DIRECTION OF CARBOHYDRATE RESEARCH IN THE NEAR FUTURE

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Abstract – Areas can be indicated where extensive carbohydrate research
work will occur in the next few years. Among these are the application
of new organic reactions, production of nucleosides and new antitumor
agents, the organization of polysaccharides, glycoproteins and proteo-
glycans in cell walls, and production of new polysaccharide structures
to serve special applications.

INTRODUCTION

It is presumptive to suggest what specific developments may occur in future carbohydrate
research but a seer is not required to indicate areas where intensive research will occur.
I wish to indicate some of these areas and to comment upon them.

Carbohydrate chemistry has grown mightily since its initial exploration in the hands of
early workers. Carbohydrates were among the first compounds analyzed when carbon and
hydrogen analysis became feasible, whereas, their molecular weight measurement needed to
await the development of methodology such as that provided by the Beckmann procedure.
Subsequently, information developed rapidly through the work of men such as Emil Fischer
and the theories of LeBel and van't Hoff. Today, there is a broad area of carbohydrate
knowledge but with surprisingly large gaps where research is needed. Then, of course,
there are the divergent probing frontiers that attract the great multitude of investigators
who concentrate in these peripheral areas, leaving a few to work on the problems left behind
or intentionally bypassed by the frontiersmen. Some of the areas have been bypassed because
appropriate instruments were not available and some because investigators simply did not
know how to solve the problems posed.

Basic chemistry
The organic chemistry of carbohydrates is well established. The principal advance in recent
years has been through the application of reactions originally established by non-carbohydrate
workers in the larger field of general organic investigations. As new reactions in organic
chemistry evolve, and new reaction mechanisms are elucidated, it is natural to expect their
application to carbohydrates. On the other hand, development of a new reaction or the
understanding of a new mechanism in carbohydrate chemistry gives the field eminence and
recognition in the minds of general chemists. One must remember that many novel reactions
have been first established in carbohydrate chemistry. For example, stereospecific displace-
ment as illustrated in the Koenigs-Knorr reaction was first presented as a carbohydrate
finding, and was later taken into general organic chemistry.

The recent regiospecific reaction of Klemer and Rodemeyer is a new and useful (1) reaction
transferred from other organic areas to carbohydrates. In the example given, methyl 2,3:4,6-
di-O-benzylidene-α-D-mannopyranoside is reacted with 2 mols of n-butyl lithium with cyclo-
elimination to methyl 4,6-O-benzylidene-α-D-erythrohexopyrano-3-ulose. Some of the reaction
proceeds to give an olefin but 70% proceeds through the enol intermediate to the ketodeoxy
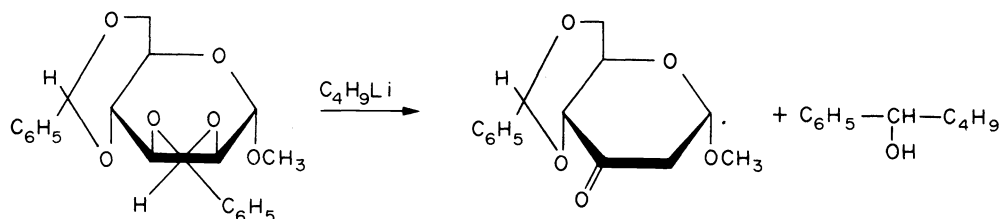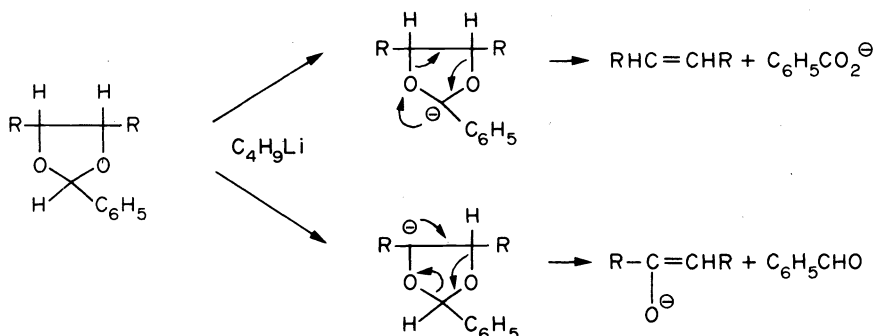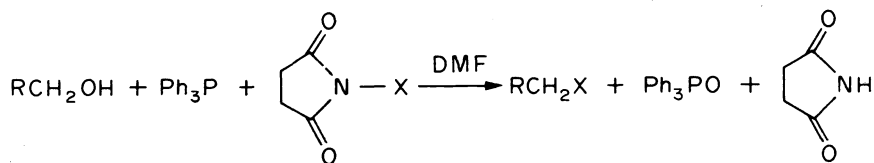sugar.

Fig. 1.

Fig. 2.

Excellent techniques for replacement of hydroxy groups with halogen have evolved from organic chemistry. Thus, primary hydroxy groups may be directly replaced by triphenyl-phosphine and N-halosuccinimides (2). Another useful approach is the opening of the dioxolane ring in a 4,6-O-benzylidene hexopyranoside (3), using N-bromosuccinimide.

$$RCH_2OH + Ph_3P + \begin{array}{c} O \\ \nearrow N-X \\ O \end{array} \xrightarrow{DMF} RCH_2X + Ph_3PO + \begin{array}{c} O \\ NH \\ O \end{array}$$
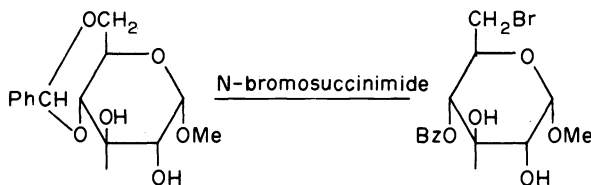
where X = Cl. Br. I

Fig. 3.

Fig. 4.

Application of physical measurements will continue to provide data from which interpretations can be made for a better understanding of conformations and reactions. From such data more information is being obtained about the anomeric effect and the preferred orientation of the O-1-aglycone bond relative to the sugar ring (4). Quantum mechanical analysis also provides a clearer insight (5). Cis-trans equilibria of analogous 2-mono-and 2,2-disub-stituted 1,3-dioxane as a function of solvent also gives valuable information (6). The

Amadori rearrangement of D-glucose is now shown to give an equilibrium mixture of the β-pyranosyl and α,β-furanosyl-amines. Lanthanide shift reagents (7) and similar nmr shift reagents will continue to yield insights into structures and reactions. Application of mass spectrometry and of advanced techniques in X-ray analysis are opening new approaches. Conformations of molecules in solutions and in gels are providing great insight into molecular behaviour and interaction.

Antibiotic and antitumor drugs

Carbohydrate components will continue to contribute important functions to antibiotic and antitumor drugs. This general area, and especially the antitumor area, may be expected to be one of the most important fields for carbohydrate research during the next ten years.

Beginning with the first nucleotide, inosinic acid, isolated from beef extract by von Liebig (8) in 1847, nucleoside and nucleotide chemistry moved forward at a substantial rate until 1950-1953 when progress leaped upward as a result of understanding the DNA and RNA structures. This opened the door to genetic chemistry. Arthur Kornberg (9) has said that genetic chemistry may transform the image of health and disease as dramatically as any advance in the history of medicine. In fact, there is hardly a major field of medicine that will not benefit from proper understanding of nucleosides and nucleotides. An especially heavy impact will be made against viral diseases and some diseases that are believed due to viral etiology such as arthritis, multiple sclerosis, infectious mononucleosis and leukemia. Nucleosides may effect control of parasites such as plasmodia, trypanosomes, schistosomes and ascaria. An excellent projection of future uses of nucleosides is given by Roland Robins as part of a recent symposium (10). T. Y. Shen (11) has pointed out interesting therapeutic agents, and Alexander Block (12) has provided insight into the design and function of nucleosides.
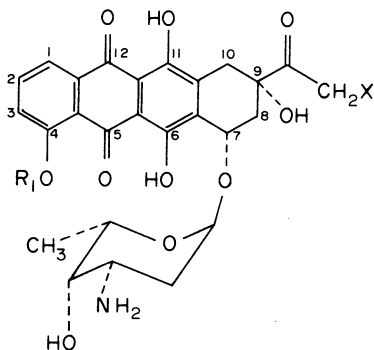

Fig. 5. Adriamycin. X = OH.

Adriamycin is perhaps the most powerful antitumor chemotherapeutic agent in use today. This drug with its near relative daunorubicin was isolated from *Streptomyces peucetius var. caesius* following mutagenic treatment of the parent culture with N-nitroso-N-methyl-urethane. There is consequently great activity synthetically, clinically and of a general biochemical nature to understand this drug's action and its toxicity, and to improve its performance, diminish side effects and reduce its cost. The importance of its effects is attracting great interest and is bringing an avalanche of investigators into every aspect of its chemistry, biochemistry and clinical medicine. Adriamycin and daunorubicin belong to the antracycline family of antibiotics (13-15). Antitumor action is presumed due to intercalation in DNA, thereby preventing replication. A side effect is cardio-myopathy but this effect, it is hoped, can be eliminated. Total synthesis by several methods has been accomplished and the sugar moiety has been modified in various ways. One of these in which the hydroxyl at C-4 has been inverted seems to be a less toxic isomer (15).

The sugar component is 3-amino-2,3,6-trideoxy-L-lyxo-hexose, the same as is present in daunorubicin, and similar to the 3-amino sugar in the antibiotic ristomycin (16) and the 3-amino sugar in the antibiotic actinoidin (17-19).

Macrolide antibiotics will continue to receive attention. Their synthesis appears promising and interesting since the chiral portions may be looked upon as a series of appropriately functionalized acyclic carbohydrate chains. This approach to synthesis has been given attention by Hanessian (20).

It is also surprising that more work has not been done on polysaccharides as antitumor agents. Very much work exists in the literature (21) to show that polysaccharides can cause tumors to regress and some polysaccharides with this property are also non-toxic. It is expected that biochemical work will be undertaken to establish how intraperatonially injected polysaccharides act upon a tumor or organize host responses to cause tumor regression.

Polymers

Since most natural carbohydrates are polysaccharides it is reasonable to expect that a large amount of work will be done in this field. Prime objectives will be the determination of molecular conformation in solution and in gels, and to determine the molecular interactions that lead to synergistic improvement in solution viscosity or gelation. A significant and earnest effort will be devoted to modification of polysaccharide structure to produce new molecules with improved industrial performance. Such new molecules will be otained by new fermentation techniques wherein polysaccharides are biosynthesized or biosynthetically modified or by forming polysaccharides or modifying molecules by enzymic means. Some effort will continue to be given to chemical modification of polysaccharides.

Surely everyone will agree that the glycoprotein area will be heavily investigated during the next few years. Such work will lead to important biochemical achievements and medical benefits. Many of these benefits will derive from an understanding of the nature of cell surface recognitions, of adhesions, or viral attachment, of membranal transport and of general cell membrane and cell surface function.

Interlocking of peptidoglycans in the surfaces of both Gram-positive and Gram-negative organisms is now recognized (22). Yet to be defined, is how the crosslinks are opened and closed during cell division and in cell transformation as a result of viral invasion of the cell or as a result of oncogenesis. The actual arrangement of the carbohydrate chains, though likely perpendicular to the cell surface, requires further assessment.
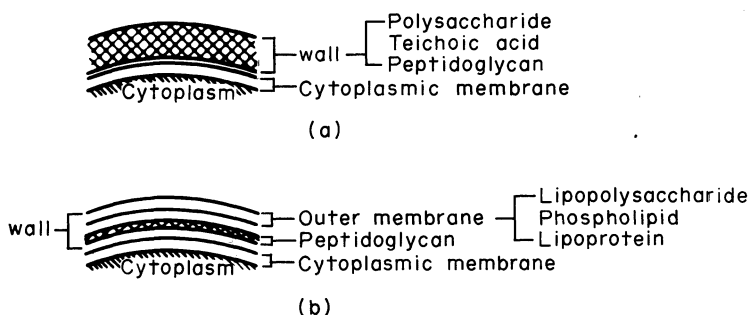

Fig. 6.   Representation of the cell was of Gram-positive (a) and Gram-negative (b) bacteria.

Additional work is required to obtain a more complete understanding of lipopolysaccharide structure and function. Transport of the lipopolysaccharide and its translocation to the outer membrane is in need of further effective work. Some excellent suggestions have been made by S. Hakomori and others (23, 25).

In addition to purely biochemical effects the glycoproteins offer interest to the physical chemist and to the practical industrial chemist. Two examples of their interests revolve on the properties of glycoproteins in aqueous solution. The first is the unusual property of a particular glycoprotein in antartic fish where an "anti-freeze" character is provided in that the polymer inhibits the phase transition of liquid water to ice, thereby protecting the fish from death by freezing. These glycoproteins, of 11,000–32,000 daltons, possess the simple repeating structure of alanine-alanine-threonine, to which is attached a di-saccharide unit (4-$\underline{O}$-β-$\underline{D}$-galactopyranosyl-2-acetamido-2-deoxy-β-$\underline{D}$-galactopyranosyl). The disaccharide side chains are essential for the significant freezing point lowering of the molecule. An understanding of the specific effects of the molecule's conformation and its effect on water structures could prove of value. Understanding of a second property could provide important basic information and possibly lead to industrial uses of similar synthetic polymers. This is the property of pituitness possessed by some glycoproteins
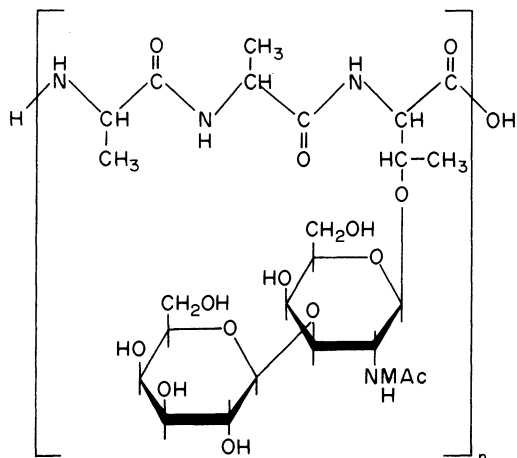
Fig. 7.   Glycoprotein of antartic fish.

particularly those with numerous generally distributed oligosaccharide side chains.   These molecules are able to form dispersions with long-stringy characteristics.   Examples are nasal mucus or salivary discharges.   Such pituitous character is desirable in certain food products and it is possible that structures with this character can be synthesized by derivation of soybean protein with oligosaccharides using either chemical or biochemical means to produce a covalent union.

Conformations of polysaccharides in solution are just becoming known.   The excellent diffraction work on polysaccharide solutions or gels by Arnott and his group and the

3 39nm

Fig. 8.   Structure of hyaluronic acid (Arnott).

experimental work of Rees and of many others is providing an understanding of viscosity, gel formation, synergistic actions and other rheeolgical behavior.   The behavior of hyaluronic acid is more (26, 27) understandable now that its structural conformation is known.

It has taken some years to work out the structure of heparin but still more work is needed
to show how the molecule functions on a molecular basis.
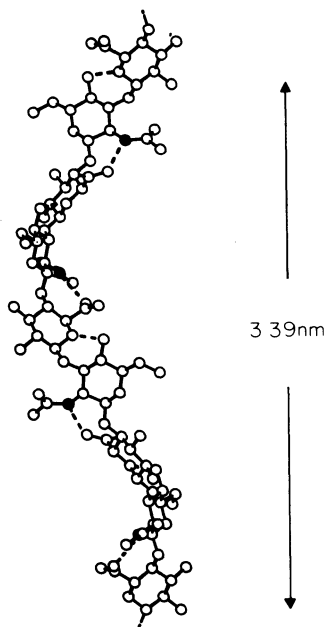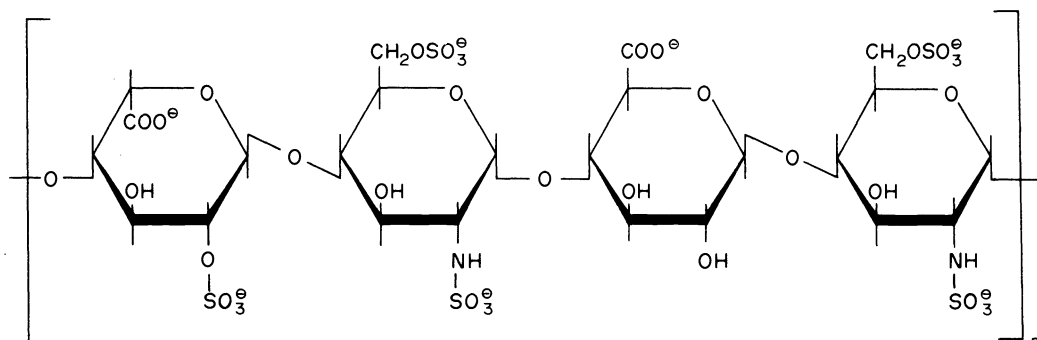
Fig. 9. Heparin.

Calcium is coordinated in an "egg crate" structure between layers of certain uronic acid
containing polysaccharides. Such complex formation can explain the tight bonding of
calcium and other multivalent ions in similar polysaccharide structures. It also explains
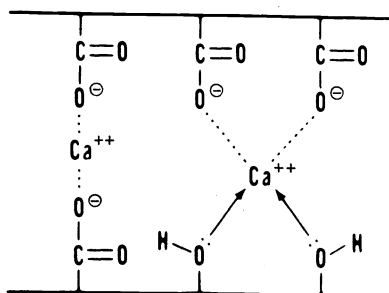
(b)

Fig. 10. Calcium complexed in polysaccharides.

how bivalent ions can cross-link such acidic polysaccharides to produce gels. The nature
of calcium complexing by alginic acid is now more clearly understood.

        Xanthan gum is known to have its side chains draped close along and spiralled
around the main chain, where they are held in this position by hydrogen bonds. An end
view of the molecules shows it to have a furry appearance. The structure and consequently
the rheological behavior of xanthan is similar to the structurally related extracellular
polysaccharide of *Escherichia coli*. The later differs in having 2-O(-4,6-O-α-carboxy-
ethylidene)-β-D-glucopyranosyl)-α-D-mannopyranosyl unit joined 1 → 4 to a 4-unit main
chain segment consisting of D-mannose, D-glucose, D-glucuronic acid and D-galactose units
as shown in D-galactose/D-mannose ratio.

Fig. 11. Xanthan conformation (Arnott).

Fig. 12. Xanthan molecules as viewed from chain-end.

-2)-α-D̲-Manp-(1→3)-β-D̲-Glcp-(1→3)-β-D̲-GlcAp-(1→3)-α-D̲-Galp-(1-
                                                            |
                                                           ⌢
                                                           4
                                                           ↑
                                                           1
                                                           ⌣
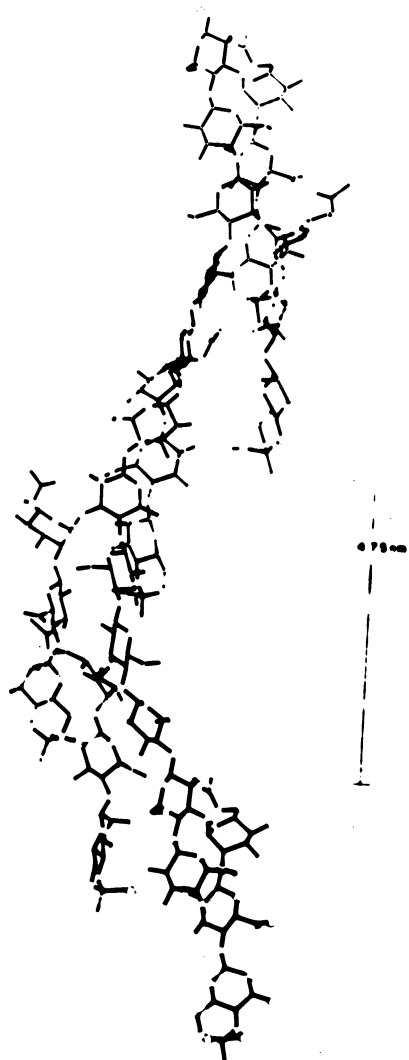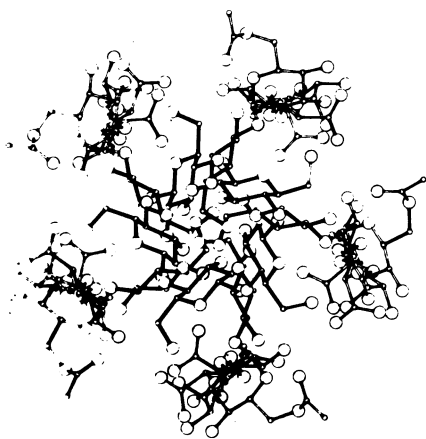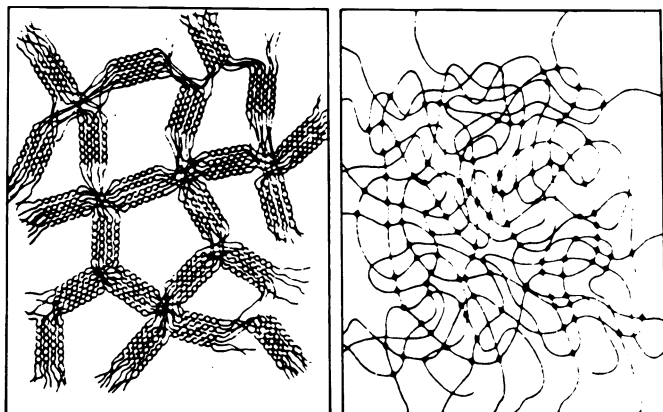 |
                        β-D̲-Glcp-(1→2)-α-D̲-Manp
                              / \
                             4   6
                              \ /
                               C
                              / \
                          H₃C·   COOH

Fig. 13. *Escherichia coli* polysaccharide.

There is a tendency from energy considerations for polysaccharides to associate inter-
molecularly. It has been known for many years that linear molecules associate in solution
and, if neutral and the polymer is uniform in repeating units and in glycosidic bonds,
the molecules will associate so high that they build groups large enough to be effected
by gravitational forces and the polymer precipitates from solution. An amylose solution
will so associate (retrograde) that some 99% precipitates from solution. This retrograda-
tion may be viewed as an attempt at crystallizations on the part of long and unwieldy
molecules.

(d)

Fig. 14.

One of the principal facts to emerge from diffraction investigations is that some poly-
saccharides in solution tend to form a spiral of varying degrees of compactness. This
is a structural form of highly thermodynamic stability. In such a spiral arrangement
both entropy and secondary valence forces are best satisfied.

Not only do most linear polysaccharides tend to form spirals in solution but in their
tendency to associate, they may form double helices as exemplified by carrageenan. Double
helices again provide structures of lower energy and high stability. Under certain con-
ditions of concentration or temperature the double helicies may associate to form gels.
Alternatively, they may associate with certain other polysaccharide molecules to form
gels. This is the case of carrageenan or of xanthan in association with locust bean gum.
In this synergestic relationship, locust bean gum can associate over part of its length
with one double helix of carrageenan or a helix of xanthan while at the same time another
portion of the locust bean gum molecule can associate with another double helix of car-
rageenan or a helix of xanthan. In this way, molecules are cross-linked to produce a
gel structure.

It was, at first, difficult to understand why locust bean gum could synergestically react
with some polysaccharides to increase viscosity more than additively, or to produce gels
as a result of cross-linking. Both locust bean gum and guaran are galactomannans differing
only in a minor way. Guaran has small or no synergestic effects on other polysaccharides.
It was only when the distribution of the D̲-galactopyranosyl units was investigated that
the behavioral difference between locust bean gum and guaran was brought fully to light.
Thus, it was established (28) that in locust bean gum the side chains are grouped together

(c)

Fig. 15.

(I)   (II)   (I)

Fig. 16. Method for location of D-galactopyranosyl units on a galactomannan

leaving long lengths of the main chain consisting of some 85 D-mannopyranosyl units,
denuded and exposed. These sections of the molecule, therefore, were free to associate
with other molecules and perform the observed cross-linking effect leading to gelation.
Guaran, on the other hand, has its α-D-galactopyranosyl side chains evenly spaced on every
second unit of the mannan backbone chain as was earlier established. Guaran molecules
can not segmentally approach uniformly close to each other so as to form associated regions.

Firmness or strength of gels depend on the strength of intersegmental association and
the water holding capacity of the gel will depend on the spacers provided in the amorphous
regions. For example, a gel of agarose is firmer than a gel of cross-linked dextran.

Fig. 17.   Gel of agarose (Agaran) and cross-linked dextran (Sephadex).

Curdlan is unusual in forming a "non-remelting" gel when its solution is heated. You
have heard at this meeting the interesting report of Dr. T. Harada (29). This polysac-
charide has the possibility of wide usefulness. A better understanding of the molecular
interactions which give rise to its rheological properties is rapidly developing and will
be most useful.

More attention has been given in recent years to water absorption by solid carbohydrate
structures. Not just to absorb a little water but to take up large quantities of water
such as 1000-2000 times the weight of the carbohydrate. Absorbers of this nature will
be of wide practical usefulness to wipe up spills, in feminine hygiene, in baby diapers,
in surgical application and in common industrial applications to prevent spilling of
aqueous solutions.

An interesting practical example of such an absorber is the grafted product wherein
polyacrylic acid-amide is grafted to starch (30).

Polysaccharide-protein interactions in plant cell walls
An interesting approach to an understanding of the relation between polysaccharides and
proteins in plant cell walls has been made by Albersheim and coworkers (31-36). Structural
work suggests the existence of a branched arabinan and linear (1→4)-linked galactan oc-
curring as sidechains on a rhamnogalacturonan. Small amounts of a xyloglucan appears
to be covalently linked to a number of galactan chains. Thus, the galactan appears to
serve as a bridge between a xyloglucan and rhamnogalacturonan components of the wall.
The rhamnogalacturonan is shown to consist of an α-D-(1→4)-linked galacturonan chain that
is interspersed with 2-linked L-rhamnosyl residues. The L-rhamnosyl residues are not
randomly distributed along the chain, but probably occur as O-L-rhamnosyl-(1→4)-O-D-
galactouronosyl-(1→2)-rhamnosyl units. This sequence appears to alternate with a homo-
galacturonan sequence containing approximately eight residues of 4-linked D-galacturonic
acid. About half of the L-rhamnosyl residues are branched, having a substituent attached
to C-4, which is thought to be the likely site of the 4-linked galactan. The xyloglucan
seems to be covalently linked to the pectic polysaccharide, but is non-covalently bound
to the cellulose fibrils of the sycamore cell wall. The structure of this polysaccharide,
on the basis of identification of oligosaccharides, is considered to be a repeating hepta-
saccharide unit consisting of four residues of (1→4)-linked β-D-glucose and three residues
of terminal D-xylose linked singly and glycosidically to C-6 of three of the D-glucosyl
residues. There is evidence for a covalent connection between the pectic polysaccharides
and the structural protein of the cell wall. Based on these interconnections and the
strong binding which occurs between the xyloglucan and cellulose, a tentative structure
of the cell wall is proposed. The model is not intended to be quantitative, but wall
components are in approximately correct proportions. A very similar xyloglucan was iso-
lated and characterized from the walls and in the extra-cellular medium of suspension-
cultures of red kidney-bean (Phaseolus vulgaris) cells (36). Although some minor dif-
ferences were found, the basic structure of the xyloglucans in the cell walls of these
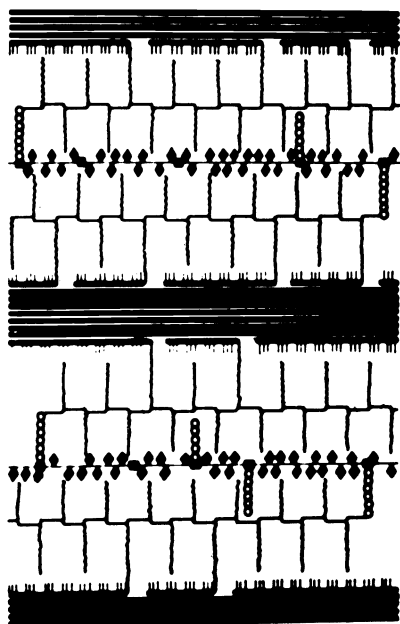distinctly related species is the same.

Fig. 18

≡≡≡ – Cellulose elementary fibril

⊔⊔⊔⊔⊔ – Xyloglucan

♦♦♦♦ – Wall protein with arabinosyl tetrasaccharides glycosidically attached

⊥⊥ – Total pectic polysaccharide

⌐⌐ – Rhamnogalacturonan main chain of the peptic polysaccharide

| – Arabinan and 4-linked galactan side-chains of the peptic polymer

T – 3,6-Linked arabinogalactan attached to serine of the wall protein

—•— – Unsubstituted seryl residues of the wall protein

It is apparent to many people that as more is learned about polysaccharide fine structure and about conformation and interaction in aqueous systems the more useful these carbohydrates will become. Consequently, industrial effort is being added to basic research work to obtain information on polysaccharides.

Coincident with this work are a growing number of projects designed to learn how to produce polysaccharide structures which will perform in expected and highly useful ways. The projects are seeking to produce new polysaccharides and to modify existing polysaccharides. The methods are chemical and biosynthetic, including microbiological and purely enzymatic. This will be an interesting and rewarding field of effort. Just to take one example, suppose a polysaccharide could be designed to work even better than xanthan in tertiary oil recovery. A polysaccharide with appropriate properties for tertiary oil recovery could be used in the United States in an estimated amount of 50 billion pounds.

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