THE CHEMISTRY OF GLYCURONOGLYCANS

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Plant polysaccharides are extremely diverse [1-15]. Up to the present time they have been divided according to a combination of properties into the following groups: 1) skeletal – celluloses [6, 14], 2) reserve polysaccharides [6, 10], 3) hemicelluloses [4, 6, 15], 4) pectin substances [1, 5, 6, 9], and 5) gums and mucilages [2, 3, 6-8, 10-13]. There are no sharp boundaries between these groups, and very frequently one and the same polysaccharide falls under the definition of several groups. This division has grown up historically and is connected mainly with the localization of the polysaccharides in plants and with their biological properties. In recent years plant polysaccharides have been divided according to their chemical characteristics into neutral, acidic, and basic [7].

We shall dwell only on the very large group of glycuronoglycans – acidic plant polysaccharides containing glycuronic acid residues.

According to their content of uronic acids, the members of the class of glycuronoglycans can be subdivided into three groups which differ appreciably in structure and properties: I – glycuronans with a carbohydrate chain consisting only of uronic acid residue; II – glycanoglycuronans, the basis of which consists of carbohydrate chains of uronic acids (in an amount exceeding 50%) but which also have neutral fragments; and III – glycuronoglycans which contain uronic acids but the amount, although it varies within wide limits, does not as a rule exceed 50%. Nevertheless, the basis of the molecule consists of neutral monosaccharides and their derivatives.

As noncarbohydrate components, acetyl, sulfate, methyl, and ester groups are not infrequently found in molecules of acidic plant polysaccharides. In nature, the glycuronoglycans are found in the form of potassium, magnesium, and iron salts; they are frequently accompanied by proteins and peptides bound to them in a definite manner and by neutral polysaccharides, and complex interrelationships of them with cellulose are observed [6-10]. The individual molecules of the glycuronoglycans are frequently bound to one another, forming a complex reticular structure, and both covalent and noncovalent bonds the nature of which not infrequently remains obscure, participate in the creation of such structures.

At the present time, there is scarcely one glycuronoglycan with a completely established structure. On the other hand, for a comparatively large number of polysaccharides the nature of the structure of the main carbohydrate chain, the skeleton of the molecule, has been determined, and this enables the glycuronoglycans to be classified according to the structure of their skeleton.

The first attempt to systematize plant polysaccharides using the structure of their basic skeleton was made by G. O. Aspinall for the case of gums and mucilages [12, 13]. This method of classification can obviously also be adapted for the whole class of acidic plant polysaccharides containing glycuronic acid residues. For this reason, the acidic plant polysaccharides concerning which sufficient information has been obtained can be assigned to the following main groups: I) glycuronans – guluronomannuronans; II) glycanoglycuronans – glycanogalacturonans; and III) glycuronoglycans – sulfated glucuronoglycans of brown algae, glucuronomannans, glucuronoxylans, and glucuronogalactans. There is no doubt that as the structures of new polysaccharides are determined the appearance of several other groups will be possible. The detection of unusual structural features in a particular polysaccharide permits the discovery of similar fragments in other polysaccharides. Furthermore, classification using the structure of the skeleton provides a convenient approach to the consideration of polysaccharides of very complex structure and serves as a method for determining the connection between different groups of plant polysaccharides [13].

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Each plant contains polysaccharides of different groups. However, the amounts of polysaccharides present in it vary greatly and by no means each plant can be a source of a particular group of polysaccharides. Thus, the wood of broadleaved and coniferous species of trees and shrubs and the stems of many cereals and annual plants are used to obtain hemicelluloses [15]. Pectin substances are found in the largest amounts in root vegetables and tree fruits [5, 9]. The source of isolation of mucilages consists of the seeds of plants and various algae [7, 8]. In particular, brown algae contain a large amount of alginic acids [11] for the industrial production of which Laminaria, Fucus, and Macrocystis seaweeds are most widely used [8].

Gums are formed by many plants [7]. Well known to everyone are cherry gum and gum arabic. But the usual source for their isolation are plants of the tropical and subtropical zones.

The isolation [6-11] of glycuronoglycans from plant raw material is generally preceded by the separation of low-molecular-weight impurities from it which not infrequently is achieved by ethanolic extraction. Many glycuronoglycans are readily soluble in water, which is used for extracting such polysaccharides. More frequently, they are extracted with aqueous solutions of salts or with alkaline agents. In some cases, highly polar organic solvents such as dimethyl sulfoxide are used.

Further purification [7-10] is performed by dialysis, and by precipitation and reprecipitation with ethanol. The polysaccharide preparations obtained in this way frequently contain more than one polysaccharide. They are fractionated by precipitation from aqueous solution with various water-miscible liquids, most frequently ethanol or acetone, and sometimes acids. In a number of cases special reagents are used for fractionation and, in particular, quaternary ammonium bases of the Cetavlon type [16]. Various types of electrophoresis [17], ultracentrifugation [18], chromatography on DEAE-cellulose [19], and gel filtration on Bio-Gels and Sephadexes [20, 21] are used to check homogeneity. In recent years, gel filtration has also come into use for the preparative fractionation of the polysaccharides.

All the usual methods of structural investigation of carbohydrate chains [7, 10, 22] are used to determine the structures of the glycuronoglycans. However, the presence in the glycuronoglycans of uronic acid residues imposes a definite imprint on the study of their structure. Fairly great attention is being devoted to the lower rate of acid hydrolysis of glycuronoside bonds as compared with ordinary glycoside bonds. This circumstance is being used widely to obtain oligouronides or aldobiuronic acids. The determination of the structure of such fragments enables very important information on the structure of the macromolecule as a whole to be obtained.

On the other hand, in the investigation of polysaccharides or their fragments, the carbohydrate chains of which consist only of uronic acid residues, the reduction of the acid polysaccharide to the corresponding neutral polysaccharide, which facilitates further structural investigation, is frequently used [23].

In recent years, enzymatic hydrolysis has been tested more widely in the determination of the structures of the glycuronoglycans [20, 24]; however, because of the difficulties connected with the preparation of specific enzymes these methods have not yet obtained wide use that is their due.

I. Glycuronans

Guluronomannuronans – Alginic Acids. The group of guluronomannuronans is represented by a large number of alginic acids. Alginic acid was first obtained by Stanford in 1883 by the treatment of brown algae of the family Laminariaceae with dilute solutions of sodium carbonate [7]. The acid gave very viscous solutions and on acidification it precipitated from the extract in the form of a jelly-like mass.

Being present in algae in the form of salts, it is usually bound by bridges of divalent cations with other polysaccharides. Such complexes apparently fulfill important biological functions in brown algae and ensure their vital activity; in particular, they regulate the water and salt regime and influence the growth and development of the algae [7, 11]. The amount of alginic acids and their nature depends appreciably on the type of algae.

Nevertheless, practically the only components of the carbohydrate chain of these acids are glycuronic acids [7, 25]. It was originally considered that the carbohydrate chain of alginic acid was constructed of D-mannuronic acids, but then it was found that it also contains an appreciable amount of L-guluronic acid [25, 26].

According to numerous analyses of alginic acids from various sources, the ratio between the mannuronic and guluronic acids varies within wide limits (from 0.5 to 3.0) [7, 10, 25]. In view of the great technical importance of alginic acid, its structural investigation is being continued at the present time [7, 10, 11].

The partial hydrolysis of alginic acid with methanolic hydrogen chloride gave a destructured alginic acid [7]. The results of a study of this fragment showed that there are 1,4-glycosidic bonds between the glycuronic acid residues. In addition, the linear nature of the carbohydrate chain of different alginic acids has been established by methylation [27]. The isolation of 4-O-β-mannosylgulose shows that alginic acids contain fragments consisting simultaneously of residues of the two glycuronic acids. The hydrolysis of alginic acid with oxalic acid forms two fractions differing sharply in solubility [29]. The insoluble fragment can be separated by acid into two fractions one of which is practically pure mannuronan and the other is guluronan, which shows the presence in the carbohydrate chain of alginic acid of sections consisting of residues of only one type of uronic acid. Thus, alginic acid is a block polymer consisting of fragments of guluronan and of mannuronan of different lengths and blocks constructed of the residues of both uronic acids simultaneously [30, 32] (Scheme 1):

Alginic Acids*

$$\rightarrow [4M]_k \xrightarrow{?} [4G]_m \xrightarrow{?} [4GM]_n,$$

Scheme 1

where M represents a D-mannuronic acid residue, G represents a L-guluronic acid residue, and k, m, n are the numbers of residues in the chain.

This structure has also been confirmed in an intensive study of alginic acids by enzymatic methods.

Although the majority of enzymes used, alginases, are in the form of crude preparations, the investigation of the fragments formed on enzymatic treatment have made a substantial contribution to the study of the structure of alginic acid. Furthermore, from abalones it has been possible to isolate two specific enzymes [33, 34] one of which cleaves β-1,4-bonds between mannuronic acid residues while the other cleaves those between guluronic acid residues. The successive action of the two enzymes has shown the presence of these types of bonds in alginic acid. It is true that the alginic acids were not completely decomposed, which may be due to steric hindrance or show the presence in them of a small number of other types of bonds. The latter circumstance has been reported previously by Hirst and his co-workers [27, 28], who assumed that there is a certain number of 1,3-bonds in the macromolecule.

Interesting information has been obtained by the oxidation of alginic acid with periodate (see Scheme 1). Under conditions excluding overoxidation, the consumption of periodate was only 0.45-0.55 mole per anhydro unit [35, 36], which contradicts the results of methylation and other structural methods showing a predominating content of 1,4-bonds [7, 11, 27, 28]. A revision of the structural methods used previously has been undertaken [37], but this only confirms established ideas on the structure of the alginic acids.

The question was solved in practical terms when it was shown that the low consumption of periodate is connected with the formation of six-membered cyclic semiacetals between neighboring monosaccharide residues [38, 39] (see Scheme 1). The formation of such fragments prevents further oxidation by periodate. When the periodate-oxidized alginate is reduced with tetrahydroborate and the resulting polyalcohol is reoxidized, the subsequent oxidation takes place smoothly and the consumption of periodate amounts to 1.0 mole per anhydro unit, as was to be expected. These results form a convincing proof of the periodate oxidation of alginic acid through the initial formation of cyclic semiacetyls. A similar state of affairs must be borne in mind in the study by periodate oxidation of other polysaccharides containing polyuronide sections in their structure.

Thus, at the present time a large amount of information has been accumulated on the structure of the alginic acids; nevertheless many details of the fine structure of their macromolecule still remain obscure.

II. Glycanoglycuronans

Glycanogalacturonans – Pectin Substances and Related Compounds. The next numerous group of acid plant polysaccharides – the glycanogalacturonans – is represented by the pectin substances and some gums and mucilages similar in structure and properties [1, 5-7, 9, 40].

Pectin substances are present in practically all higher land plants [6, 9] and seaweeds [41-43] and in a number of fresh-water algae [44, 45]. They are present in the composition of the cell walls and fulfill

*Scheme as in Russian original – Publisher.

important biological functions [9, 45]: They affect the germination of the seeds and the growth of the cells, protect the plants from drying out, increase their resistance to drought and frost, fulfill a protective function in the interrelationships of plants with phytopathogens. The pectins are widely used as gelling agents in the food industry, in perfumery, and in medicine [9, 46].

The biological functions of gums and mucilages have been investigated inadequately [7]. There is information that gums play a determining role in the mechanical damage to plant tissue. Mucilages serve as a medium in which biochemical processes take place. Gums and mucilages are required in various fields of industry and agriculture [7, 8].

Pectin substances were discovered as definite compounds by Braconnot [47] in 1825; he gave them this name. The beginning of the chemistry of the pectin substances dates to 1917 [48], when it was shown that they were based on D-galacturonic acid partially esterified with methanol.

According to the nomenclature of pectin substances that exist at the present time [49] they are considered as galacturonan derivatives. In this connection several terms used in the modern literature on pectin substances are distinguished. Protopectin is an insoluble high-molecular-weight pectin complex present in plants and giving the soluble pectin that is usually extracted from plant material on treatment with dilute acids. Pectin is a complex glycanogalacturonan. Pectinic acid is a galacturonan containing carboxy groups partially esterified with methanol. Pectic acid is a galacturonan with substantially all its carboxy groups unesterified.

There is a large number of publications dealing with the structural investigation of pectin substances [9, 10, 40]. It may be considered as established today that pectins are complex glycanogalacturonans which are frequently accompanied by neutral glycans, most usually galactans, arabans, and arabinogalactans. Many obscure points remain in the structure of the pectins, just as in the case of other, related, glycanogalacturonans. Very little is known about the structure of protopectin [50].

The partial hydrolysis of glycanogalacturonans forms galacturonan [51]. The hypothesis of the linear structure of galacturonan as the main carbohydrate chain of pectin substances was put forward at the beginning of the 30's [52] and was subsequently developed by various authors [5]. The hypothesis [1] has been put forward according to which in galacturonan the galacturonic acid residues are connected by a 1,4-glycosidic bond as in cellulose. The structure of galacturonan was determined by means of all the existing methods of carbohydrate chemistry. As a result, it was shown that galacturonan is a linear polysaccharide with α-1,4-bonds between D-galacturonic acid residues [5, 9, 40]. The conclusion concerning the α-configuration of the glycosidic bonds was based on the high positive rotation of galacturonan.

The pyranose form of the galacturonic acid ring was assumed on the basis of the extremely high resistance of galacturonan to hydrolysis, although at the present time there is no strict proof of this assumption.

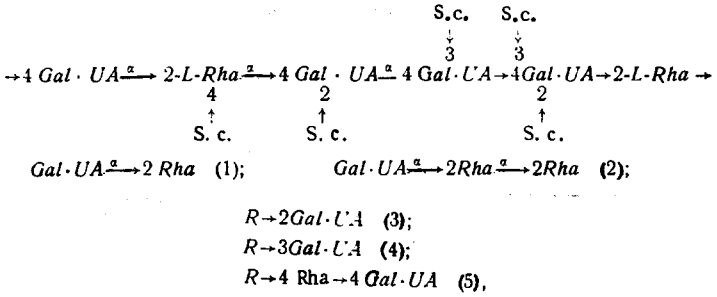
As early as the end of the 40's, it was established [1] that the carbohydrate chain of galacturonan includes L-rhamnose residues. The hypothesis was put forward that rhamnose plays a fundamental role in the creation of the fine structure of the pectin substances and of other glycanogalacturonans. Numerous subsequent investigations using partial acid and enzymatic hydrolysis of this group of compounds led to the isolation of an aldobiuronic acid (1) and a trisaccharide (2) (Scheme 2), and then of oligosaccharides containing residues of galacturonic acid, rhamnose, and other neutral monosaccharides (5). This permitted the conclusion that the rhamnose residues are included in the carbohydrate chain of the galacturonan by 1,2-bonds, connect individual blocks of the galacturonan with one another, and serve as points of branching of the carbohydrate chain of the glycanogalacturonans [53, 54] (see Scheme 2). As a rule, the pectins contain a small percentage of rhamnose residues [7]. However, in gums of this type the relative amounts of galacturnoic acid and rhamnose may vary within fairly wide limits and, in addition to gums containing a small amount of rhamnose residues, glycanogalacturonans are found in which considerable sections of the main chain consist of alternating galacturonic acid and rhamnose residues [13]. A very small amount of rhamnose residues is found in gum tragacanth and in the pectin-like mucilages of soya beans, while a somewhat larger amount of it has been found in the gum of the eucalyptus tree, Khaya sp. On the other hand, the gums from various species of Sterculia and from Cochlospermum gossypium belong to the second group.

It has been shown that the carbohydrate chain of glycanogalacturonans contains residues of neutral monosaccharides, especially D-galactose and L-arabinose [5]. Other monosaccharides are usually found as minor components. The ratio of the monosaccharides in pectins and other glycanogalacturonans varies appreciably according to the source of isolation [40]. At the present time, the results of fractionation and of

the determination of homogeneity permit us to consider as reliably established the presence of a covalent bond between the neutral and acid fragments of the pectins and other glycanogalacturonans, which is also confirmed by the production of various aldobiuronic acids on partial hydrolysis [53, 54, 56, 57] and by the results of a study of the Smith degradation of the glycanogalacturonans [58].

Furthermore, in addition to the rhamnose residues the galacturonic acid residues may also serve as branching points for the main chain through the C_2 and C_3 positions. Consequently, the general scheme of the construction of glycanogalacturonans can be represented in the following way (Scheme 2):

Glycanogalacturonans (general scheme)



Scheme 2

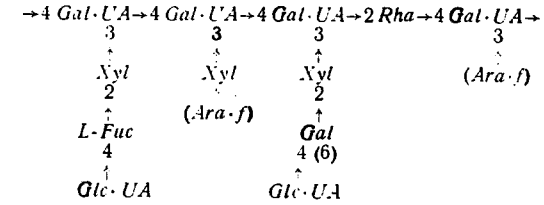
where S.c. represents carbohydrate side chains, and
R represents Xyl, Araf, Gal, Glc, UA, etc., residues.

In spite of the intensive structural investigation of glycanogalacturonans, at the present time there is not one representative of this class of compounds with a fully determined structure.

The pectin substances of apples have long attracted the attention of workers [59], but comparatively little about their structure has become known [59, 60]. On partial hydrolysis, in addition to the aldobiuronic acids usual in such cases, two other acid oligosaccharides were obtained: Gal→Gal·UA and Xyl→Gal·UA. The isolation of these oligosaccharides shows that the carbohydrate chain of apple pectin is branched and there is a covalent bond between the neutral and the acid components. The periodate oxidation of the pectin showed the presence of branching at C_2 and C_3 of the galacturonic acid.

Aspinall has made a fairly detailed study of the glycanogalacturonans of group I, which includes the pectins of lucerne [61, 62] and of lemon peel [53], gum tragacanth [63], and the glycanogalacturonans of soya beans [56, 64, 66] (Scheme 3). Partial hydrolysis of these substances gave a number of acidic and neutral oligosaccharides which were separated and investigated (see Scheme 3). The close structural similarity of all the glycanogalacturonans mentioned above was shown. This was also confirmed by the results of methylation.

Glycanogalacturonans of Group I



Oligosaccharides	Tragacanth	Lemon	Luceme	Soya beans
Xyl→3 Gal·UA	—	+	—	+
Ara·f→3 Gal·UA	...	...	—	...
Glc·UA→4-L-Fuc	—	+	+	+
Glc·UA→4 Gal	—	...	...	...
Glc·UA→6 Gal	—	+	+	+
L-Fuc→2 Xyl	—	...	...	+
Gal→2 Xyl	—	...	...	+

Scheme 3

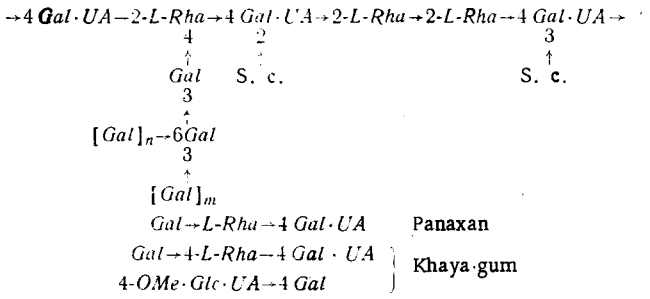
In addition to the usual oligosaccharides composed of residues of galacturonic acid and rhamnose, great interest is presented by oligosaccharides composed of residues of xylose and galacturonic acid: Xyl→3Gal·UA and Xyl→2Gal·UA. The appearance of such fragments shows the presence of branchings at C_2 and

C_3 of galacturonic acid. An interesting fact is the presence of glucuronic acid residues in the macromolecule, although the number of such residues is very small. We may note that in all the cases considered the glucuronic acid is bound to fucose and galactose residues. The positions of these fragments in the side chain has been established only in the case of gum tragacanth, but there are grounds for assuming that these fragments occupy the same position in other glycanogalacturonans. Arabinofuranose residues are found mainly at the ends of the side chains. It is not completely clear whether there is branching at a comparatively small number of rhamnose residues and what is the nature of these branchings. A preliminary structure of this group of glycanogalacturonans is shown in Scheme 3.

A similar structure is obviously possessed by the pectin substances of sisal [23], coltsfoot [67], and the plantain (plantaglucide, studied by A. G. Gorin [68]), although the experimental facts for a definitive conclusion are few.

The pectin of ginseng – panaxan – is apparently very close to this group [69, 70] (Scheme 4). However, a feature of this pectin is the presence of a branched side chain constructed of 1,3- and 1,6-bound galactose residues. Such a carbohydrate chain is probably attached to rhamnose residues, as is shown by the isolation of the acidic oligosaccharide shown in Scheme 4.

Glycanogalacturonans of Group II

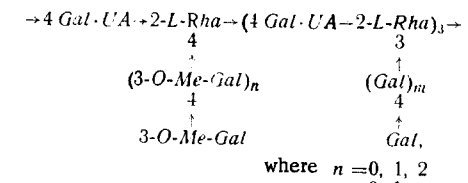


Scheme 4

The gum of the eucalyptus tree Khaya belongs to this group of glycanogalacturonans [13]. In this case, the addition of a galactan chain to rhamnose residues is confirmed by the isolation and determination of the structure of a trisaccharide containing galactose, rhamnose, and galacturonic acid residues (see Scheme 4). An interesting feature of this glycanogalacturonan is the presence in it of terminal 4-O-methylglucuronic acid residues attached by a 1,4-bond to galactose residues. A similar fragment is also characteristic for the glycanogalacturonan (mucilage) of opium poppy heads studied by Norwegian workers [71]. In all these cases, moreover, the terminal residues are arabinofuranose residues.

Pectin substances have been isolated repeatedly from the bark of the fir [51], the spruce [72], the birch [73], the elm [74], etc. These polysaccharides have been studied inadequately. More detailed information has been obtained only for the glycanogalacturonan of elm bark [74] (Scheme 5). A feature of this polysaccharide is the presence of 3-O-methylgalactose residues in the side chains of a 1,3-bond between the galactose and rhamnose residues.

Glycanogalacturonans of the Bark of the Elm Ulmus fulva

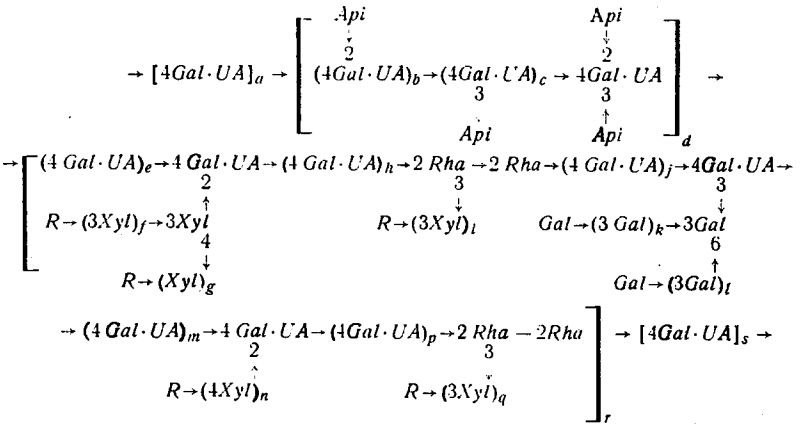


Scheme 5

A pectin has been obtained from the cell walls of duckweed, Lemna sp., which has a sugar fraction of unusual composition [75, 76]. It consists of a mixture of similar apiogalacturonans the D-apiose content of which varies from 7.9 to 38.1%. Apiogalacturonans with a comparatively high content of apiose are stable to the action of pectinase. The results of a study of their structure have permitted the conclusion that disaccharide side chains exist which are connected to the galacturonan and consist of 1,3-bound apiose residues [77].

A very peculiar pectin is contained in seaweeds. It was first isolated from White Sea eelgrass in 1940 by V. I. Miroshnikov [41], who called this substance zosterin and showed that it belonged basically to the class of pectin substances. A structural investigation of the zosterin isolated from various seaweeds of the sea of Japan has been performed quite recently [43, 78]. A characteristic feature of the carbohydrate chain of zosterin is the presence in it for a fairly large number of D-apiose residues. On the basis of the results obtained, the following shceme of the structure of the zosterin molecule has been suggested (Scheme 6):

Zosterin



Scheme 6

where R represents the terminal residues (L-Arap, L-Araf, D-Xylp, and 2-O-Me-Xylp), and the coefficients *a*, e-n, and p-s = 0, 1, 2, 3,..., b and c = 1, 2, d = 10-12, and r = 5.

By the partial hydrolysis, enzymatic hydrolysis, and acetolysis of zosterin high-molecular-weight fragments have been isolated: a galacturonan, a rhamnogalacturonan, an apiogalacturonan, etc. Furthermore, very diverse oligosaccharides have been obtained, and the structures of the fragments have been studied in detail.

It can be seen from the scheme given that zosterin has a block structure and consists of the following main fragments: galacturonan, apiogalacturonan, and a heteroglycanogalacturonan, which are connected to one another by galacturonic acid or rhamnose residues. Such a structure of zosterin undoubtedly reflects its polyfunctional nature in the manifestation of biological properties. The apiogalacturonan fragment, which is distinguished by a higher resistance to the action of pectolytic enzymes, probably fulfills protective functions, protects the seaweeds from the action of phytopathogens, and is responsible for the resistance [79] of these plants to natural processes of putrefaction and decomposition. It has been shown previously [80] that zosterin tends to form high-molecular-weight aggregates in aqueous and aqueous-salt solutions. In acid solutions zosterin shows gelling properties, which are the more distinct the more complex the neutral part of its molecule [81]. In this connection, the galacturonan and heteroglycanogalacturonan fragments, which readily undergo the action of hydrolytic enzymes, possibly play an important role in maintaining the water and water-salt regime of the seaweeds in the growth and development of these plants.

III. Glycuronoglycans

1. Sulfated Glucuronoglycans – Mucilages of Brown Algae. The presence in brown algae of acidic polysaccharides containing glucuronic acid residues was shown a comparatively long time ago [7, 25]. In the middle of the 60's, Norwegian workers systematically studying the polysaccharides of brown algae isolated from the alga Ascophyllum nodosum a sulfated glucuronoglycan containing a strongly bound polypeptide component [82, 83]. They called it ascophyllan. Its carbohydrate chain contains residues of D-glucuronic acid (19.2%), L-fucose (25.2%), and D-xylose (26%). In addition, 12.9% of sulfate and about 12% of a peptide were found. From the same alga another glucuronoglycan, related to ascophyllan, was isolated [84]. A similar polysaccharide has been obtained from the brown algae Laminaria hyperborea [85] and Fucus vesiculosus [86], which shows the presence of sulfated glucuronoglycans in other brown algae, as well. A systematic investigation [87] of the brown algae of the sea of Japan has shown that 13 of the main types include sulfated glucuronoglycans containing 4-5% of a strongly bound polypeptide component, which are similar to ascophyllan [87]. Very similar in composition is a sulfated glucuronoglycan isolated recently from the brown alga Sargassum linifolium [88].

However, up to the present time only preliminary information on the structure of the majority of these compounds has been obtained. In particular, it has been established that they are based on a carbohydrate chain consisting of residues of glucuronic acid alternating with residues of neutral monosaccharides [83, 84, 89], and the side chains are constructed of xylose and fucose residues connected by β-1,3-bonds [89, 90]. In addition to this, there are 1,4-bound xylose residues and 1,2-bound fucose residues containing sulfate groups [89]. It has also been found that these polysaccharides are distinguished by a high degree of branching [83, 84, 89].

The sulfated polysaccharides from the algae Sargassum pallidum [91] and Pelvitia wrightii [92], called respectively, sargassan and pelvecyan, have been studied in more detail. These polysaccharides each contains 4-5% of a strongly bound polypeptide component and they are similar to one another in their main characteristics. Their carbohydrate chains consist of residues of the same monosaccharides: D-galactose, D-mannose, D-xylose, L-fucose, and D-glucuronic acid, which is present in an amount of 25%.

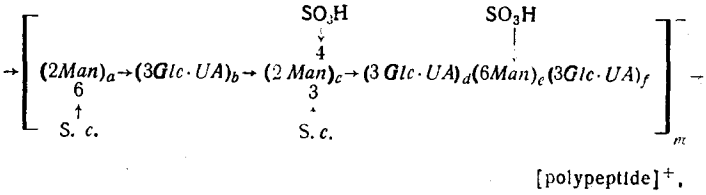
The polypeptide component consists of the same 16 amino acids with a predominance of histidine. When sargassan and pelvecyan are subjected to ion-exchange chromatography on cation-exchange resins, a considerable decrease in the amount of protein is observed and, moreover, both compounds can be separated in this way into a carbohydrate and a peptide fraction. These results show that the polypeptide is not a structural element of the carbohydrate chain. Sargassan and pelvecyan probably consist of carbohydrate-protein complexes in which the polysaccharide and the polypeptide are bound to one another by ionic bonds with the polysaccharide component playing the role of a polyanion and the polypeptide component that of a polycation.

It is quite possible that such complexes in algae serve as regulators of the water-salt metabolism and fulfill other specific biological functions the elucidation of which presents undoubted interest.

The results of structural investigations of sargassan and pelvecyan [93-99] have already permitted a determination of the basic nature of the structures of their molecules. The structures of the side chains has not definitively established. On partial hydrolysis it was possible to isolate a number of oligosaccharide fragments forming parts of the side chains: Xyl → 3Fuc, Xyl → 4Gal, Fuc → 2Xyl, Xyl → 6Gal → 6Man, Xyl → 6Gal, Fuc → 2Xyl → Gal, etc.

The structure of sargassan and pelvecyan is shown in Scheme 7.

Sargassan and Pelvecyan



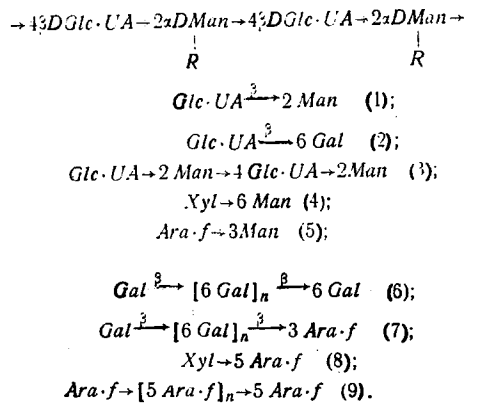
Scheme 7

where S.c. represents carbohydrate side chains, [polypeptide] represents a polypeptide component of an established structure, and the coefficients have the values: a-f = 0, 1, 2, while m is unknown.

2. Glucuronomannans. Many gums belong to the class of glucuronoglycans [13] and of these the polysaccharides of cherry gum (Prunus sp.), leiocarpan A, and the gum of Anogeissus sp., Encephulartos longifolius and Virgilia oroboides have been studied in some detail.

The aldobiuronic acids (1) and (2) have been isolated as products of the partial hydrolysis of all the polysaccharides of this type (Scheme 8). In the case of the gums from Anogeissus sp., the acidic tetrasaccharide (3) was isolated, which enabled the sequence of monosaccharide residues in the main carbohydrate chain to be determined. The side chains are attached to the mannose residues and in a number of cases have a fairly acidic nature. Side chains consisting of a single D-xylose or L-arabinofuranose residue attached to the mannose residues of the main chain by 1,6- and 1,3-bonds, respectively, are the simplest. This is shown by the disaccharides (4) and (5). The structure of the more complex side chains is reflected by the oligosaccharides (6)-(9). There are also more complex branched side chains the structure of some of which have been established, but in the majority of cases they are far from having been fully elucidated. A general scheme of the structure of the glucuronomannans is given below (Scheme 8).

Glucuronomannans

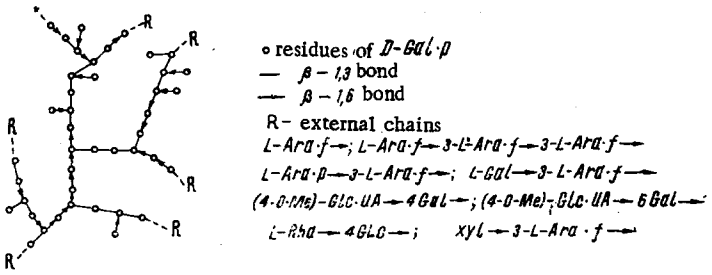


Scheme 8

3. Glycuronogalactans. The largest group of gums consists of the glycuronogalactans [13]. It includes many gums such as mesquite gum, Khaya gum, araucaria gum, lemon gum, chollu gum, jeol gum, and others, and also the widely known gum arabic, which is obtained on an industrial scale. These polysaccharides contain a skeleton consisting of D-galactopyranose residues connected by 1,3- and 1,6-bonds, D-glucuronic acid residues present at the nonreducing ends or close to the end positions, and side chains consisting of L-arabinofuranose and D-xylopyranose or, sometimes, L-rhamnopyranose residues. In their monosaccharide compositions, their structures, and their properties, some hemicelluloses [15] of coniferous and broad-leaved species of trees (pine – Pinus sp.; larch – Larix sp.; poplar – Populus), which are, in fact, fairly few in number, are very similar to this group of gums.

Information on the determination of the structure of the glycuronogalactans is given in many publications [7, 13]. Particular attention has been devoted to determining the structure of the skeleton, since in this case it has a very complex reticular structure. The individual elements of this structure have been refined in recent years. An example of the skeletal structure is shown in Scheme 9.

Glycuronogalactans



Scheme 9

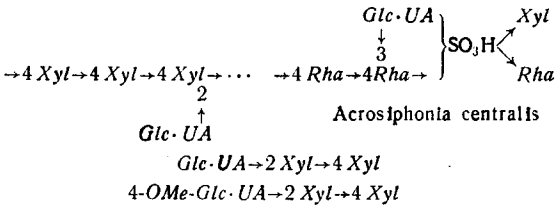
The formation of degraded polysaccharides of this group by partial hydrolysis includes the predominant decomposition of the arabinofuranose and rhamnopyranose bonds. It must be assumed that these residues are present exclusively in external side chains. The isolation of various oligosaccharides in the process of fragmentation has enabled the structures of a number of external side chains to be determined.

We may mention that this group of acidic plant polysaccharides is distinguished by the greatest complexity, and many structural features of them are still obscure.

It is interesting to recall that recently Percival et al. [100] have isolated from the green alga Acetobularia crenulata a glucuronogalactan which probably belongs to this group and is distinguished by the presence of sulfate groups bound to galactose residues in the skeleton.

4. Glycuronoxylans. Comparatively the largest number of gums belongs to the group of glycuronoxylans, but only two polysaccharides have been studied in fairly great detail [13]. These gums are structurally related to the hemicelluloses, the majority of which belong just to this group of acidic plant polysaccharides [15]. They are characterized by the formation of partial hydrolysis of one of two acidic trisaccharides: (1) or (2). One of the latter contains glucuronic acid and the other its 4-O-methyl ether (Scheme 10).

Glucuronoxylans

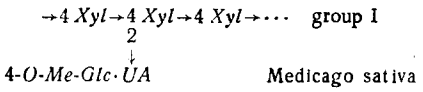


Scheme 10

In this connection we may consider two types of structures here: glucuronoxylans and 4-O-methylglucuronoxylans. A small group of glucuronoxylans is composed of the hemicelluloses of some cereals (maize stumps and wheat, straw and bran) and annual herbs (jute). They almost all have a fairly simple structure (see Scheme 10). An exception is the acidic sulfated polysaccharide isolated from the green alga Acrosiphonia centralis [101]. The structure of this glucuronoxylan is distinguished by greater complexity and has not yet been definitively elucidated.

It is desirable to divide the 4-O-methylglucuronoxylans into two groups. In the polysaccharides of the first group, the side chains consist only of 4-O-methyl-D-glucuronic acid. The structure of such compounds is shown in Scheme 11.

4-O-Methylglucuronoxylans

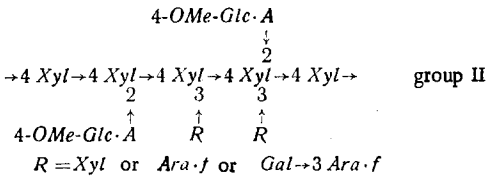


Scheme 11

Similar saccharides are found in considerable amount (10-30%) in the wood of broad-leaved trees. Thus, the birch (Betula sp.) contains 14-27% of gum, the poplar (Populus sp.) 19-21%, the maple (Acer sp.) 12-22%, the beech (Fagus sp.) 13-18%, the alder (Alnus sp.) 14-24%, and the willow (Salix sp.) 13-15% [15].

Glucuronoxylans are not present in coniferous species. Some grasses and annual plants, such as hemp, sisal hemp, and cotton pods have a fairly high percentage (10-20%) of this type of compound. A distinguishing feature of the hemicelluloses of alfalfa, which belongs to this type, is the presence in the main carbohydrate chain of a small number of 1,3-bound L-rhamnopyranose residues.

The second group of 4-O-methylglucuronoxylans may be represented generally by Scheme 12.



Scheme 12

The side chains of these polysaccharides consist not only of individual 4-O-methyl-D-glucuronic acid but of individual residues of xylopyranose or arabinofuranose attached by 1,2- and (or) 1,3-bonds to the xylose residues of the main carbohydrate chain. Such polysaccharides are absent from broad-leaved species of trees [75]. The source of their production consists of coniferous species (3-9%) and also some grasses and annual herbs [75]. In particular, reeds contain up to 16% of glucuronoxylans. The structure of these compounds is comparatively simple in the majority of cases and has been studied fairly well [15].

In conclusion, we must emphasize once more the desirability of classifying acidic plant polysaccharides on the basis of the structure of their sekeleton [13]. This enables the glycuronoglycans isolated from various sources to be combined into groups, comparisons to be made between them, and their affinities, similarities, and differences to be elucidated. This approach provides the possibility of predicting the main structural features of new representatives of a particular group and, to some extent, is a guide to their structural investigation. Of course, the structures of a very large number of glycuronoglycans have still

not been studied or have been studied inadequately. There is no doubt that in future the appearance of new groups of acidic polysaccharides unified by the common structure of their skeleton will be possible.

It is impossible, and unnecessary, to mention all those working in the field of the structural investigation of glycuronoglycans. Their number is very large. However, it must be mentioned that in the last 2 or 3 years the number of communications on glycuronoglycans has decreased considerably. As is well known, interest in the structural study of a particular class of compounds is due to a number of factors, including the possibility of their practical application, the importance of their biological functions and physiological activity, and the development of structural methods of investigation. The range of the practical use of the glycuronoglycans attracted the attention of workers to them long ago.

The investigation of the dependence of the technical properties of polysaccharides on their nature required the determination of the main features of the structures of individual representatives, and this was done. The development of modern methods of investigation at the end of the 50's and, particularly, in the 60's gave a new impetus to the study of the glycuronoglycans and enabled individual features of their fine structure to be determined. This, in its turn, provided the possibility of connecting individual aspects of the biological activity of the polysaccharides with their structure. Unfortunately, the study of the biological function and the physiological activity of the glycuronoglycans is being performed inadequately.

At the end of the 60's, the existing methods of structural investigation proved to be largely exhausted. They could no longer give basic information on the fine structure of the glycuronoglycans. As a result, at the beginning of the 70's a situation arose in the chemistry of the glycuronoglycans which required a new impetus in the investigation of the fine structure, which is a far from simple task.

Consequently, at the present time it appears most desirable to pay particular attention to the study of the biological function and physiological activity of the glycuronoglycans and also to the development of new approaches to the structural investigation of the carbohydrate chain and, particularly, to the development of methods for its specific fragmentation.

The time has begun for approaching the study of the molecular structure of the glycuronoglycans and their secondary, tertiary, and quaternary structures, and the determination of the nature of their bond and of their interaction with other components of the cell, which requires the urgent development of still more powerful methods of investigation: chemical, biochemical, and, particularly, physicochemical, with the computer processing of the results obtained. Only in this way will it be possible to speak of approaches to the elucidation of the connection between the structure and biological function of the glycuronoglycans.

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