THE CHEMISTRY AND BIOCHEMISTRY OF PECTIC SUBSTANCES

H. G. J. WORTH

Royal Holloway College, University of London, Englefield Green, Surrey, Great Britain

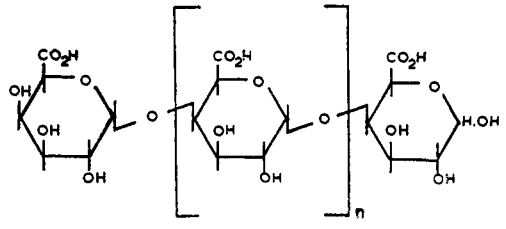
Received March 21, 1967

CONTENTS

I.	Introduction	465
II.	Chemistry	465
	A. Nomenclature and Analysis	465
	B. Fractionation	466
	C. Hydrolysis	467
	D. Methylation	468
	E. Periodate Oxidation	468
III.	Biosynthesis	469
	A. Introduction	469
	B. Inositols	469
	C. Methionine	470
	D. Nucleotides	470
IV.	Physiology	471
V.	Conclusions	471
VI.	References	472

I. INTRODUCTION

The group of heterogeneous polysaccharides known as pectic substances occurs in all higher plants but not in animals. The biological role of this material is that of an intercellular adhesive. The main component within this group appears to be poly-D-galacturonic acid chains with α-1,4 linkages (I). In addition,



I

chains of D-galactose and L-arabinose units are found together with L-rhamnose and traces of other neutral sugars. Some of these neutral sugars are bonded to the polygalacturonic acid, although the exact relationship between the neutral and acid fractions is unknown.

The early work on pectic substances is reviewed in a publication by the Department of Scientific and Industrial Research (21) and is primarily of historical interest although it deals with the isolation of galacturonic acid (34) and some of the neutral monosaccharides (11–13).

The earlier structural chemistry of pectic substances has been reviewed (48, 59, 113), and the knowledge to date suggests that pectic substances consist of a triad, α-1,4-D-galacturonan, a highly branched L-arabinan, and a β-1,4-D-galactan.

This present survey covers the more recent advances in the chemical field and the biosynthesis and physiological importance of pectic substances.

II. CHEMISTRY

A. NOMENCLATURE AND ANALYSIS

During the early work on pectic substances so many different names appeared in the literature for one single structure, that a committee was set up by the American Chemical Society (27) giving a strict definition for the terms necessary in the pectin field. These definitions are as follows.

Pectic Substances.—A group designation for those complex, colloidal carbohydrate derivatives which occur in or are prepared from plants and contain a large proportion of anhydrogalacturonic acid units which are thought to exist in a chain-like combination. The carboxyl groups of polygalacturonic acids may be partly esterified by methyl groups and partly or completely neutralized by one or more bases.

Protopectin.—Applied to the water-insoluble parent pectic substance which occurs in plants and which upon restricted hydrolysis yields pectin or pectinic acids.

Pectinic Acids.—Used for colloidal polygalacturonic acids containing more than a negligible proportion of methyl ester groups. Pectinic acids, under suitable conditions, are capable of forming gels with sugar and acid or, if suitably low in methoxyl content, with certain metallic ions. The salts of pectinic acids are either normal or acidic pectinates.

Pectin.—Designates those water-soluble pectinic acids of varying methyl ester content and degree of

neutralization which are capable of forming gels with sugar and acid under suitable conditions.

Pectic Acids.—Applied to pectic substances mostly composed of colloidal polygalacturonic acid and essentially free from methyl ester groups. The salts of pectic acids are either normal or acid pectates.

The report lists the more common obsolete terms with their present designation.

No major advances other than determining the major components of pectic substances were made until the advent of paper partition chromatography (29, 87, 88) and powdered cellulose chromatography (54). With the aid of these techniques, D-galacturonic acid, D-galactose, L-arabinose (7, 8, 25, 26, 77), L-rhamnose (7, 8, 25, 26, 77), D-xylose (7, 25, 26, 77), L-fucose (8), D-glucose (7), 2-*O*-methyl-L-fucose, and 2-*O*-methyl-D-xylose (7, 8) have been isolated and characterized.

The presence of methoxyl groups (see Table I) was realized by the earlier workers and confirmed by Schneider and Bock (99) who showed that these existed as esters of the carboxylic groups of the uronic acid residues. The presence of acetyl groups in pectic substances was claimed by some workers (35, 36, 79), but disputed by others (75, 100), until McComb and McCready conclusively showed the existence of small amounts (see Table II) using a colorimetric determination (76).

Optical rotation and molecular weight measurements have been made and typical results are shown in Tables III and IV, respectively.

TABLE I
METHOXYL CONTENT OF PECTIC SUBSTANCES

Source of pectic substance	% methoxyl	Ref
Orange	9.4–9.6	83
Apple	10.0–11.4	15,83
Strawberry	0.2	15
Fresh hops	3.7–7.8	83
Beet	8.3	83

TABLE II
ACETYL CONTENT OF PECTIC SUBSTANCES

Source of pectic substance	% acetyl	Source of pectic substance	% acetyl
Raspberry	0.25	Strawberry	1.43
Apricot	1.36	Cherry	0.18
Citrus	0.24	Sugar beet	2.50

TABLE III
OPTICAL ROTATION OF PECTIC SUBSTANCES

Source	$[\alpha]_D$ in H_2O, deg (temp, °C)	Ref
Strawberry	+251 (20)	15
Apple	+230 (23)	15
Citrus	+250 (19)	74
Cottonwood	+216 (25)	3
Cotton fiber	+225[a] (28)	41

[a] In 0.1 *N* NaOH.

TABLE IV
MOLECULAR WEIGHT OF PECTIC SUBSTANCES

Source	Molecular weight	Ref
Citrus	23,000–71,000	86
Apple and lemon	50,000–90,000	98
Apple and lemon[a]	200,000–360,000	98
Apple, pear, and plum	25,000–35,000	107
Orange	40,000–50,000	107

[a] Pectin leached out without boiling.

B. FRACTIONATION

The modern concept of the basic structure of pectic substances is that of a triad consisting of polygalacturonans, galactans, and arabinans. Until recently separation of these constituents proved difficult. It was even considered that they might be linked together covalently. Although the separation of the arabinan had been achieved (14, 45–47, 49–51), by prolonged extraction with alcohol, and instances of the isolation of a galactan are recorded (51, 78), many workers were unsuccessful (2, 4, 7, 8).

With new techniques available, in particular the use of basic celluloses and gel filtration, a number of fractionations have been achieved by several workers. Deuel (82), using a column of diethylaminoethyl (DEAE) cellulose, eluted an arabinan from the column with phosphate buffer at pH 6.1, while the main acidic polysaccharide was not eluted until sodium hydroxide was passed down the column. However, this work was carried out on a pectic substance of very high arabinose content (*ca.* 50%) and only 14% of the total arabinose was eluted with the buffer. The remainder appeared with the acidic polysaccharide, which itself was fractionated into three components, although these were qualitatively similar. More recently a number of pectic substances have been fractionated on DEAE cellulose (96) and have yielded up to five fractions. In one case a fraction was obtained which contained only galactose, and in most cases at least one fraction containing galactose and arabinose free from galacturonic acid.

The isolation of a galacturonan free from neutral sugars has been reported (16). The pectic substance was passed down a cation ion-exchange column (H^+ form) and the eluent ultracentrifuged. This resulted in a precipitate which was shown to contain only galacturonic acid, while the supernatant liquid contained galacturonic acid and the neutral sugars.

The most comprehensive fractionation of a pectic material has been made by Barrett and Northcote with apple pectin (10). An initial fractionation with ethanol yielded two fractions, an acidic one and a neutral one, the latter containing 41% arabinose and 46% galactose. The acidic fraction was further fractionated by gel filtration to give two fractions, one containing

98% galacturonic acid and the other 31% galacturonic acid and 51% arabinose.

C. HYDROLYSIS

Hydrolysis or partial hydrolysis of a polysaccharide may be achieved either by treatment with a base or mineral acid, or enzymatically.

Base-catalyzed hydrolysis of a polysaccharide is often a hazardous procedure as it frequently results in total breakdown or rearrangement. Under alkaline conditions aldehydes containing β-hydroxyl groups readily undergo a reversible change to a conjugated form.

$$CH_3CH(OH)CH_2CHO \overset{OH^-}{\rightleftharpoons} CH_3CH{=}CHCH{=}O + H_2O$$

The majority of oligo- and polysaccharides possess β-hydroxyaldehyde groupings. In a 1,3-linked saccharide the glycosidic bond is cleaved, as it is in the β position to the reducing (aldehyde) end. A similar cleavage often occurs with 1,4-linked saccharides where, although the glycosidic bond is γ to the reducing end, it is labile and results in the formation of saccharinic acids. A considerable amount of work has been done on the complex mechanism involved (30, 94). 1,4-Linked polyhexuronic acids are not only susceptible to this stepwise degradation from the reducing end, but, by virtue of the carboxylic group at C-6, the glycosidic linkage is in a β position to a carbonyl group, and so degradation may be random. It has been shown that when the carboxylic group is esterified the glycosidic bond is more labile to alkali attack and that the degree of degradation is directly proportional to the degree of esterification (114). Presumably the free acid is so highly charged that it hinders the hydroxyl attack.

Acid hydrolysis of saccharides involves attack on the glycosidic oxygen by a proton followed by the addition of water to the resulting carbonium ion (33); see Figure 1.

Uronic acid polymers are more difficult to hydrolyze with acid than the corresponding neutral glycans. This was thought to be due to the inductive effect of the oxygen atoms in the carboxylic group removing electrons from the glycosidic oxygen. This would involve the transmission of an inductive effect capable of inhibiting hydrolysis through one oxygen and two carbon atoms which now seems unlikely (110). It has also been shown that there is a considerable difference between the entropies of activation of methyl α-D-galactoside and methyl α-D-galacturonide (32), suggesting that the mechanisms of hydrolysis are different. The conjugated carbonium ion postulated in Figure 1 must exist in a half-chair conformation (33), and the formation of this would be hindered by increasing the size of the group attached to C-5 (24), *e.g.*,

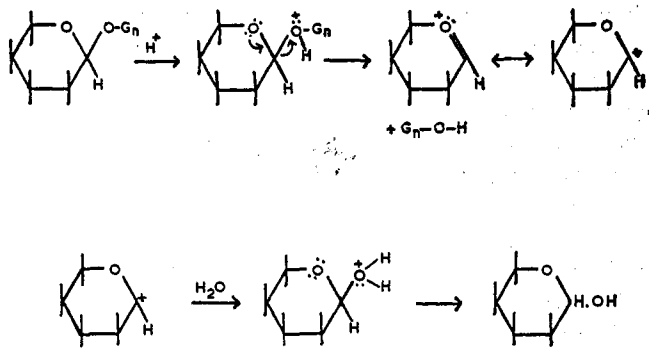


Figure 1.—Acid hydrolysis of glycosidic bonds.

by replacing the primary alcohol group at C-6 with a carboxylic acid group. Feather and Harris (36a) have investigated the acid-catalyzed hydrolysis of many glycopyronosides and suggest that steric hindrance causing resistance to rotation, particularly about C-2–C-3 and C-4–C-5 bonds, may be a major rate-determining factor. Acid hydrolysis of 2-naphthyl β-D-glucuronide (23) has shown that this is hydrolyzed by 1 *N* HCl $^1/_{45}$th as fast as 2-naphthyl β-D-glycoside, but at pH 4.79 the glucuronide is hydrolyzed 35 times faster. It is presumed that this is because the glucuronide is ionized at pH 4.79 but not in 1 *N* HCl. Increased hydrolysis of polyuronides compared with the corresponding glycan at pH 4.79 would be expected, but no work has been reported on this.

In view of these recent advances, the mechanism shown in Figure 1 seems valid for the hydrolysis of neutral glycosides but not uronides, although there is insufficient evidence to postulate an alternative mechanism.

Pectic enzymes have been reviewed in recent years (31, 60, 68) and only need a brief mention here. The hydrolases fall into two major categories, those which deesterify (the pectin esterases) and those which split the glycosidic bond (the polygalacturonases). Crude pectinase preparations also hydrolyze the arabinan and galactan present (19, 93). It seems likely that this is due to the presence of separate arabinases and galactanases (93), although these have yet to be isolated.

Apart from the obvious importance of hydrolysis in the liberation of the constituent monosaccharides from a polysaccharide, the production of oligosaccharides is often the only way of detecting and characterizing the branch points as well as adding conclusive evidence to the structure of the main chain. Acidic and enzymic hydrolyses of various pectic substances have been carried out by many workers. A series of oligogalacturonic acids has been isolated (8, 16, 19, 57, 58) ranging from di- to pentagalacturonic acid, and characterized as containing α-1,4 linkages only. A number of di- and trisaccharides containing galacturonic acid and neutral sugars have been isolated and are

thought to have arisen from branch points in the polysaccharide. 2-*O*-(α-D-Galactopyranosyluronic acid)-L-rhamnose (8, 10, 16) has been characterized, and a galacturonosyl rhamnosyl rhamnose (8, 16) isolated. Other oligosaccharides have been isolated but not fully characterized, *e.g.*, disaccharides containing galacturonic acid and fucose (8), galacturonic acid and galactose (8, 10, 19), galacturonic acid and xylose (10), and a trisaccharide containing galacturonic acid, galactose, and rhamnose (19).

The isolation of neutral oligosaccharides from pectic substances has not been recorded.

D. METHYLATION

Methylation has proved to be the most useful single technique in the elucidation of the basic structure of polysaccharides, particularly the more complex heterogeneous ones such as pectic substances. It has been possible to determine the structure of the arabinan and galactan as well as the galacturonan, without previous knowledge of the relationship between these polymers.

The most usual methods of methylation are the Purdie (56), Haworth (42), and Kuhn (64) or modifications of these (20, 105, 112). With the application of vapor phase chromatography to the analysis of methylated sugars (6, 17, 63), it has been possible to carry out identification on very small quantities. Methylation has been used in the characterization of many of the oligosaccharides mentioned in section C (8, 19, 58).

The methylation of pectic arabinans has been mentioned in an earlier review (48). The isolation of 2,3,5-tri-*O*-methyl-L-arabinose, 2,3-di-*O*-methyl-L-arabinose, and 2-*O*-methyl-L-arabinose or 3-*O*-methyl-L-arabinose in the ratio 1:1:1 together with a negative specific rotation has led to the postulation of a highly branched structure (see Figure 2) for the arabinan (45, 49, 50). This structure is supported by later workers (8). It is interesting to note that Hirst and Jones, who derived the structure in Figure 2, were working with arabinans fractionated from the main acidic polymer whereas Bhattacharjee and Timell (16) methylated an unfractionated pectic substance and obtained the mono-, di-, and trimethylated arabinoses in the ratio of 1:1:5.5 approximately. This suggested that some of the arabinose may be incorporated in the acidic chain, as fractionation studies indicate (10).

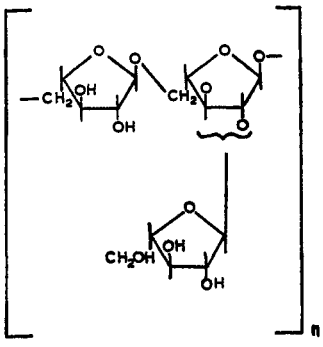


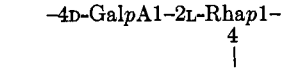
Figure 2.—Repeating unit of pectic arabinan.

Methylation of the pectic galactan is mentioned in an earlier review (48) which postulates a structure of a β-1,4-linked straight-chain polymer of approximately 120 units. This has been supported by later workers (55), although they also obtained small amounts of 2,3,6-tri-*O*-methyl-D-galactose and 2,4-di-*O*-methyl-D-galactose which suggested a small degree of branching.

Complete methylation of acidic polysaccharides is difficult (44), but this has been carried out (48, 74) and yielded mainly the methyl ester of 2,3-di-*O*-methyl-D-galacturonoside. Bhattacharjee and Timell regarded this as an over simplification in the case of some pectic substances (16). By direct methylation they obtained galacturonic acid, 2- and 3-*O*-methyl-D-galacturonic acids, 2,3-di-*O*-methyl-D-galacturonic acid, and 2,3,4-tri-*O*-methyl-D-galacturonic acid in appreciable quantities although the dimethylated uronic acid was the largest constituent (45.3%). Bhattacharjee and Timell suggested that some of the neutral sugar components were attached to the uronic acid chain, a theory not without other support, which would account for the quantities of partially methylated uronic acids.

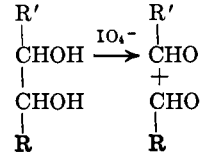
Reduction of the galacturonan prior to methylation yields 2,3,6-tri-*O*-methyl-D-galactose as the major component (7, 19).

3-*O*-Methyl-L-rhamnose (8, 16), 3,4-di-*O*-methyl-L-rhamnose (8), and 2,3,4-tri-*O*-methyl-L-rhamnose (7, 8) have been isolated from the methylation products of pectic substances but not from fractionated pectic arabinans or galactans, and it is thought that L-rhamnose occurs in the acidic chain, and as single unit branches off this chain. It may even occur as the nonreducing end unit in some cases (8). Aspinall postulates a structure which allows for the inclusion of L-rhamnose in the acidic chain (8).



E. PERIODATE OXIDATION

Periodate oxidation will occur in any polyol system where there are hydroxyl groups on two or more adjacent carbon atoms, resulting in the cleavage of the carbon–carbon bond and the formation of aldehyde groups. The application of this technique to carbo-



hydrates has already been reviewed (18). Its importance is twofold. Firstly, the measurement of periodate uptake gives an indication of the number of adjacent carbon atoms bonded to free hydroxyl groups, and hence the position of the glycosidic bond; secondly, the analysis of the oxidation products, usually after further treatment, yields useful data in assigning a structure to the polysaccharide. Measurement of periodate uptake by pectic substances is of little importance as the polysaccharide is too heterogeneous for the value to have much meaning.

The periodate oxidation of a pectic material with a high uronic acid content (92%) followed by oxidation with bromine and acid hydrolysis (48, 67) yielded D-threaric acid ((−)-D-tartaric acid) which, assuming a pyranose ring form, could have arisen from a 1,4 linkage only.

Few other applications of periodate oxidation in pectin chemistry are recorded, although there is one example of the use of the Smith degradation (1, 40, 102), *e.g.*, periodate oxidation followed by borohydride reduction and hydrolysis, of a pectic arabinan (55). The products were glycerol and tetritol.

III. BIOSYNTHESIS

A. INTRODUCTION

The precise pathway of the biosynthesis of pectic substances is as yet unknown and may remain so until a greater knowledge of the finer structure of the polysaccharide is available. Chemically D-galactose may be oxidized to D-galacturonic acid and this decarboxylated to give L-arabinose. In theory this triad could be formed by the initial synthesis of the galactan (44). The galactan exists as a β-linked polymer (51), while the galacturonan is α-linked (74). The highly branched nature of the arabinan (49) indicates that it is unlikely to arise from the decarboxylation of a straight-chain galacturonan.

By feeding radioactively labeled precursors to plants, some general observations have been made. The use of D-glucose-1-^{14}C, D-glucose-2-^{14}C, D-glucose-6-^{14}C, and D-galactose-1-^{14}C resulted in the detection of ^{14}C in pectic D-galacturonic acid and L-arabinose to varying degrees (102). Furthermore, when D-glucose-1-^{14}C was fed to plants, virtually all the radioactivity in the pectic D-galacturonic acid and L-arabinose residues was found at C-1 (101), suggesting that the biosynthetic pathway from hexose to D-galacturonic acid did not involve cleavage of the carbon chain and that the L-arabinose was produced by the removal of C-6 from the hexose.

The absorption of labeled carbon dioxide ($^{14}CO_2$) by the leaves of Victoria plums (52), followed by the extraction of the leaf polysaccharides (assumed to contain pectic substances), showed on hydrolysis that all the resulting monosaccharides contained ^{14}C. When D-glucose-1-^{14}C and D-glucose-6-^{14}C were fed to plum leaves (53), the former gave rise to radioactivity in D-glucose, D-galactose, L-rhamnose, L-arabinose, and D-xylose, mostly at C-1, while the latter, D-glucose-6-^{14}C, resulted in labeling at C-6 in the hexoses, but at C-1 in the pentoses. In this case the activity in the pentoses was considerably less than that in the hexoses. It appears that biosynthesis may occur directly from carbon dioxide, probably *via* glucose, and that the skeletal carbon chain is not broken, although there is some indication that the pentoses may not be derived simply by the removal of C-6 from a hexose molecule.

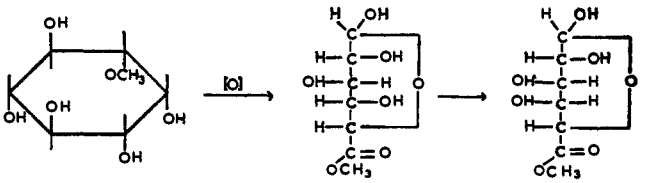


Figure 3.—Conversion of L-bornesitol to the methyl ester of D-galacturonic acid.

B. INOSITOLS

It has been shown that the route to D-galacturonic acid in pectic substances may lie *via* inositol or one of its derivatives. L-Bornesitol (1-*O*-methylmyoinositol), if cleaved between C-1 and C-6 and oxidized, yields the methyl ester of D-glucuronic acid; see Figure 3 (70). A similar reaction has been shown to occur enzymatically in the metabolism of camphor (28). The methyl glucuronide on epimerization would give the methyl ester of D-galacturonic acid, which could be incorporated into the pectin molecule. Both the epimerization (38) (*via* uronic acid nucleotide) and the incorporation of the uronic acid into the polysaccharide (61, 71) are known reactions. When *meso*-inositol-2-^{14}C is fed to plants *in vivo*, D-galacturonic acid-5-^{14}C is obtained (69, 73). This biosynthetic pathway is supported by the occurrence of methylated glucuronic acids in other allied polysaccharides, whose precursors could also be inositols. For instance, 4-*O*-methyl-D-glucuronic acid occurs in hemicelluloses (5) and gums (103, 115) for which D-bornesitol (3-*O*-methylmyoinositol) could be a precursor.

It has also been shown that D-glucose is a metabolite of myoinositol (39, 62, 72) and hence L-bornesitol. The pathway does not involve rupture of the skeletal carbon chain and therefore is not contrary to the work with ^{14}C-labeled glucose and galactose, described in the previous section (53, 101). On the basis of this evidence a tentative pathway to the biosynthesis of the galacturonan in pectic substances is postulated in Figure 4 (70).

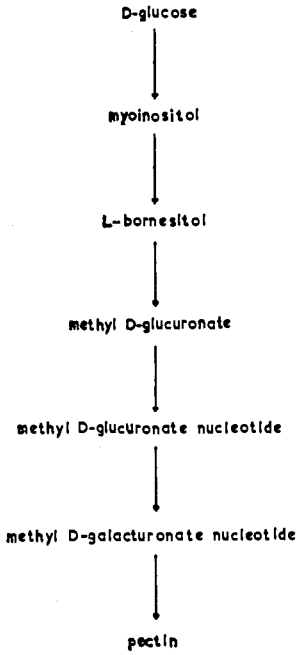


Figure 4.—Formation of pectin *via* inositol.

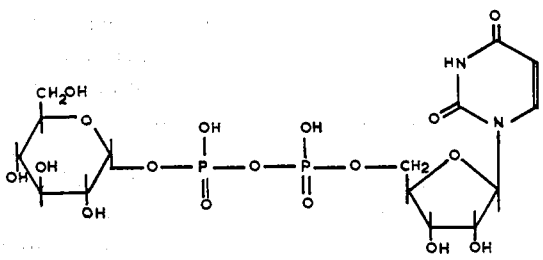
C. METHIONINE

^{14}C-Labeled methionine, a methyl transferring agent, acts as a precursor for the formation of the methyl ester of pectic acid (97). By treating pectic acid with ^{14}C-labeled methionine, over 90% of the radioactivity which appeared in the pectic substance occurred in the methyl ester.

This work is incompatible with that on inositols, as the latter requires the methyl ester to be present in the molecule before the formation of the uronic acid (see Figures 3 and 4), and any free acid groups that may be present in the pectic substance are presumable due to deesterification after biosynthesis of the main uronic acid chain.

D. NUCLEOTIDES

The discovery of nucleotides as coenzymes in carbohydrate biosynthesis is the most important single advance in this field and has been reviewed recently (65, 80). The first sugar nucleotide to be discovered (43) was α-D-glucopyranosyl (uridine 5-pyrophosphate), or as it is more commonly referred to, uridine diphosphate glucose (UDPG) (II).



II

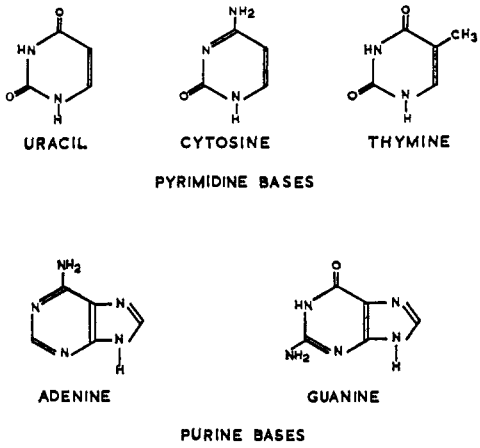


Figure 5.—Purine and pyrimidine bases involved in carbohydrate biosynthesis.

The pyrimidine base uracil is one of five bases usually involved in carbohydrate synthesis; the others are cytosine and thymine (pyrimidines) and adenine and guanine (purines); see Figure 5.

Biosynthetic reactions involving nucleotides may be classified as follows.

Nucleotidyl Transferases.—(i) Combination of a nucleotide triphosphate with a glycosyl phosphate to give a sugar nucleotide and inorganic phosphate, *e.g.*

$$\text{UTP} + \text{D-Glc-1-P} \xrightarrow{\text{UTP-D-Glc-1-P uridylyl transferase}} \text{UDPG} + \text{inorganic phosphate (81)}$$

(ii) Transfer of a nucleotide moiety from one glycosyl phosphate to another, *e.g.*

$$\text{UDPG} + \text{D-Gal-1-P} \xrightarrow{\text{UDPG-D-Gal-1-P uridylyl transferase}} \text{UDP-Gal} + \text{D-Glc-1-P}$$

There is no evidence for the occurrence of this reaction in higher plants.

Isomerases (Epimerase).—Steric inversion of one of the sugar hydroxyl groups of the sugar nucleotide, *e.g.*

$$\text{UDPG} \xrightarrow[\text{UDPG-4-epimerase}]{} \text{UDP-Gal (81)}$$

Glycosyl Transferases.—Transfer of a glycosyl moiety from a sugar nucleotide to a carbohydrate acceptor molecule, *e.g.*

$$\text{UDPG} + \text{G-G}_n\text{-G} \xrightarrow{\text{UDPG-glucan glucosyl transferase}} \text{G-G}_{n+1}\text{-G} + \text{UDP (66)}$$

Using UDPG as a precursor, a scheme may be postulated (see Figure 6) for the biosynthesis of pectic substances. Most of the reactions involved have already been demonstrated in plants, and in some cases in the biosynthesis of pectin.

Hassid has shown by labeling techniques that both D-glucuronic acid and D-galacturonic acid can act as precursors to pectin (61), and suggests that D-glucuronic

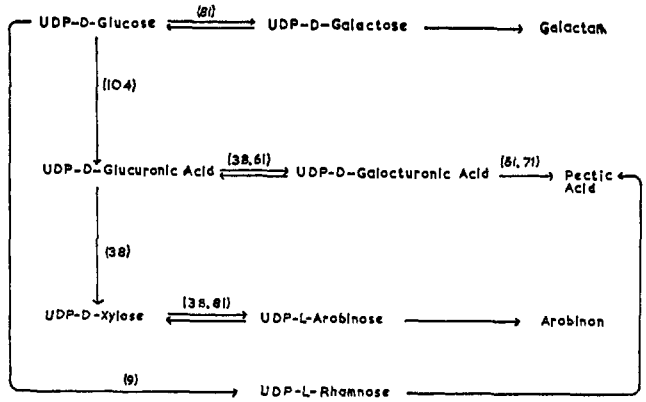


Figure 6.—Scheme for the formation of pectic substances from UDP D-glucose.

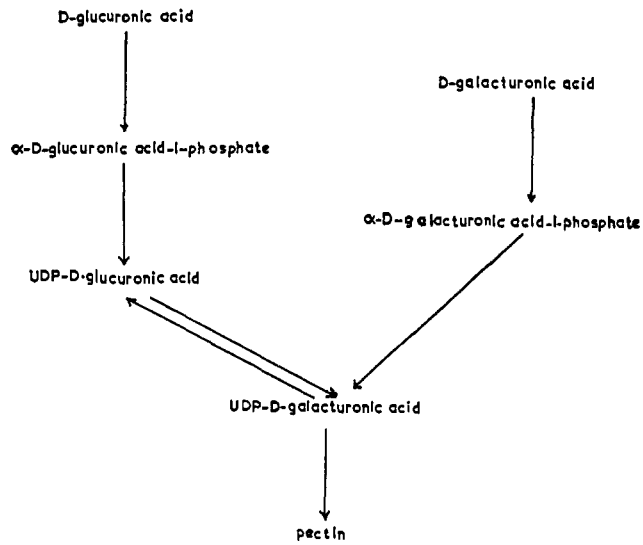


Figure 7.—Formation of pectin from D-glucuronic and D-galacturonic acids.

acid arises from UDPG, as shown in Figure 7. Each individual stage in this figure has been shown to occur in plants (37, 38, 106, 111).

IV. Physiology

Pectic substances are found in the cell wall and middle lamella (see Figure 8), associated with cellulose, hemicelluloses, and lignins. Little or none is found in the secondary cell wall. Pectic substances are hydrophilic and therefore have certain adhesive properties, whose importance in the middle lamella is obvious. They may also be a means for the translocation of water (92). Until maturation the pectin in the middle lamella is insoluble, giving rigidity to the tissue. During the ripening of fruit the pectin is solubilized; this results in softening until the fruit is overripe and the cells are completely separated. The cellulose in the cell walls is laid down as long fibrils of crystalline micelles (90, 91) running parallel to each other on a given axis. A number of such layers are

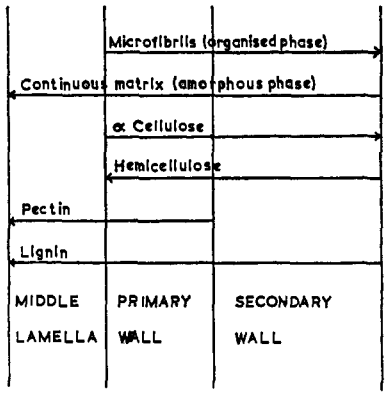


Figure 8.—Distribution of cell wall polysaccharides.

present having their axes at specific angles to each other, although this orientation may differ from plant to plant. The interspaces of the fibrils and the different layers are filled with lignins, hemicelluloses, and pectic substances. It is suggested that the polygalacturonic acid chains of the pectic substances are held in a network by the presence of Ca^{2+} or Mg^{2+} (116) which form ionic bonds with two polygalacturonan chains. A more detailed analysis of the situation is beyond the scope of this article, but further information may be obtained from other recent reviews (95, 116).

The absence of any pectic substance in the secondary wall led workers to believe that it was a precursor of lignins and hemicelluloses, but on closer examination this seemed unlikely (22). Recent work indicates that hemicelluloses are biosynthesized simultaneously with pectic substances (61).

Northcote supports this theory (108, 109) and has shown that when the secondary wall is laid down there is little change in pectic content. In later work (85, 89, 117, 118) Northcote has demonstrated, by using D-glucose-1- and -6-^{3}H, that the cell wall materials are synthesized in the Golgi bodies within the cell and are then transferred to the cell wall. He suggests that the Golgi bodies act as a metabolic pool for the cell wall polysaccharide precursors and that during the maturation of the cell the synthesis of pectic substances ceases while that of hemicelluloses continues as the secondary thickening takes place (84, 85). Northcote indicates that the site of cellulose synthesis may be elsewhere in the cell (85).

V. Conclusions

In summary it may be stated that the basic structure of pectic substances has been elucidated and consists of three polymers, a highly branched arabinan and a straight-chain galactan and galacturonan with some indication of a small degree of branching in these chains. Neutral sugars are associated with the galacturonan, notably rhamnose; the galacturonan may exist in two types: one in association with neutral

sugars, the other purely acidic. Fractionation studies show that these polymers are separable but the structural analysis carried out by various workers on the resulting fractions are not always in agreement. This may be because pectic substances isolated from different plants are structurally different, or the fractionation may depend upon the method used.

Partial hydrolysis and enzymic studies have produced much of the evidence available concerning the finer structure of the polysaccharide, and it would seem that future investigations must lie in this direction. The isolation of arabinases and galactanases could throw much light on the finer structure of the arabinan and galactan.

It has been shown that the galacturonan may be synthesized purely by the use of nucleotides and sugar interconversions, although biosynthesis from cyclitols replaces many of these stages. However, methionine as a precursor is contrary to the cyclitol pathway. Much of this work has been carried out *in vitro*, and a pathway which can be demonstrated *in vitro* may not necessarily occur *in vivo*. It may also be true that different plants favor different pathways, or that different pathways are favored in different stages of growth.

ACKNOWLEDGMENTS.—The author wishes to express his grateful thanks to the American Chemical Society for permission to reproduce the definitions of pectic substances which appeared in *Chemical and Engineering News* (27) and Table 1 from *Anal. Chem.*, **29**, 820 (1957), and also to the Academic Press Inc. for permission to reproduce Table III and part of Table IV from Chapter VII of "Polysaccharide Chemistry" (113), pp 185 and 188.

VI. REFERENCES

(1) Abdel-Akher, M., Hamilton, J. K., and Smith, F., *J. Am. Chem. Soc.*, **73**, 4691 (1951).
(2) Anderson, D. M. W., and King, N. J., *J. Chem. Soc.*, 5333 (1961).
(3) Anderson, E., Kaster, R. B., and Seeley, M. G., *J. Biol. Chem.*, **144**, 767 (1942).
(4) Andrews, P., Hough, L., Powell, D. B., and Woods, B. M., *J. Chem. Soc.*, 774 (1959).
(5) Aspinall, G. O., *Advan. Carbohydrate Chem.*, **14**, 433 (1959).
(6) Aspinall, G. O., *J. Chem. Soc.*, 1676 (1963).
(7) Aspinall, G. O., and Canas-Rodriguez, A., *J. Chem. Soc.*, 4020 (1958).
(8) Aspinall, G. O., and Fanshawe, R. S., *J. Chem. Soc.*, 4215 (1961).
(9) Barber, G. A., *Biochem. Biophys. Res. Commun.*, **8**, 204 (1962).
(10) Barrett, A. J., and Northcote, D. H., *Biochem. J.*, **94**, 617 (1965).
(11) Bauer, R. W., *Landw. Vers. St.*, **38**, 319 (1891).
(12) Bauer, R. W., *Landw. Vers. St.*, **41**, 477 (1892).
(13) Bauer, R. W., *Landw. Vers. St.*, **43**, 191 (1894).
(14) Beavan, G. H., Hirst, E. L., and Jones, J. K. N., *J. Chem. Soc.*, 1865 (1939).
(15) Beavan, G. H., and Jones, J. K. N., *J. Chem. Soc.*, 1218 (1947).
(16) Bhattacharjee, S. S., and Timell, T. E., *Can. J. Chem.*, **43**, 758 (1965).
(17) Bishop, C. T., and Cooper, F. P., *Can. J. Chem.*, **38**, 388 (1960).
(18) Bobbitt, J. M., *Advan. Carbohydrate Chem.*, **11**, 1 (1956).
(19) Bourne, E. J., Pridham, J. B., and Worth H. G. J., *Phytochem.*, **6**, 423 (1967).
(20) Bouveng, H. O., and Linberg, B., *Advan. Carbohydrate Chem.*, **15**, 59 (1960).
(21) Branfoot, M. H., "A Critical and Historical Study of the Pectic Substances of Plants," Her Majesty's Stationery Office, London, 1929.
(22) Buston, H. W., *Biochem. J.*, **29**, 196 (1935).
(23) Capon, B., and Ghosh, B. C., *Chem. Commun.*, 586 (1965).
(24) Capon, B., and Overend, W. G., *Advan. Carbohydrate Chem.*, **15**, 37 (1960).
(25) Carras, A., *Ann. Sper. Agrar.* (Rome), 1675 (1954).
(26) Coleman, R. J., Lenny, J. F., Coscia, A. T., and DiCarlo, F. J., *Arch. Biochem. Biophys.*, **59**, 157 (1955).
(27) Committee for the Revision of the Nomenclature of Pectic Substances, *Chem. Eng. News*, **22**, 105 (1944).
(28) Conrad, H. E., DuBus, R., and Gunsalus, I. C., *Biochem. Biophys. Res. Commun.*, **6**, 293 (1961).
(29) Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, **38**, 224 (1944).
(30) Corbett, W. M., and Kenner, J., *J. Chem. Soc.*, 2245 (1953).
(31) Deuel, H., and Stutz, E., *Advan. Enzymol.*, **20**, 341 (1958).
(32) Easty, D. B., *J. Org. Chem.*, **27**, 2102 (1962).
(33) Edward, J. T., *Chem. Ind.* (London), 1102 (1955).
(34) Ehrlich, F., *J. Soc. Chem. Ind.*, **36**, 562 (1917).
(35) Ehrlich, F., and Kosmahly, A., *Biochem. Z.*, **212**, 162 (1929).
(36) Ehrlich, F., and von Sommerfeld, R., *Biochem. Z.*, **168**, 263 (1926).
(36a) Feather, M. S., and Harris, J. F., *J. Org. Chem.*, **30**, 153 (1965).
(37) Feingold, D. S., Neufeld, E. F., and Hassid, W. Z., *Arch. Biochem. Biophys.*, **78**, 401 (1958).
(38) Feingold, D. S., Neufeld, E. F., and Hassid, W. Z., *J. Biol. Chem.*, **235**, 910 (1960).
(39) Fernandez, O., de Mingo, M., and Martinez, E., *Farm. Nueva.* (Madrid), **10**, 541 (1945).
(40) Hamilton, J. K., and Smith, F., *J. Am. Chem. Soc.*, **78**, 5907 (1956).
(41) Harris, S. A., and Thompson, H. J., *Contrib. Boyce Thompson Inst.*, **9**, 1 (1937).
(42) Haworth, W. N., *J. Chem. Soc.*, 8 (1915).
(43) Hill, A. C., *J. Chem Soc.*, **73**, 634 (1898).
(44) Hirst, E. L., *J. Chem. Soc.*, 70 (1942).
(45) Hirst, E. L., and Jones, J. K. N., *J. Chem. Soc.*, 496 (1938).
(46) Hirst, E. L., and Jones, J. K. N., *J. Chem. Soc.*, 452 (1939).
(47) Hirst, E. L., and Jones, J. K. N., *J. Chem. Soc.*, 454 (1939).
(48) Hirst, E. L., and Jones, J. K. N., *Advan. Carbohydrate Chem.*, **2**, 235 (1946).
(49) Hirst, E. L., and Jones, J. K. N., *J. Chem. Soc.*, 1221 (1947).
(50) Hirst, E. L., and Jones, J. K. N., *J. Chem. Soc.*, 2311 (1948).
(51) Hirst, E. L., Jones, J. K. N., and Walder, W. O., *J. Chem. Soc.*, 1225 (1947).
(52) Hough, L., and Andrews, P., *J. Chem. Soc.*, 4483 (1958).
(53) Hough, L., Andrews, P., and Picken, J. M., *Biochem. J.*, **94**, 75 (1965).
(54) Hough, L., Jones, J. K. N., and Wadman, W. H., *J. Chem. Soc.*, 2511 (1949).
(55) Hough, L., and Powell, D. B., *J. Chem. Soc.*, 16 (1960).
(56) Irivine, J. C., and Purdie, T., *J. Chem. Soc.*, 1021 (1903).

(57) Jermyn, M. A., and Tomkins, R. G., *Biochem. J.*, **47**, 437 (1950).
(58) Jones, J. K. N., and Reid, W. W., *J. Chem. Soc.*, 1361 (1954).
(59) Kertesz, Z. I., "The Pectic Substances," Interscience Publishers Ltd., London, 1951.
(60) Kertesz, Z. I., and McColloch, R. J., *Advan. Carbohydrate Chem.*, **5**, 79 (1950).
(61) Kessler, G., Neufeld, E. F., Feingold, D. S., and Hassid, W. Z., *J. Biol. Chem.*, **236**, 308 (1961).
(62) Kindl, H., Scholda, R., and Hoffmann-Ostenhof, O., *Angew. Chem., Intern. Ed. Engl.*, **5**, 167 (1966).
(63) Kircher, H. W., *Anal. Chem.*, **32**, 1103 (1960).
(64) Kuhn, R., Trisohmann, H., and Löw, I., *Angew. Chem.*, **67**, 32 (1955).
(65) Leloir, L. F., and Cardini, C. E., *Perspectives Biol. Coll. Papers*, 496 (1963).
(66) Leloir, L. F., Rougine de Fekete, M. A., and Cardini, C. E., *Nature*, **187**, 918 (1960).
(67) Levene, P. A., and Kreider, L. C., *J. Biol. Chem.*, **120**, 591 (1937).
(68) Lineweaver, H., and Jansen, E. F., *Advan. Enzymol.*, **2**, 267 (1951).
(69) Loewus, F. A., and Kelly, S., *Federation Proc.*, **21**, 88 (1962).
(70) Loewus, F. A., *Nature*, **203**, 1175 (1964).
(71) Loewus, F. A., and Kelly, S., *Arch. Biochem. Biophys.*, **95**, 483 (1961).
(72) Loewus, F. A., and Kelly, S., *Biochem. Biophys. Res. Commun.*, **7**, 204 (1962).
(73) Loewus, F. A. and Kelly, S., *Arch. Biochem. Biophys.*, **102**, 96 (1963).
(74) Luckett, S., and Smith, F., *J. Chem. Soc.*, 1106 (1940).
(75) Ludtke, M., and Felser, H., *Ann.*, **549**, 1 (1941).
(76) McComb, E. A., and McCready, R. M., *Anal. Chem.*, **29**, 819 (1957).
(77) McCready, R. M., and Gee, M., *Agr. Food Chem.*, **8**, 510 (1960).
(78) Miyama, R., *J. Dept. Agr. Kyushuhup. Univ.*, **4**, 195 (1936).
(79) Nelson, E. K., *J. Am. Chem. Soc.*, **48**, 2945 (1926).
(80) Neufeld, E. F., and Hassid, W. Z., *Advan. Carbohydrate Chem.*, **18**, 309 (1963).
(81) Neufeld, E. F., Ginsburg, V., Putman, E. W., Fanshier, D., and Hassid, W. Z., *Arch. Biochem. Biophys.*, **69**, 602 (1957).
(82) Neukom, H., Deuel, H., Heri, W. J., and Kündig, W., *Helv. Chim. Acta*, **43**, 64 (1960).
(83) Norris, F. W., and Resch, C. E., *Biochem. J.*, **31**, 1945 (1937).
(84) Northcote, D. H., *Biochem. J.*, **100**, 19P (1966).
(85) Northcote, D. H., and Pickett-Heaps, J. D., *Biochem. J.*, **98**, 159 (1966).
(86) Owen, H. S., Lotzkar, H., Schultz, T. H., and Maclay, W. D., *J. Am. Chem. Soc.*, **68**, 1628 (1946).
(87) Partridge, S. M., *Nature*, **158**, 270 (1946).
(88) Partridge, S. M., *Biochem. J.*, **42**, 238 (1948).
(89) Pickett-Heaps, J. D., and Northcote, D. H., *J. Exptl. Botany*, **17**, 20 (1966).
(90) Preston, R. D., "Vistas in Botany," Vol. III, Pergamon Press, Oxford, England, 1963, p 255.
(91) Preston, R. D., Symposium on "Biosynthetic Pathways in Higher Plants," Academic Press Inc., New York, N. Y., 1965, p 123.
(92) Reed, H. S., *Am. J. Botany*, **17**, 971 (1930).
(93) Reid, W. W., *J. Sci. Food Agr.*, **1**, 234 (1950).
(94) Richards G. N., *et al.*, *J. Chem. Soc.*, 3019 (1957), and earlier papers.
(95) Roelofsen, P. A., *Advan. Botan. Res.*, **2**, 69 (1965).
(96) Rosik, J., Zitko, V., and Vasatko, J., *Collection Czech. Chem. Commun.*, **27**, 1346 (1962).
(97) Sato, C. S., Byerrum, R. U., and Ball, C. D., *J. Biol. Chem.*, **224**, 717 (1957).
(98) Säverborn, S., "Almquist and Wiksells Boktryckeri A. B., Uppsala, 1945; *Chem. Abstr.*, **41**, 396 (1947).
(99) Schneider, G. G., and Bock, H., *Ber.*, **71**, 1353 (1938).
(100) Sloep, A. C., Dissertation, Delft, 1928; Whistler, R. L., and Smart, C. L., "Polysaccharide Chemistry," Academic Press Inc., New York, N. Y., 1953, p 182.
(101) Seegmiller, C. G., Axelrod, B., and McCready, R. M., *J. Biol. Chem.*, **217**, 765 (1955).
(102) Seegmiller, C. G., Jang, R., and Mann, W., Jr. *Arch. Biochem. Biophys.*, **61**, 422 (1956).
(103) Smith, F., *J. Chem. Soc.*, 2646 (1951).
(104) Smith, F., and van Cleve, J. W., *J. Am. Chem. Soc.*, **77**, 3091 (1955).
(105) Srivastava, H. C., and Harshe, S. N., *Indian J. Chem.*, **1**, 304 (1963).
(106) Strominger, J. L., and Mapson, L. W., *Biochem. J.*, **66**, 567 (1957).
(107) Svedberg, T., and Garlen, H., *Nature*, **142**, 261 (1938).
(108) Thornber, J. P., and Northcote, D. H., *Biochem. J.*, **81**, 449 (1961).
(109) Thornber, J. P., and Northcote, D. H., *Biochem. J.*, **81**, 455 (1961).
(110) Timell, T. E., *Chem. Ind.* (London), 503 (1964).
(111) Villemez, C. L., Lin, T.-Y., and Hassid, W. Z., *Proc. Natl. Acad. Sci. U. S.*, **54**, 1626 (1965).
(112) Wallenfels, K., Bechtler, G., Kuhn, R., Trischmann, H., and Egge, H., *Angew. Chem., Intern. Ed. Engl.* **2**, 515 (1963).
(113) Whistler, R. L., and Smart, C. L., "Polysaccharide Chemistry," Academic Press Inc., New York, N. Y., 1953, Chapter VII.
(114) Whistler, R. L., and BeMiller, J. N., *Advan. Carbohydrate Chem.*, **13**, 313 (1958).
(115) White, E. V., *J. Am. Chem. Soc.*, **70**, 367 (1948).
(116) Wilson, K., *Intern. Rev. Cytol.*, **17**, (1964).
(117) Wooding, F. B. P., and Northcote, D. H., *J. Cell Biol.*, **23**, 327 (1964).
(118) Wooding, F. B. P., and Northcote, D. H., *J. Cell Biol.*, **24**, 117 (1965).