucuronic acid residues, but also of single xylopyranose and arabinofuranose residues attached by 1,2- and/or 1,3-linkages to the xylose residues of the core.

It is noteworthy that such polysaccharides are absent from hardwoods[75]. They are obtained from conifers, as well as from certain cereals and annual weeds[75]. Their structures are mostly rather simple and have been adequately studied[15].

V. CONCLUSIONS

In conclusion, the expediency of classifying plant acidic polysaccharides in terms of their basal core structures[13] should again be emphasized. Such a classification would make it possible to group and compare the glycuronoglycans isolated from various sources, and to clarify their relationships,

similarities and differences. This, in turn, would allow us to predict the main structural features of new representatives of the respective groups and, to some extent, serve as a guide to a structural study. Naturally, the structure of very many glycuronoglycans is still obscure and insufficiently studied, and new groups of acidic polysaccharides with common basal structures may, no doubt, appear in the future.

There is neither possibility nor need to enumerate all the people involved in the study of glycuronoglycan structures. However, it should be noted that over the past two or three years the number of works devoted to the study of glycuronoglycans has markedly decreased. It is common knowledge that the interest in structural investigations of various classes of compounds is conditioned by several factors, namely the possibility for commercial use; the importance of biological functions: the physiological activity; and the development of structural methods of investigation.

The possibilities for wide practical use of glycuronoglycans has long attracted the attention of investigators.

The clarification of the dependence of polysaccharide specifications on their nature has introduced the need to elucidate the main structural features of individual representatives, and this work was subsequently completed. The development of modern methods of structural analysis at the end of the 1950s and, especially, in the 1960s gave a new impetus to the study of glycuronoglycans and allowed us to clarify certain features in their precise structure. This, in turn, made it possible to associate some properties of polysaccharides with their structure. Unfortunately, the study of the biological function and physiological activity of glycuronoglycans is still obviously insufficient. At the same time, the possibilities of the available methods for providing basically novel information on the precise structure of plant acidic polysaccharides appeared to be exhausted by the end of the 1960s. As a result, by the beginning of the 1970s, the chemistry of glycuronoglycans was confronted by a situation which demanded a new stimulus in structural studies, but the implementation of this task proved to be far from simple.

For this reason, it would appear to be most expedient at present to give special attention to the study of the biological function and physiological activity of glycuronoglycans and to the development of new approaches for a structural investigation of the carbohydrate chain, particularly involving the development of techniques for its specific fragmentation.

The time has come to start the examination of the molecular structure of glycuronoglycans; their secondary, tertiary and quaternary structures; and the nature of their interrelation and interaction with other cell components. This would essentially require persistent development of even more effective structural methods. Only in this way would it be possible to speak about approaches for elucidation of the relationships between the structures and biological functions of glycuronoglycans.

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