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THE SELECTIVE DEGRADATION OF CARBOHYDRATE POLYMERS

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Abstract - Methods for the selective degradation of polysaccharides and glycoproteins are reviewed. Emphasis is placed on procedures giving information on sugar sequences and on the nature of linkages undergoing modification. Special attention is given to a variety of base-catalyzed degradations, to the introduction of acid-sensitive units within carbohydrate chains, and to nitrous acid deaminations of glycosaminides.

INTRODUCTION

The failure of established methods to provide complete solutions to a number of structural problems in polysaccharide and glycoprotein chemistry has led to the development of new selective and, in some cases, functionally-specific fragmentation procedures (1). In order to ensure that reactions proceed in high yield with the desired selectivity and to establish adequate procedures for product characterization, it is wise to carry out model compound studies before attempting applications to polysaccharides and other carbohydrate polymers. These preliminary studies are important in seeking to define appropriate experimental conditions provided that it is borne in mind that direct extrapolation to the polymer is not always a simple matter. Aside from problems of solubility, which may be far from trivial, competing side-reactions, which are apparently of little importance for the model compounds, may be highly significant in the reactions of the corresponding polymers.

Table 1 shows the main methods used and the types of information obtained in the determination of the primary or covalent structure of carbohydrate polymers. Although the main emphasis here will be given to partial fragmentations and stepwise degradations, the methylation method remains the single most important procedure. This method alone never furnishes a complete solution to a structural problem in that it gives no information on the configuration of glycosidic linkages, it does not always define ring size uniquely, and for carbohydrate polymers containing more than one type of linkage it provides no evidence on the sequence of sugar residues other than indicating end groups, chain units, and branching points. Nevertheless, the method, far from becoming obsolete, has been even more widely used during the past 14 years since the development of rapid and more efficient methylation techniques by Kuhn (2) and Hakomori (3), and through the widespread use of gas chromatography alone (4) and in combination with mass spectrometry (5) for the identification of volatile carbohydrate derivatives. The whole sequence of operations, from alkylation to the analysis of partially alkylated sugar derivatives, may now be achieved so rapidly that the isolation and fractionation of a polysaccharide may be monitored by methylation analysis. Until quite recently the controlled partial depolymerization of methylated polysaccharides has been of limited application for lack of suitable fragmentation procedures and lack of adequate methods for the separation and rapid characterization of partially methylated oligosaccharides. The coupling of alkylation methods with selective fragmentations now provides an extremely powerful approach to structure determination. Thus Fig. 1 shows how an hydroxyl group exposed by selective cleavage of a glycosidic linkage in a methylated polysaccharide may be recognized by further O-trideuteriomethylation (or O-ethylation).

TABLE 1. Structural information derived from methods of general application in polysaccharide chemistry*

Information	Method	Remarks
Ring size and substitution pattern of individual sugar residues	Methylation	May give quantitative data
	Periodate oxidation	Without supplementary information results are rarely unambiguous; within these limitations may give quantitative data
Sequence of sugar residues	Partial depolymerizations leading to oligosaccharides, including partial acid hydrolysis, acetolysis etc., enzymic hydrolysis	Value of a particular procedure dependent on adequacy of separation techniques and extent to which products may be fully characterized
	Stepwise degradations, including sequential enzymic hydrolysis by exo glycosidases, Smith degradation, Svensson degradation of methylated polysaccharides	Progress of reaction followed either by product liberation or by changes in composition of residual polymer
Configuration of glycosidic linkage	Specific enzymic hydrolysis, fragmentations leading to fully characterized oligosaccharides,	
	N.m.r. (^{1}H and ^{13}C) of intact polymers	Extremely valuable for many structurally regular polysaccharides; may give quantitative data
	Selective oxidation of equatorially oriented acetylated glycosides	Valuable small-scale technique, generality not yet established
Sequence at a higher level of structure, e.g., regularity or irregularity of branching and other repeating structures	No generally applicable procedure	

*Methods for compositional analysis are not listed here

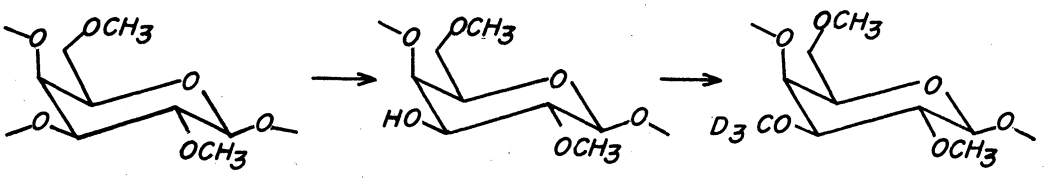


Fig. 1 Selective cleavage of a glycosidic linkage followed by O-trideuteriomethylation of exposed hydroxyl group

In this lecture, which will be concerned with chemical rather than enzyme-catalyzed reactions, distinctions between certain terms are best exemplified by reference to enzyme action. Specificity, as applied to enzymes, implies a measure of uniqueness in the substrate attacked or product formed. Specificity of chemical transformations normally refers to the reactivity of particular functional groups, but in practice uniqueness in structure is not always accompanied by uniqueness in reactivity. Selectivity in chemical or enzyme-catalyzed reactions refers rather to differences in rates which are sufficiently great to allow one reaction to be performed under conditions where competing reactions are of minor significance. Specificity and selectivity are not always easily distinguished, and it may be noted that enzymes, whilst displaying specificity with regard to some structural feature, e.g., the stereochemical configuration of a glycosidic linkage, may be used selectively to break bonds in one structural environment much more rapidly than those in a different location. Some of the degradations to be discussed later are functionally specific, but the overall transformations can only be specific if the required functional group is present in the nature polysaccharide or glycoprotein or may be introduced by a specific reaction or series of reactions. In a number of cases the introduction of the required functionality involves a chemical operation that is selective rather than specific. The terms exo and endo are used to distinguish the modes of action of glycan hydrolases which attack the substrate in a stepwise manner, usually from the non-reducing termini, from those which attack in a random manner. These terms may also be used operationally to indicate the directions in which chemical depolymerizations proceed even if the preferred points of attack are as much a consequence of the fortuitous location of particular functional groups as of the nature of the attacking reagents.

SELECTIVITY IN ACID-CATALYZED DEPOLYMERIZATIONS

The commonest examples of selective partial acid hydrolysis of polysaccharides are those which make use of the readier hydrolysis of furanosides than of pyranosides, and of the relative resistance to hydrolysis of glycosiduronic acids and unprotected glycosaminides. It was only after the development of column chromatographic techniques for the preparative separations of mixtures of oligosaccharides that it became possible to look for and take advantage of the low degree of selectivity afforded by relatively small differences in rates of hydrolysis. Even today rather few systematic studies have been undertaken of relative rates of hydrolysis or acetolysis of different glycosidic linkages. Nevertheless, a number of useful observations have been made in the course of carrying out 'hit or miss' partial depolymerizations, and their importance has been extended by the recognition that certain differences in rates of cleavage of glycosidic linkages may be reversed by using alternative procedures, e.g., acetolysis in place of partial hydrolysis in aqueous acid.

Four examples may be cited of reversals in relative susceptibilities towards acetolysis and partial acid hydrolysis. Whereas (1 → 6)-linkages are generally the most resistant to acid hydrolysis, these linkages are most readily split during the acetolysis of oligomers of D-glucose and D-mannose of both α-D- and β-D-configuration (6).

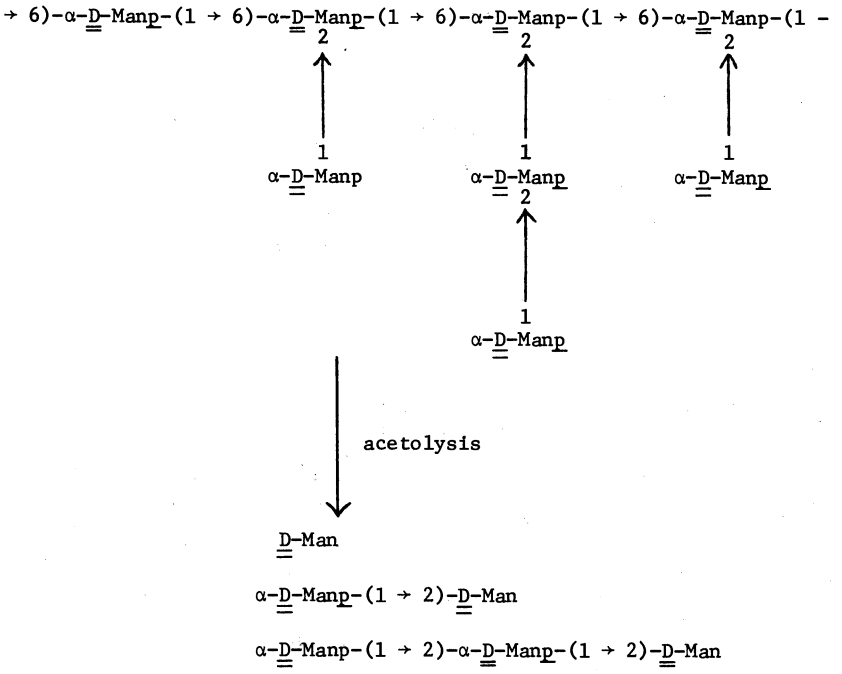


Fig. 2 Selective cleavage of (1 → 6)-linkages during acetolysis of yeast mannan [(1 → 3)-linkages are omitted for simplicity]

Another useful reversal of relative rates of cleavage of different linkages was observed by Rees et al. (10) in the partial depolymerization of desulfated λ-carrageenan where the (1 → 3)-linkages are much more readily split by acid hydrolysis than the (1 → 4)-linkages, whereas the reverse situation holds for acetolysis (Fig. 3).

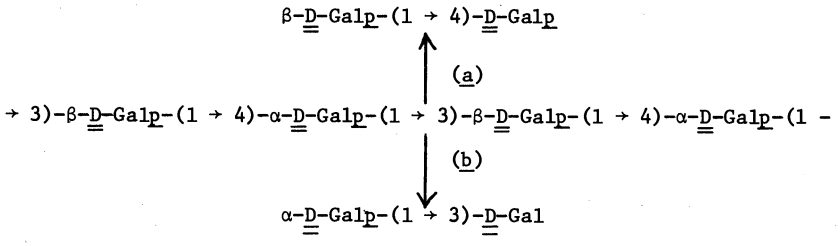


Fig. 3 Selective depolymerizations of desulfated λ-carrageenan during (a) partial acid hydrolysis and (b) acetolysis

Acetolysis is also the preferred procedure for depolymerization for the isolation of oligosaccharides with intact 6-deoxyhexopyranosyl linkages. Thus whereas the L-fucopyranosyl linkages in tragacanthic acid (11) are selectively hydrolyzed in aqueous acid, acetolysis permits the isolation of 2-O-α-L-fucopyranosyl-D-xylose (Fig. 4). Further examples of acetolysis, in these cases for the isolation of oligosaccharides with intact L-rhamnopyranosyl linkages, are provided with polysaccharides which have undergone prior structural modification by carboxyl-reduced gum arabic [Fig. 5 (12)] and carboxyl-reduced Sterculia urens gum [Fig. 6 (13)].

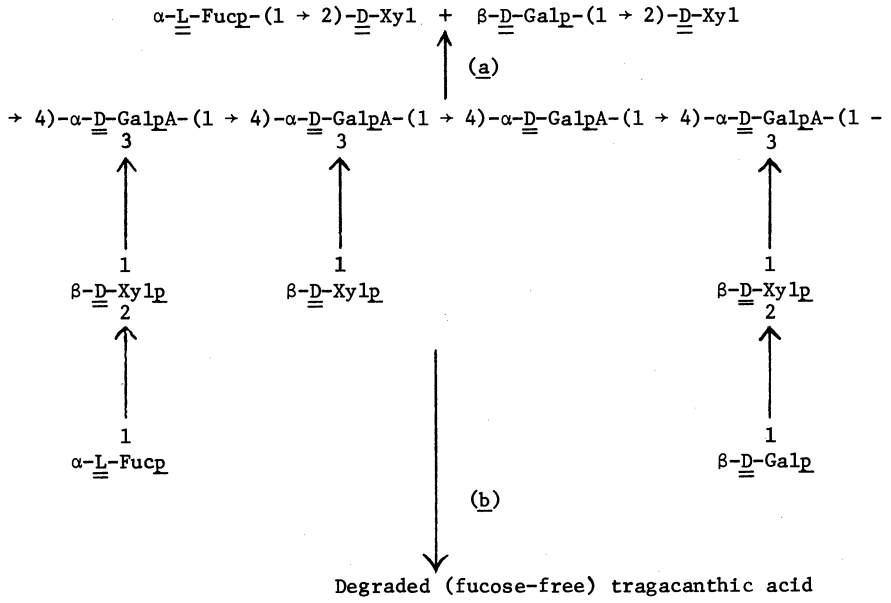


Fig. 4 (a) Retention during acetolysis and (b) selective scission during partial acid hydrolysis of L-fucopyranosyl bonds in tragacanthic acid

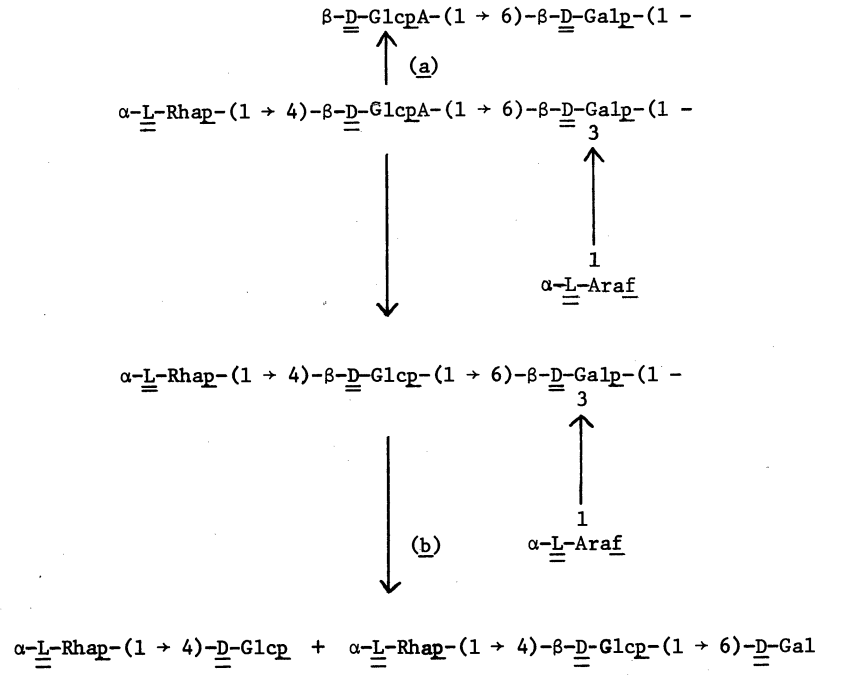


Fig. 5 Cleavage of outer chains of gum arabic, (a) with selective liberation inter alia of L-rhamnose during partial acid hydrolysis and (b) with retention of L-rhamnopyranosyl bonds during acetolysis of the carboxyl-reduced polysaccharide

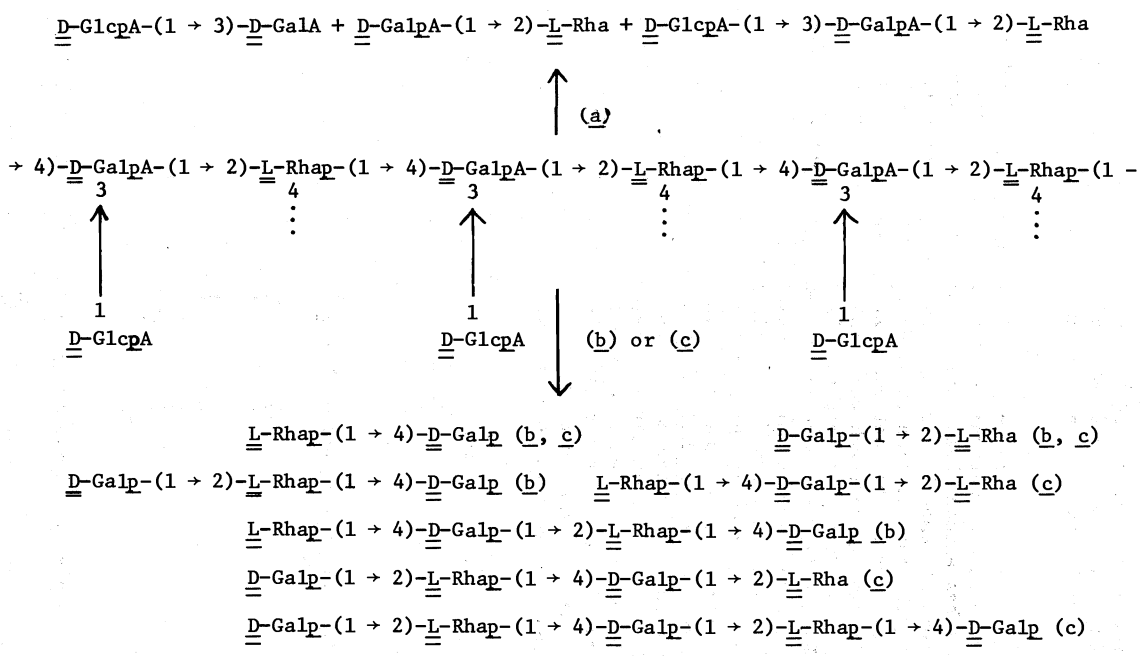


Fig. 6 Oligosaccharides from the galacturonorhamnan region of Sterculia urens gum formed (a) with liberation of L-rhamnose reducing groups from partial acid hydrolysis, and with retention of L-rhamnopyranosyl linkages from acetolysis of (b) the carboxyl-reduced gum and (c) the degraded polysaccharide from Smith degradation of the carboxyl-reduced gum

In studies on glycosaminoglycans, glycoproteins and glycolipids, in addition to minimizing N-deacetylation, acetolysis finds its most striking application in permitting the isolation of sialic acid-containing oligosaccharides; in contrast, sialic acids are generally extremely labile to aqueous acid. Examples of the isolation of such oligosaccharides (Fig. 7) are furnished in the acetolysis of brain ganglioside G_1 (14) and of glycopeptides derived from human serotransferrin (15).

(a) α-NANA-(2 → 3)-D-Gal

(b) α-NANA-(2 → 6)-β-D-Galp-(1 → 4)-D-GlcpNAc

α-NANA-(2 → 6)-β-D-Galp-(1 → 4)-β-D-GlcpNAc-(1 → 2)-α-D-Manp-(1 → 3)-D-Man

Fig. 7 Oligosaccharides containing intact sialic acid residues formed on acetolysis of (a) brain ganglioside GM_1 and (b) glycopeptides derived from human serotransferrin [NANA = neuraminic acid or sialic acid]

In seeking to achieve further selectivity in partial fragmentation a further approach is to try to alter or even to reverse the 'natural' selectivity by structural modification of the polysaccharide. Reference has already been made to the reduction of hexuronic acid to hexose residues with consequent removal of acid-resistant glycosiduronic acid linkages. The opposite process involving the introduction of acid-resistant linkages by the selective oxidation of primary hydroxyl groups has not been extensively used due to lack of efficient methods of oxidation. The general approach was first demonstrated in the oxidation of terminal L-arabinofuranose residues in an arabinoxylan (16) and Fig. 8 shows the different patterns of depolymerization before and after structural modification. 3,6-Anhydrohexose residues, e.g., those of 3,6-anhydro-D(or L)-galactose in algal polysaccharides, are extremely labile to acid, and in addition for isolation the cleavage products must be protected against further degradation by the formation of glycosides or acyclic dialkyl acetals or thioacetals. Figure 9 shows the selective introduction of 3,6-anhydrohexose residues into a glucomannan from Ceratocystis brannea (17), followed by controlled hydrolysis of the modified but not of the unmodified glycosidic linkages. Another type of structural modification to which more detailed reference will be made later involves the formation and ready hydrolysis of hex-5-enopyranosides. The formation of such modified sugars residues is selective rather than specific, but since the hydrolysis probably proceeds by a different mechanism from that of normal glycosides, the approach may be considered with other functionally specific degradations.

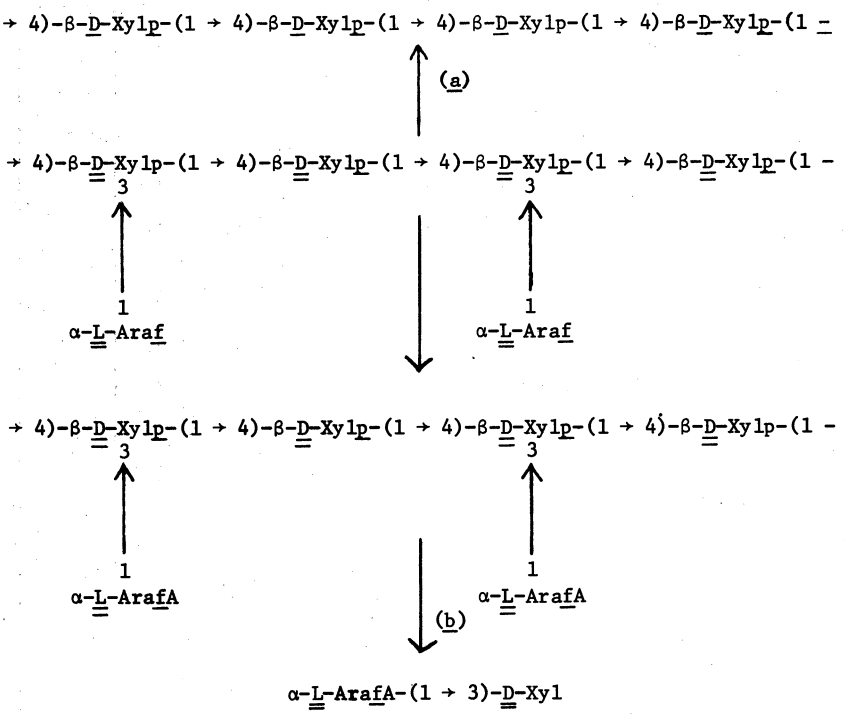


Fig. 8 Susceptibility of L-arabinofuranosyl linkages during (a) partial hydrolysis of arabinoxylan and (b) resistance to partial hydrolysis after selective oxidation in modified polysaccharide

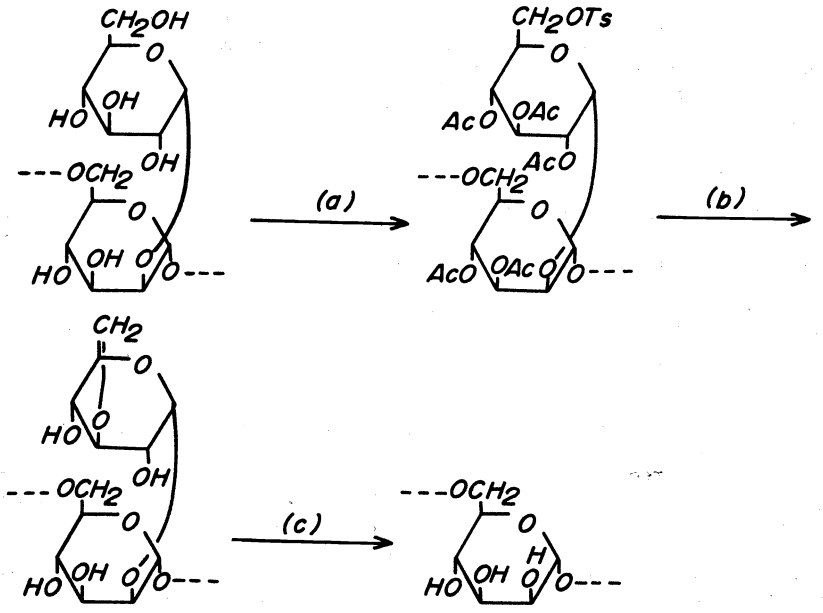
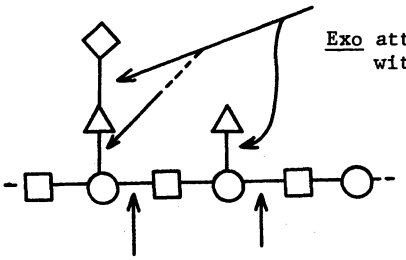


Fig. 9 Structural modification of Ceratocystis brannea glucomannan by (a) successive tritylation, acetylation, detritylation and p-toluenesulfonylation, (b) treatment with sodium methoxide, and (c) mild acid hydrolysis

FUNCTIONALLY SPECIFIC DEGRADATIONS

The majority of methods, whether specific or merely selective, for degradation of branched polysaccharides proceed by exo attack. These methods, which are summarized in Fig. 10, include those based on periodate oxidation, such as the Smith degradation (18), since exposed glycol units are most frequently concentrated in exterior chains. Furthermore, since many polysaccharides contain relatively acid-sensitive glycosidic linkages in peripheral positions, partial acid hydrolysis and related reactions preferentially remove these outer residues so that it is often possible to isolate structurally simpler degraded polysaccharides with minimum cleavage of interior linkages. Information on the mode of attachment of the acid-labile peripheral units is therefore often limited to a 'difference' approach in which the parent and partially degraded polysaccharides are compared, for example, by methylation analysis. In principle, endo attack should provide the means whereby fragments with intact side-chains still attached could be derived from the rupture of interior chains. Enzymic hydrolysis would be ideal, but unfortunately rather few enzymes have yet been isolated, which have the required specificity and action pattern to approach sufficiently close to the branch points, to give conveniently isolable oligosaccharide fragments. A number of laboratories, especially those of Professor Lindberg in Stockholm and Professor Kochetkov in Moscow, are presently very active in looking for selective chemical procedures for the controlled fragmentation of polysaccharides and glycoproteins. Our attention has been directed primarily to branched polymers with the objective of isolating sugar residues, or recognizable degradation products, from interior chains with side-chains still attached.



Exo attack, e.g. Smith degradation, hydrolysis with exo enzymes, partial acid hydrolysis

Endo attack, e.g. hydrolysis with endo enzymes, base-catalyzed β-elimination of 4-O-substituted hexuronates, structural modifications leading to acid-sensitive linkages

Fig. 10 Degradations of polysaccharides by exo and endo attack

Table 2 shows a number of functionally specific degradations. The largest group includes four types of base-catalyzed processes. It is well known that in the alkaline degradation of reducing carbohydrates β-eliminations result in the exposure of new reducing groups, and that the preferentially formed products are characteristic of the sites of substitution of the reducing unit. Table 3 outlines the main transformation products from variously substituted reducing groups. Whilst the substitution pattern of the reducing group determines the nature of the major degradation product, degradations are rarely specific. Consequently, the well known 'peeling' reaction, in which stepwise erosion of a polysaccharide takes place from its reducing terminus with repetitive exposure of new reducing groups, rarely, if ever, proceeds to completion. Like all chain processes the reaction sequence is particularly susceptible to interception by competing reactions. A familiar example of the so-called 'stopping' reaction, without which many wood pulping companies would be bankrupt, is that in cellulose (19) which leads to the formation of an alkali-stable metasaccharinic acid end group (Fig. 11). If such is the case for linear homopolysaccharides the prospects for achieving complete stepwise degradation of more complex polysaccharides are very limited.

TABLE 2. Some functionally specific degradations

Base-catalyzed degradations
(1) from reducing groups
(2) from 4-O-substituted hexuronates
(3) from carbonyl groups (from oxidation of isolated hydroxyl groups)
(4) from 6-C-sulfonyl derivatives
Hypochlorite degradation of glycosiduronamides
Acid hydrolysis of hex-5-enopyranosides
Nitrous acid deamination of equatorially oriented 2-amino-2-deoxyglycosides

TABLE 3. Alkaline degradation of reducing sugar derivatives

Site of substitution	Products from reducing unit	New reducing groups exposed for further degradation
2-O-	None at room temperature	
3-O-	Metasaccharinic acids (3-deoxyaldonic acids)	3-O-substituent
4-O-	Isosaccharinic acids (3-deoxy-2-C-hydroxymethylaldonic acids)	4-O-substituent
6-O-	Metasaccharinic acids, fragments from dealdolization	6-O-substituent (when dealdolization occurs)
2,3-di-O-	2-O-Substituted hex-2-enopyranose (from which the 2-O-substituent is readily liberated by acid)	3-O-substituent
3,4-di-O-	various deoxyaldonic acids	3-O- and 4-O-substituents
3,6-di-O-	6-O-glycosylmetasaccharinic acids	3-O-substituent
4,6-di-O-	5-O-glycosylisosaccharinic acids	4-O-substituent
2,3,4,6-tetra-O-	2,4,6-tri-O-substituted hex-2-enopyranose (from which 3- and 4-O-substituents are readily liberated by acid with formation of 5-O-glycosyloxymethyl-2-furaldehyde)	

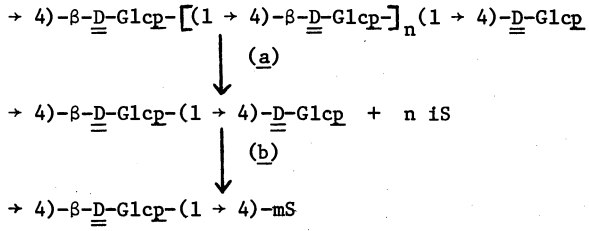


Fig. 11 Alkaline degradation of cellulose, (a) stepwise erosion by the 'peeling' reaction, (b) formation of alkali-stable product by the 'stopping' reaction [iS = isosaccharinic acid, mS = metasaccharinic acid]

Branched polysaccharides may be degraded from the reducing end along interior chains with the formation of recognizable products carrying intact exterior glycosidic linkages. However, since reaction does not proceed to completion, valid structural conclusions may only be drawn for those portions of the polysaccharide chain undergoing degradation. In (1 → 3)- and (1 → 4)-linked glycans with branches at C-6 the 'peeling' reaction proceeds past the branch point with the formation of saccharinic acids carrying intact side-chains (20, 21). A complicating factor, however, in saccharinic acid formation is the production of diastereomeric pairs of compounds. In our hands attempts to degrade such an epimeric pair of 6-O-glycosylsaccharinic acids derived from Sclerotium rolfsii β-D-glucan (20) to a single transformation product was only partially successful. In the event the epimeric acids were lactonized and reduced with sodium borohydride at pH 3-4 to to O-glycosyl-3-deoxyhexoses (Fig. 12).

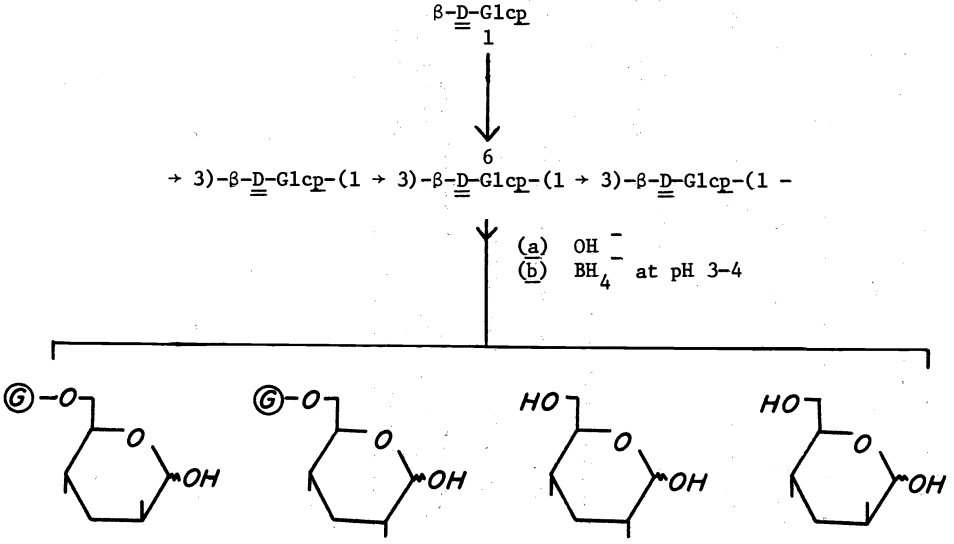


Fig. 12 Alkaline degradation of β-D-glucan from Sclerotium rolfsii (~56% complete)
[G = β-D-glucopyranosyl, | = -OH]

Alkaline degradations initiated at reducing groups will probably be of limited value in structural studies on polysaccharides. The procedure, however, offers much greater promise for oligosaccharides of relatively low molecular weight where degradation reactions diagnostic for substitution pattern are likely to occur with few detectable side reactions. Such oligosaccharides may be those formed by selective cleavage of interior linkages in polysaccharide chains, e.g., by base-catalyzed β-elimination of 4-O-substituted hexuronate residues, or by liberation from base-sensitive O-glycosidic linkages to serine or threonine residues in glycoproteins. The latter type of degradation deserves special mention. Since the reducing sugar units of oligosaccharide chains in such glycoproteins are liberated in the presence of base, further degradation is liable to occur. One or both of two approaches may be used in product characterization. If alkaline degradation is allowed to proceed until an alkali-resistant linkage is encountered, the final stable product may be isolated and characterized or the sequential formation of degradation products typical of various modes of linkage may be monitored. Both procedures have been used in studies on blood group substances (22, 23). Alternatively intact oligosaccharide chains may be trapped as in the method developed by Carlson and Iyer (24) where, in the presence of

adequate concentrations of sodium borohydride, the initially formed reducing group is converted into an alditol residue more rapidly than it is degraded by alkali.

The second main type of base-catalyzed degradation is that of 4-O-substituted hexuronates. This reaction, was first recognized as a competing reaction in the saponification of highly esterified pectins in aqueous solution. Scission of glycosidic linkages occurs with formation of hex-4-enopyranosiduronates and exposure of reducing groups. The degradation is performed most effectively in non-aqueous, and preferably non-hydroxylic solvents, with polysaccharide derivatives carrying base-stable substituents, which may be permanent (e.g., methyl ethers) or subsequently removable (e.g., methoxyethyl acetals [25]), and using bases of low nucleophilicity towards ester functions so that the driving force of the carboalkoxy group is maintained. The first general procedure satisfying these criteria was developed and has been extensively used by Lindberg and his collaborators (26). The method, which involves treatment of the polysaccharide derivative with sodium methylsulfinylmethylide in methyl sulfoxide, is particularly suitable for the study of methylated acidic polysaccharides which are available in only limited quantities. The course of the degradation is readily monitored by analysis of the unmodified sugar residues, usually by hydrolysis, reduction and acetylation, and identification of partially methylated alditols by gas chromatography-mass spectrometry. Furthermore the newly formed non-reducing termini are those of hex-4-enopyranosiduronates which may be easily hydrolyzed by very weak acid, usually with a high degree of selectivity. The exposed hydroxyl groups to which uronic acid residues were formerly attached are readily recognized by re-O-alkylation with trideuteriomethyl iodide or ethyl iodide. The overall procedure is illustrated in Fig. 13 for methylated Klebsiella type 52 polysaccharide (27).

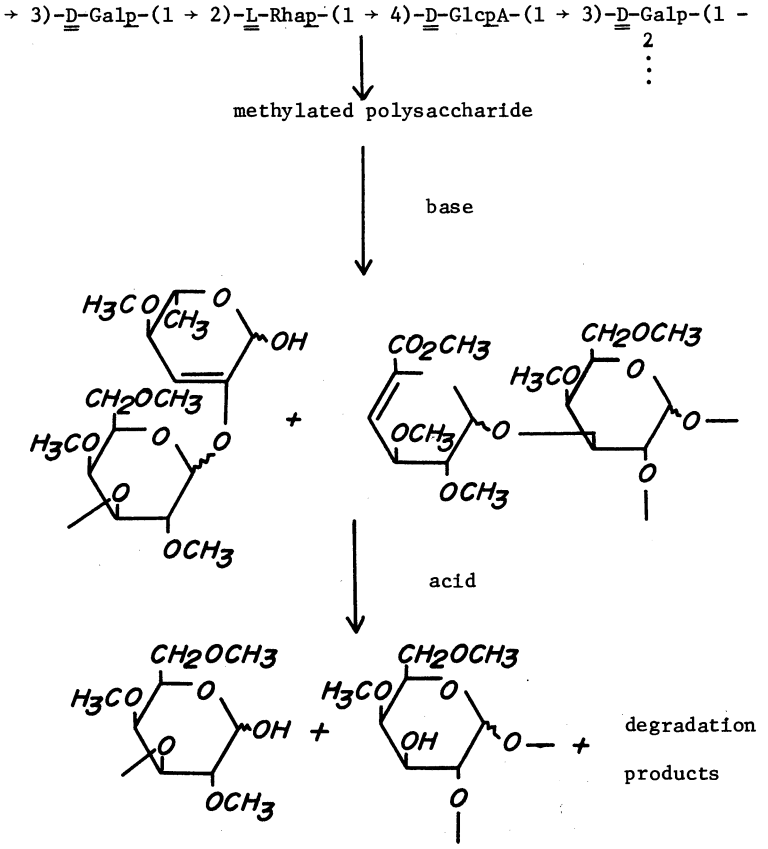


Fig. 13 Base-catalyzed degradation of methylated Klebsiella type 52 polysaccharide

Despite the strongly basic conditions Hakomori's method has been used successfully for the methylation of several acidic polysaccharides containing 4-O-substituted hexuronate residues. A surprising observation, however, has been made recently by Shimizu (28) during the methylation and subsequent partial hydrolysis of birch xylan. One of the products has been characterized as the partially methylated unsaturated aldobiouronic acid, 3-O-methyl-2-O-(4-deoxy-2,3-di-O-methyl-β-L-threo-hex-4-enopyranosyluronic acid)-D-xylose (Fig. 14).

That β-elimination could take place during the methylation is hardly surprising, but there is no simple explanation for the survival of the acid-labile hex-4-enopyranosiduronate residue during the partial hydrolysis.

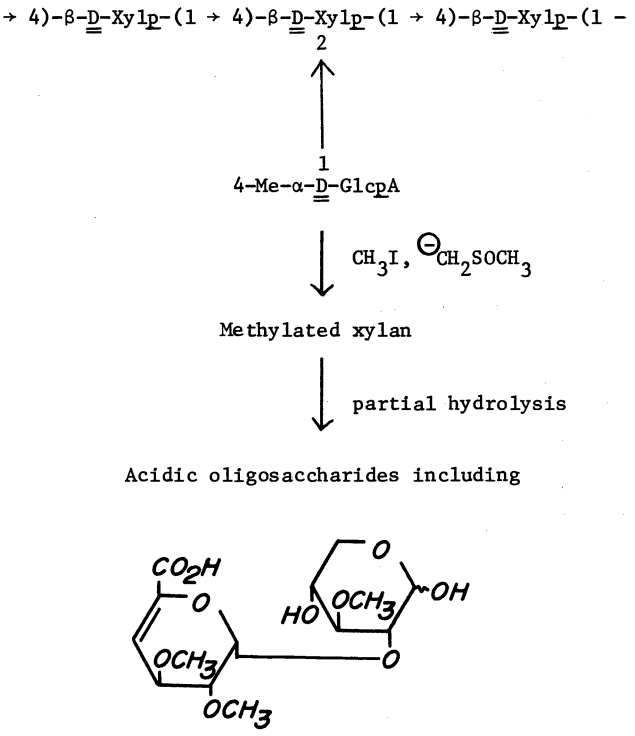


Fig. 14 Formation of unsaturated aldobiouronic acid from methylated birch xylan

A further consideration to be noted in the Lindberg method is that the exposed reducing units of the outer chains arising from the β-elimination are not protected from further reaction, and that degradation will proceed until some base-stable unit, such as a 2-O-substituted 3-deoxy-hex-2-enopyranose, is formed. These acid-labile units are destroyed during the analysis of methylated sugars and the disappearance of one or more constituents provides evidence for sequence in placing such a unit (or units) immediately external to the degraded hexuronate residue. There are, however, situations where it is desirable to obtain maximum information on the fragments arising from β-eliminations, and for this purpose the behaviour of model compounds was studied. Thus treatment of the maltose derivative, methyl (methyl 4-O-[2,3,4,6-tetra-O-methyl-α-D-glucopyranosyl]-2,3-di-O-methyl-β-D-glucopyranosid)uronate, with DBU (1,5-diazabicyclo[5.4.0]undec-5-ene), a base of low nucleophilicity towards esters, led to the formation of the known unsaturated hexuronate with virtually complete scission of the inter-residue bond. The exposed reducing sugar was protected from further degradation by acetylation when the elimination was performed in the presence of added acetic anhydride (29). Products from both sides of the bond cleavage were thus identified (Fig. 15). In a similar manner conditions were confirmed for the selective acid hydrolysis of hex-4-enopyranosiduronates (Fig. 15). A study of the β-elimination of methylated degraded leiocarpan A showed that cleavage of interior linkages with protection of exposed reducing groups could be used to isolate segments of the interior chain with side-chains intact (Fig. 16). Reduction of the disaccharide with sodium borohydride followed by further O-alkylation with trideuteriomethyl iodide furnished a derivative suitable for characterization by g.l.c.-mass spectrometry. In this manner it was possible to obtain otherwise inaccessible structural information on this polysaccharide (30), namely, direct confirmation of the site of attachment and evidence for the configuration of the glycosidic linkage.

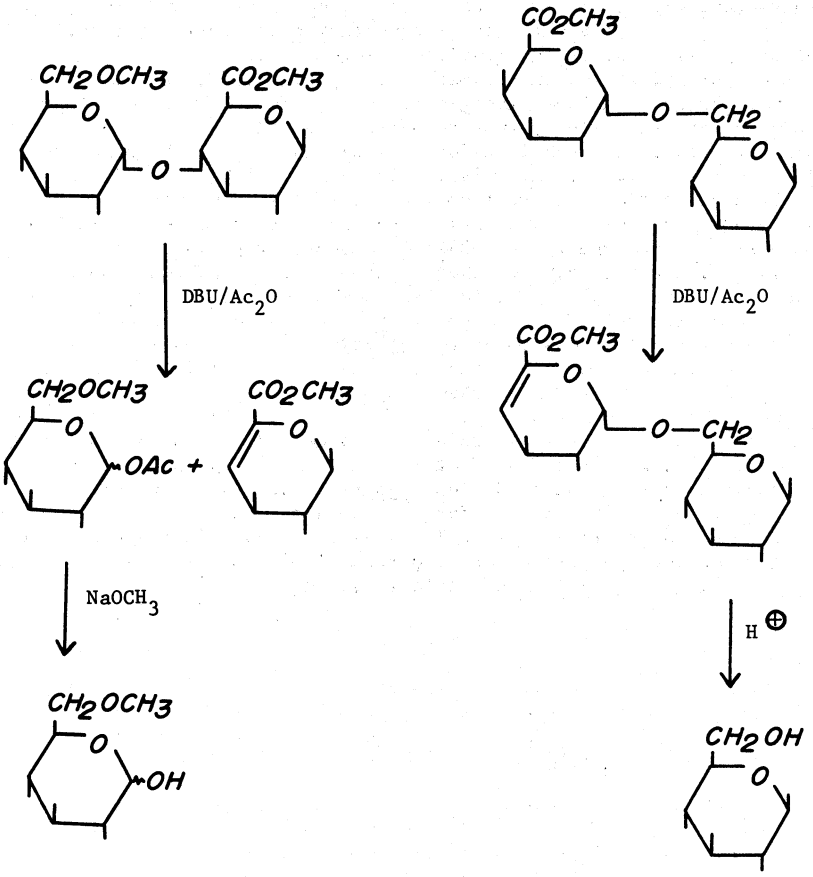


Fig. 15 Base-catalyzed β-eliminations of methylated acidic disaccharides [| = OCH_3]

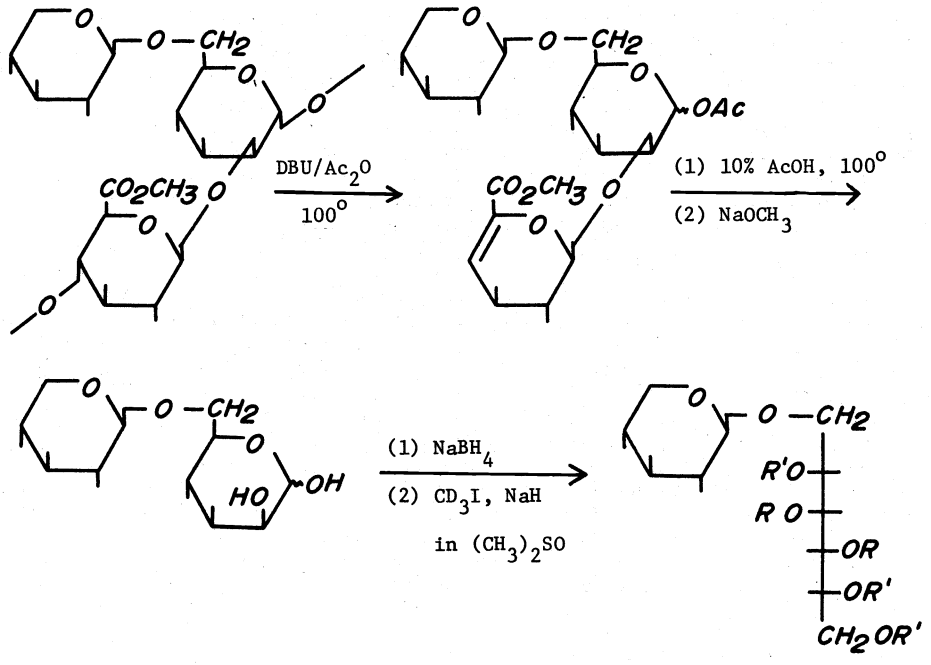


Fig. 16 Isolation of side-chain units by fragmentation of methylated degraded leiocarpan A [| = OCH_3, R = CH_3, R' = CD_3]

The third base-catalyzed degradation is that developed by Svensson and his collaborators and it provides a stepwise procedure for the fragmentation of partially methylated carbohydrate polymers (31). The method requires the generation of a limited number of specifically placed hydroxyl groups in the methylated polymer, for example, from the controlled hydrolysis of an acid-sensitive glycosidic linkage or by base-catalyzed β-elimination of 4-O-substituted glycopyranosiduronates. Specific oxidation to carbonyl groups, preferably with chlorine-methyl sulfoxide (32), is followed by base treatment and results directly, or after mild acid hydrolysis, in the splitting of a glycosidic linkage and the exposure of a new hydroxyl group from which the whole operation may be repeated. Furthermore, the location of the new hydroxyl group may be ascertained by re-O-alkylation. On the basis of model compound reactions (33-36) the degradation may be initiated from 2-, 3-, 4- or 6-hydroxyl groups, and from the 4- and 6-hydroxyl groups exposed on removal of cyclic ketals, such as those of pyruvic acid. Examples are shown in Fig. 17. Figure 18 shows the application of the method to the methylated polysaccharide from Klebsiella type 59 (37). In this and other applications to bacterial polysaccharide derivatives glycosyl substituents have been placed adjacent to the generated keto function so that the glycosidic linkage has been split by acid after the base-catalyzed elimination. When reducing groups are liberated directly under basic conditions further degradation presumably occurs, and probably proceeds until an alkali-stable product is formed (38). The degradative procedure has been applied to the stepwise erosion of outer chains, and, as in the example cited in Fig. 18, to the scission of internal bonds in regularly repeating structures where essentially only one product is given. As far as I am aware, the method has not yet been used for the cleavage of interior glycosidic linkages in polysaccharide chains containing non-repetitive sugar sequences where different oligomeric fragments would be formed externally and internally to the points of chain scission.

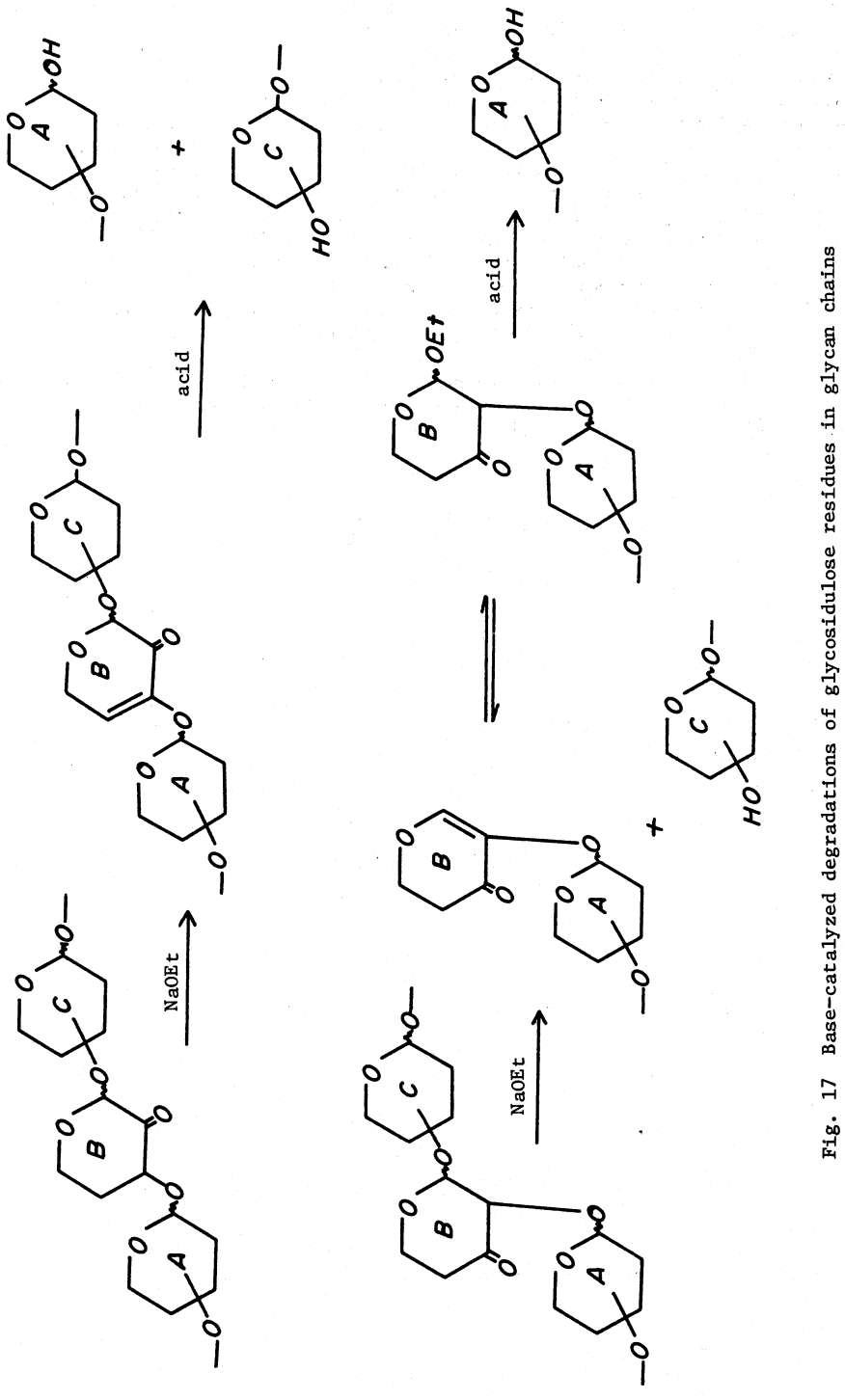


Fig. 17 Base-catalyzed degradations of glycosidulose residues in glycan chains

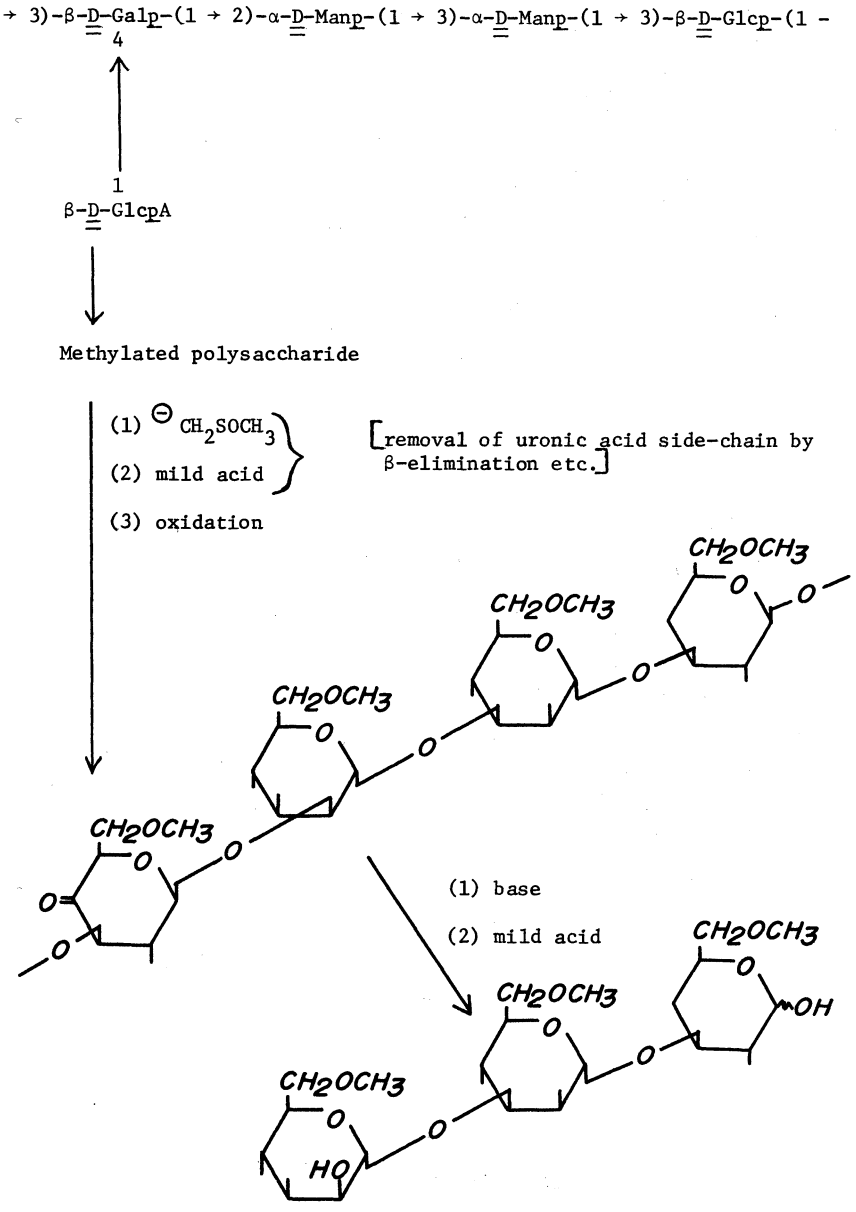


Fig. 18 Stepwise degradation of methylated Klebsiella type 59 polysaccharide

The sulfone degradation (39) was the first base-catalyzed degradation to be applied to an otherwise fully methylated polysaccharide. The method requires the introduction of a p-tolylsulfone function from primary hydroxyl groups in a series of selective substitutions by conversion into the 6-deoxy-6-iodo derivative and reaction with sodium p-toluenesulfinate Base-catalyzed β-elimination is then performed with sodium methylsulfinylmethylide with exposure of an hydroxyl group on the next internal sugar residue. Fig. 19 shows an application of the method to the stepwise degradation of methylated Pneumococcus type 2 polysaccharide in which the residues in the two unit side-chains have been successively eliminated by uronic acid and sulfone degradations (40). The method clearly requires relatively large quantities of material for the selective substitution steps. The fate of the liberated sulfone moiety, to which glycosyl substituents may be attached, is not known, so the potential of the procedure for providing detailed information on sugar sequences external to the site of cleavage has still to be ascertained.

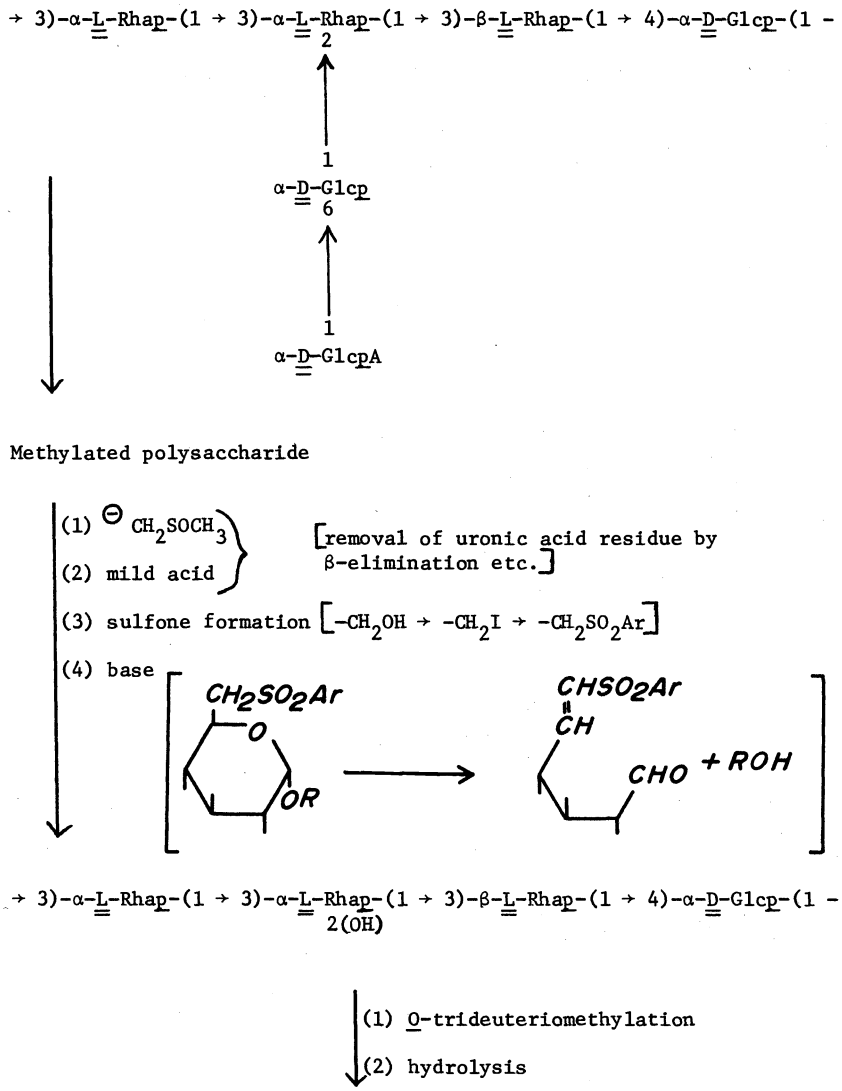


Fig. 19 Sulfone degradation in the stepwise depolymerization of methylated Pneumococcus polysaccharide

In a strategically different approach Baker and Whistler have elegantly employed the sulfone degradation to assess the distribution of side-chains in galactomannans (41). The modification of unsubstituted primary hydroxyl groups allows the side-chains and unbranched units in the main chains to be stripped away with the liberation of fragments containing mannose residues formerly involved in branching and terminated by modified sugar sulfone residues (Fig. 20). The unmodified sugar residues were suitable for methylation analysis. Figure 21 shows how the method has been used to detect regular branching on alternate mannose units in guaran, but blocks of branched units interspersed with unbranched regions in locust-bean gum.

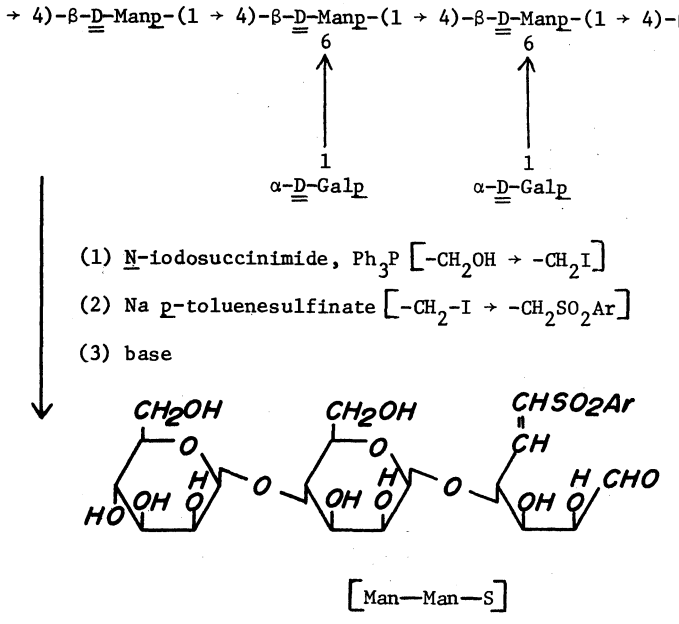


Fig. 20 Sulfone degradation of galactomannans

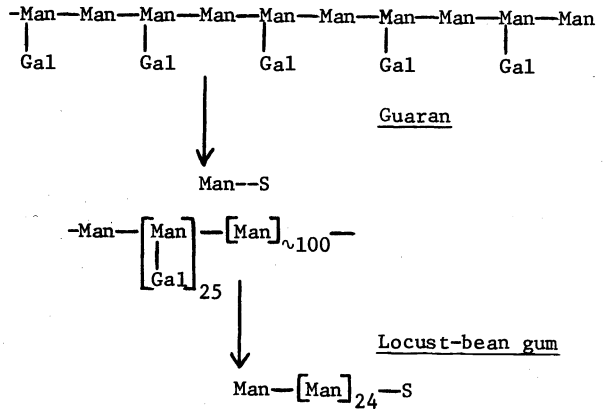


Fig. 21 Isolation of branch points by sulfone degradation of guaran and locust-bean gum [S = modified sugar sulfone unit]

The next degradative technique is selective rather than specific and has yet only been developed for model compounds, but it may be considered here since the method is designed to give structural information on both fragments when interior linkages are split. The introduction of acid-sensitive hex-5-enopyranoside units may be achieved from primary hydroxyl groups by reaction with triphenylphosphite methiodide followed by treatment with DBU. The formation of otherwise substituted hexose residues by selective blocking and deblocking is cumbersome, but our use of such groups is primarily for carboxyl-reduced methylated uronic acid-containing polysaccharides. Model compound studies (Fig. 22) have shown that hex-5-enopyranosides are hydrolyzed under extremely mild conditions with no detectable hydrolysis of normal glycosidic linkages (42, 43). External chains are terminated by 6-deoxyglycos-5-ulose residues and an unsatisfactory feature of our present characterization procedure, which involves reduction with sodium borohydride and further O-alkylation, is the formation of C-5 epimers. Nevertheless, the epimeric products in the maltose series were separable by gas chromatography and mass spectrometry confirmed their structural identity in other respects including the site of the O-glycosyl substituent. Furthermore the ^{1}H n.m.r. spectrum of the mixture of epimers confirmed the configuration of the intact glycosidic linkage. Inasmuch as this reaction sequence may be applied, after reduction of hexuronic acid units, to methylated acidic polysaccharides, it is complementary to the base-catalyzed degradation of 4-O-substituted hexuronates. Whereas the base-catalyzed reaction cleaves the polysaccharide chain externally to the 4-O-substituted hexuronic acid, the hex-5-enopyranoside procedure would furnish a fragment in which the entire external chain is attached to a modified sugar residue arising from the original hexuronic acid unit.

Furthermore the fragmentation method would not be restricted to hexuronic acid residues carrying glycosyl substituents at 0-4.

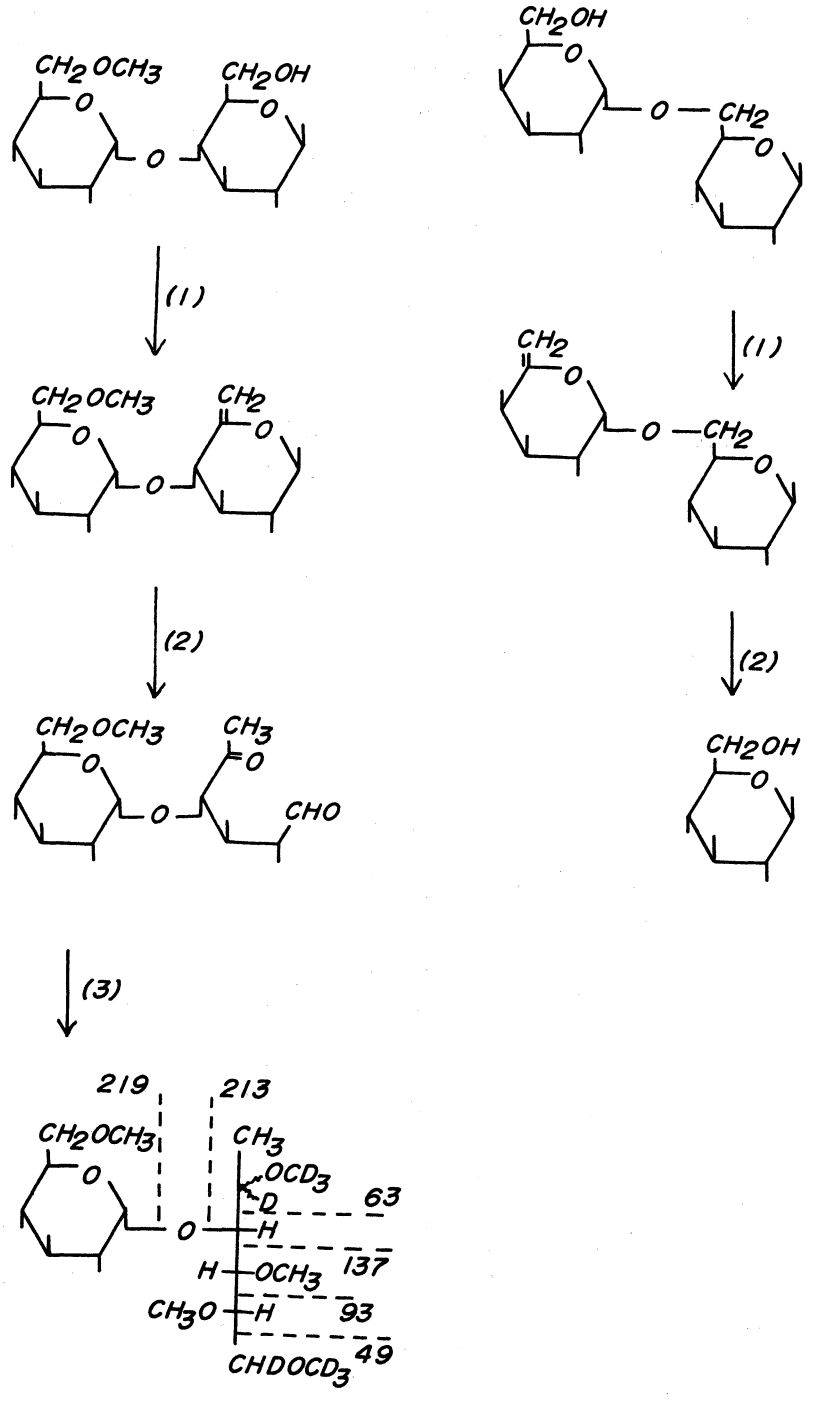


Fig. 22 Selective hydrolysis of hex-5-enopyranosides in disaccharide model compounds. (1) $Ph_3P\text{-}CH_3^{\oplus}\ I^{\ominus}$, DBU; (2) $H^{\oplus}$; (3) $NaBD_4$, $CD_3I/^{\ominus}CH_2SOCH_3$. [| = OCH_3]

Another degradation initiated at hexuronic acid residues is that developed by Kochetkov and co-workers (44) in which the Hofmann reaction with sodium hypochlorite is applied to hexopyranosiduronamides (Fig. 23). The degradative sequence has been applied to the selective removal of the 4-O-methyl-D-glucuronic acid side-chains from birch xylan (Fig. 24). To my knowledge, however, the reaction has not yet been applied successfully for the scission of interior glycosidic linkages, and procedures have not been developed for the characterization of sugar sequences external to the site of glycoside cleavage. In principle the method probably has greater potential for obtaining structural information than some of the procedures already discussed, provided that the substituted pentodialdose is not further degraded under the alkaline conditions of the reaction.

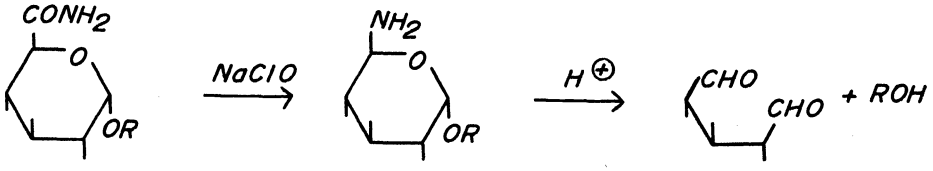


Fig. 23 Degradation of hexopyranosiduronamides with selective glycoside cleavage

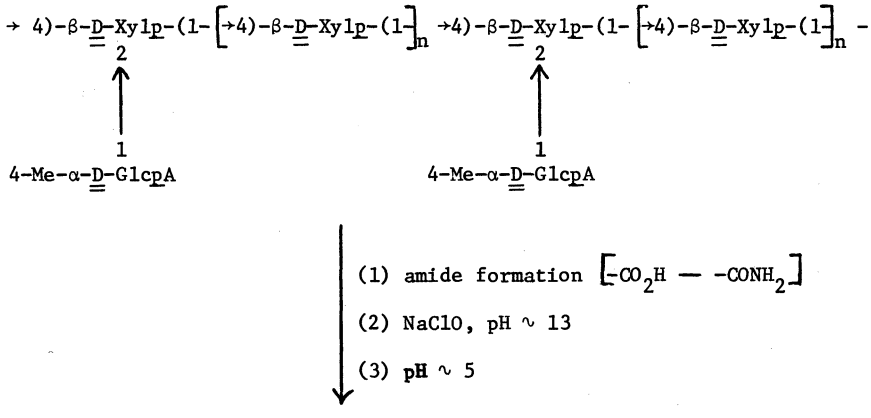


Fig. 24 Degradation of birch xylan with removal of 4-O-methyl-D-glucuronic acid side-chains

The last technique for the selective degradation of carbohydrate polymers to be discussed is that of nitrous acid deamination of 2-amino-2-deoxy glycosides (45). My comments will be mainly restricted to the reactions of the two 2-amino-2-deoxyhexoses of widespread occurrence, namely, D-glucosamine and D-galactosamine, which carry equatorially oriented amino functions. We may observe in passing that the deamination of axially oriented aminodeoxy glycosides, as in derivatives of D-mannosamine, is a complex reaction but that, at least in polymers, the dominant reaction involves the conversion of D-mannosamine into D-glucose residues without scission of glycosidic linkages. Thus Matsushima and his collaborators (46) have converted a polysaccharide, a 2-acetamido-2-deoxy-D-mannurono-D-glucan, from the cell walls of Micrococcus lysodeikticus into a D-glucan by nitrous acid deamination of the N-deacetylated carboxy-reduced polysaccharide (Fig. 25). That deamination of D-glucosamine units in polymers occurs with predominant formation of 2,5-anhydro-D-mannose and with rupture of glycosidic linkages was observed many years ago for N-deacetylated chitin, and the reaction has been used extensively in recent studies on heparin, notably by Lindahl (47) and Perlin (48) and their co-workers. The potential of the procedure in sequence determination in heteropolysaccharide chains was clearly demonstrated by Baddiley and Buchanan and their collaborators in the reactions of the dephosphorylated repeating units from the specific substances from Pneumococcus types 10A (49) and 29 (50). Figure 26 shows alternative points of cleavage in the type 10A substance from nitrous acid deamination on the one hand and partial acid hydrolysis on the other hand.

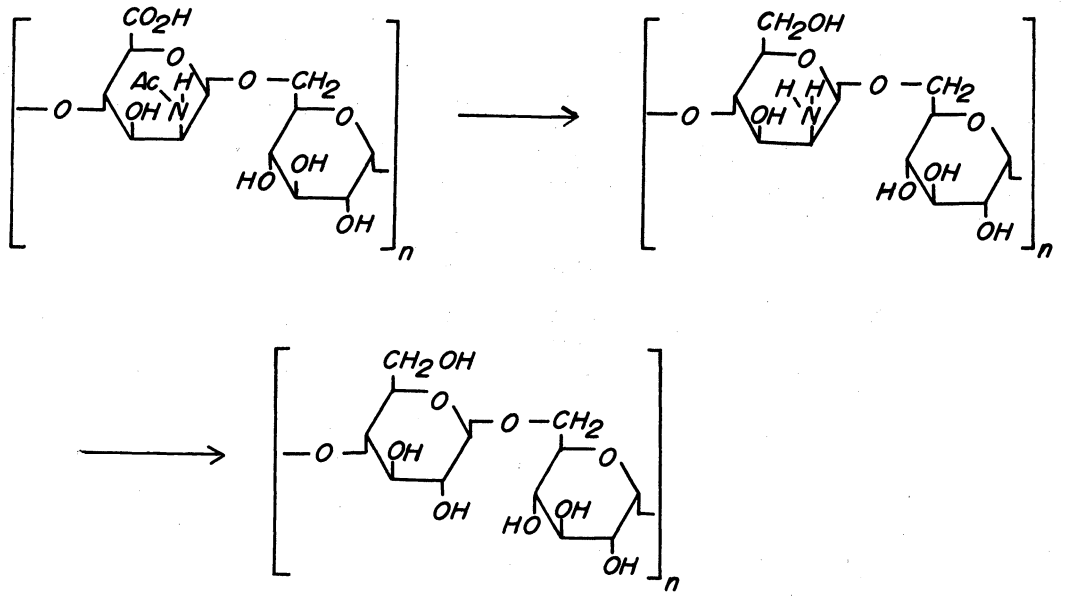


Fig. 25 Conversion of a 2-acetamido-2-deoxy-D-mannurono-D-glucan from Micrococcus lysodeikticus cell walls into a D-glucan by nitrous acid deamination of the N-deacetylated carboxy-reduced polysaccharide

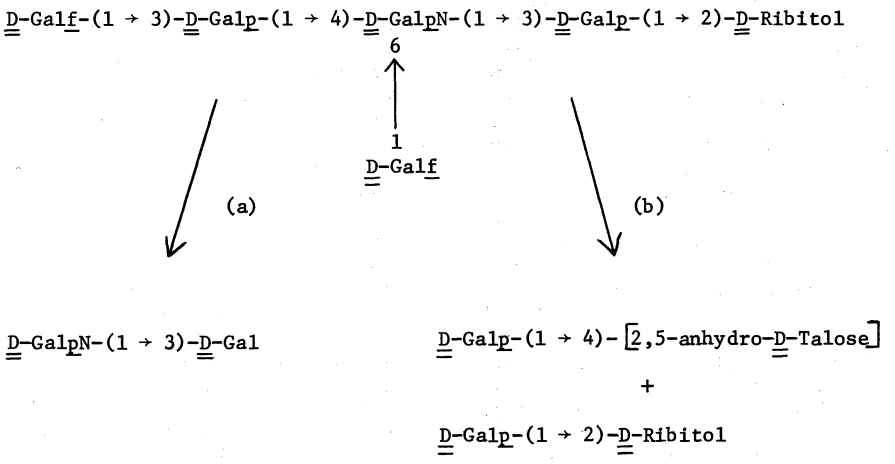


Fig. 26 Fragmentation of N-deacetylated hexasaccharide from Pneumococcus type 10A specific substance, (a) by partial acid hydrolysis and (b) by nitrous acid deamination (with accompanying hydrolysis of galactofuranosyl bonds).

In turning to some recent more sophisticated examples of the use of nitrous acid deaminations in polysaccharide and glycoprotein structure determinations, and to some relevant model compound experiments, I wish to comment on the three aspects of the overall reaction sequence shown in Table 4. Since the commonly occurring amino sugars are found most frequently as their N-acetyl derivatives, N-deacetylation must be achieved as efficiently as possible with minimum degradation of the carbohydrate substrate. Hydrazinolysis, preferably in the presence of catalytic quantities of hydrazine sulfate (51), is a commonly used procedure but complete N-deacetylation is not always achieved. Recently Lindberg et al. (52) have reported an improved method using sodium hydroxide in methyl sulfoxide for dealing with intransigent materials. Whichever method is used for the necessary N-deacetylation it is clear that base-labile functional groups must be immobilized. Thus in studies on the oligosaccharide chains of glycoproteins of the mucin type, in which these chains are attached as base-labile O-glycosides to serine or threonine units, a desirable approach would be to liberate the oligosaccharide units with minimum degradation by reductive elimination, and then to de-N-acylate N-acetylhexosamine residues in the stabilized chains.

TABLE 4. Some problems in the application of the N-deacylation-nitrous acid deamination reaction sequence to the fragmentation of glycosaminoglycans and glycoproteins.

(1) Can complete N-deacylation be achieved without degradation?
(2) What is the importance of side-reactions during deamination?
(3) How can the maximum structural information be obtained from the 2,5-anhydrohexose-forming reaction?

The major reaction pathway in the deamination of equatorially oriented amino glycosides is that leading to 2,5-anhydrohexose formation with concomitant glycoside scission (Fig. 27). Recently Erbing, Lindberg, and Svensson (53) have demonstrated an alternative ring contraction, with the same stereoelectronic requirements, leading to the formation of 2-deoxy-2-C-formylpentofuranosides. Two aspects of this competing reaction are of particular significance for the degradation of glycosaminoglycans. Firstly, the initial reaction does not itself lead to the scission of glycosidic linkages, although, as pointed out by Lindberg (53), it is probable that the 2-deoxy-2-C-formylpentofuranosides or the corresponding 2-C-hydroxymethyl derivatives after reduction may be selectively cleaved by acid. Secondly, a special situation is encountered when the amino glycoside carries a 3-O-substituent. Figure 28 shows the products formed on deamination, followed by reduction, of methyl 2-amino-2-deoxy-3-O-β-D-galactopyranosyl-α-D-glucopyranoside (54). Kochetkov and his collaborators (55) first studied the deamination of the corresponding benzyl glycoside and established the structure of the main reaction product, 2,5-anhydro-3-O-β-D-galactopyranosyl-D-mannose, by reduction with sodium borohydride followed by conversion into the acetylated derivative. The formation of galactitol together with an unidentified compound as by-products was attributed to base-catalyzed β-elimination during reduction with sodium borohydride. Lindberg et al., however, proposed that these by-products were derived from the alternative ring contraction (53). The correctness of this proposal has now been confirmed in my laboratory as shown in Fig. 28 (54). In order to minimize the possibility of β-elimination during reduction of the deamination products from the disaccharide methyl glycoside, this reaction was performed at pH 3.5 using sodium cyanoborohydride. The major product was again characterized as 2,5-anhydro-3-O-β-D-galactopyranosyl-D-mannose. The formation as by-products of galactitol and, in approximately equimolar proportions, a mixture of methyl 2-deoxy-2-C-hydroxymethyl-α-D-ribo-(and -D-arabino-) pentofuranosides was shown by gas chromatography. Furthermore the mass spectrum of the 2-C-hydroxymethyl compounds confirmed their identity with the compounds similarly derived from methyl 2-amino-2-deoxy-α-D-glucopyranoside.

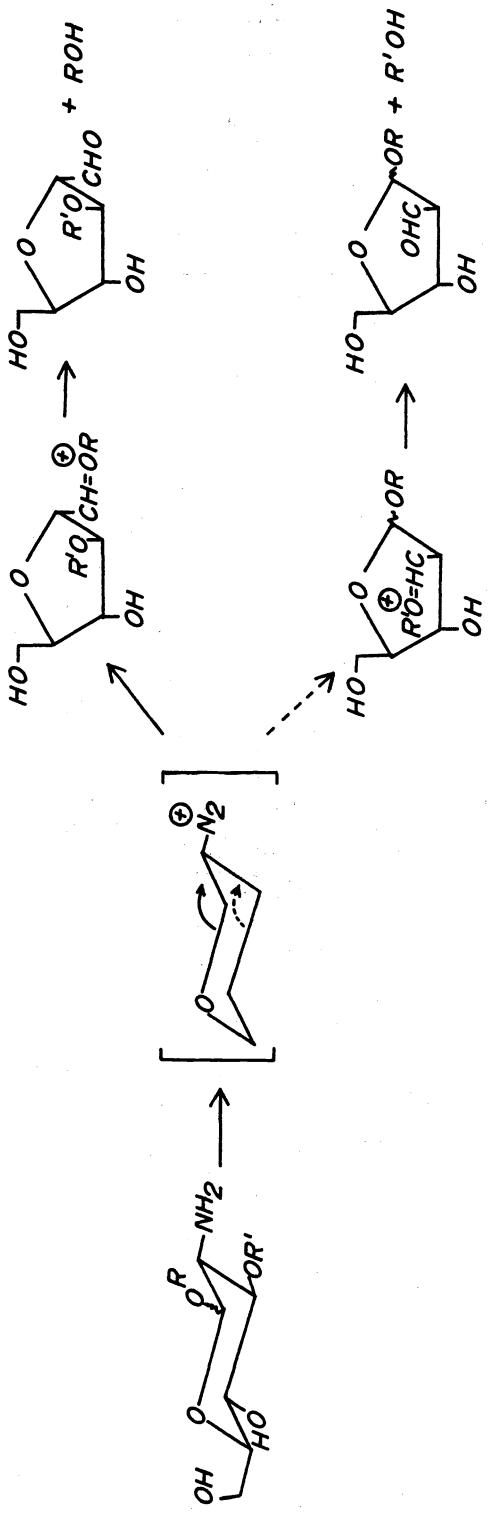


Fig. 27 Alternative ring contractions during the nitrous acid deamination of equatorially oriented 2-amino-2-deoxyglycosides

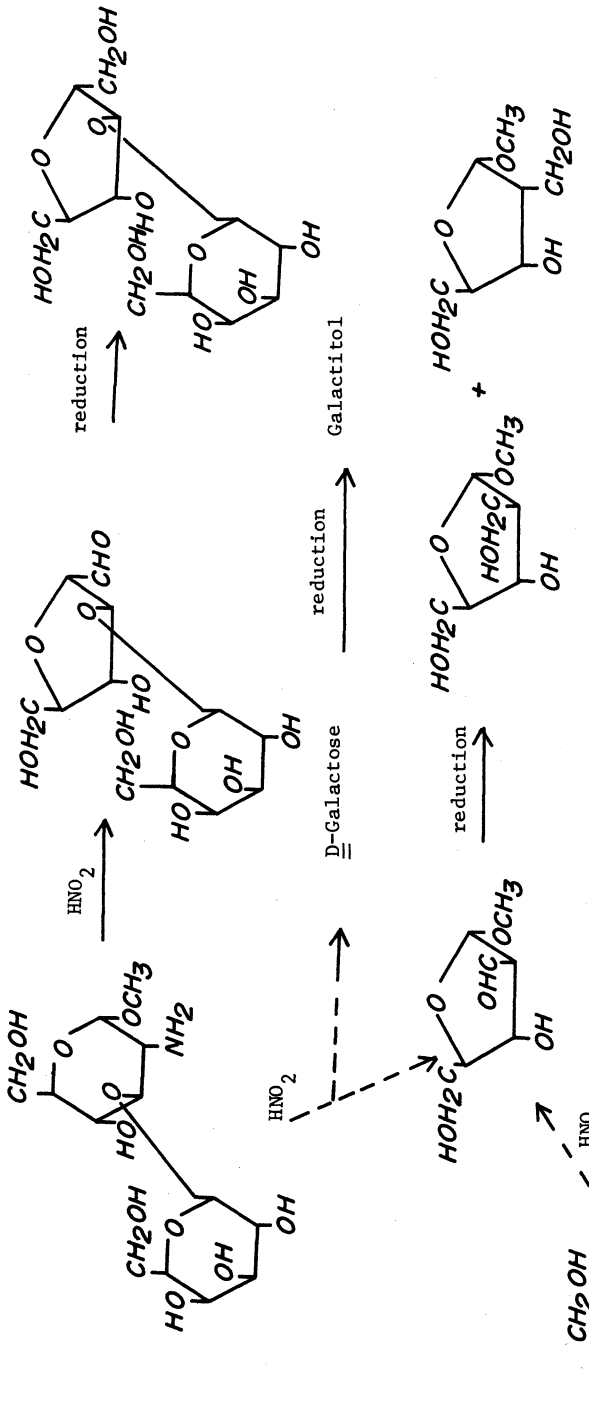


Fig. 28 Deamination, followed by reduction, of methyl 2-amino-2-deoxy-3-O-β-D-galactopyranosyl-α-D-glucopyranoside

Kochetkov and his collaborators have recently described the use of this fragmentation technique in structure determination in their studies on the polysaccharide components of the antigenic lipopolysaccharides of Shigella dysenteriae serotypes, (56, 57). Figure 29 shows the degradation of the type 6 polysaccharide (57). It is noteworthy that the deamination of this polysaccharide, which contains a 3-O-substituted N-acetyl-D-galactosamine residue, furnishes a by-product which probably arises from the alternative ring contraction.

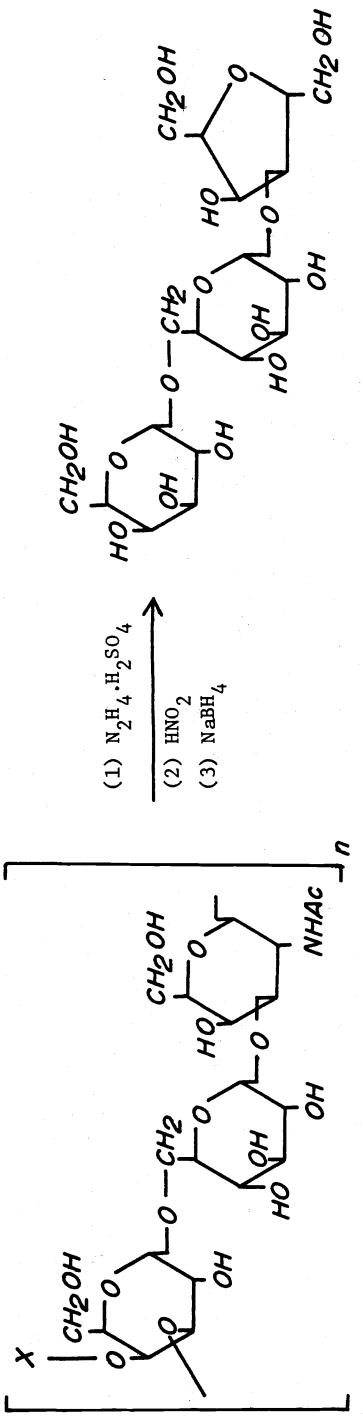


Fig. 29 Formation of trisaccharide (with loss of unknown sugar substituent X) from the polysaccharide component of Shigella dysenteriae type 6 lipopolysaccharide by N-deacetylation, deamination, and reduction

In the glycoprotein field the most complete study of the deamination procedure has been that of Bayard and co-workers on serum-type glycoproteins containing the N-acetyl-D-glucosamine-asparagine linkage (58-60). Figure 30 shows the formation of 2,5-anhydro-D-mannose-terminated oligosaccharides from asialoglycopeptides after hydrazinolysis and nitrous acid deamination. Methylation and other studies established the structures of the two main products. These results provided an important part of the evidence for the structure of the carbohydrate inner core of several serum glycoproteins. When the degradation is performed on sialic acid-containing glycopeptides additional products may be obtained in which modified sialic acid residues are present (60).'

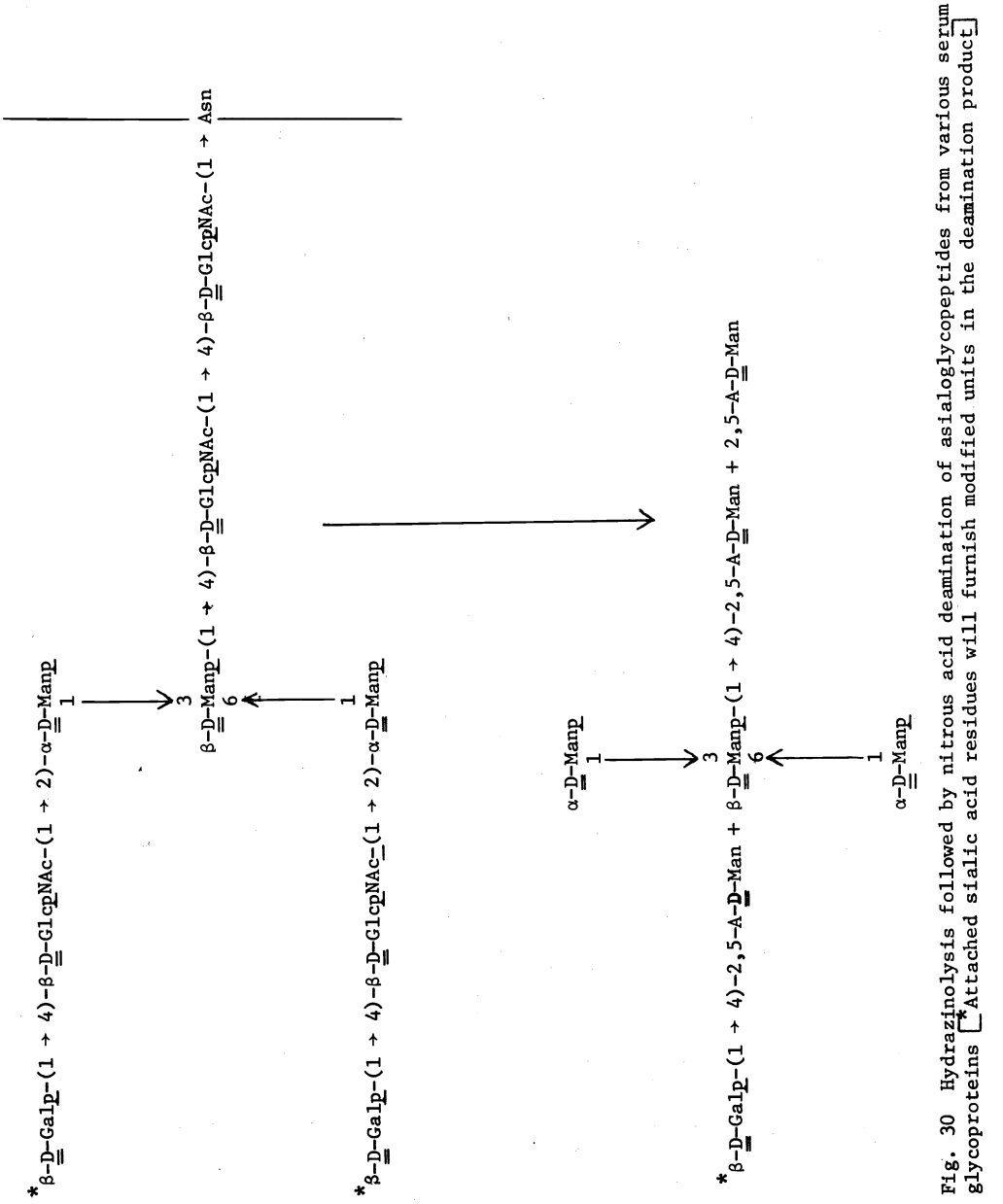


Fig. 30 Hydrazinolysis followed by nitrous acid deamination of asialoglycopeptides from various serum glycoproteins [*Attached sialic acid residues will furnish modified units in the deamination product]

ADDENDUM

Very recently we have shown that the Kochetkov application of the Hofmann reaction may be used for the scission of non-terminal glycosiduronic acid linkages with isolation of the fragment external to the point of cleavage (43). Figure 31 shows the sequence of reactions leading to the characterization of a disaccharide alditol from methylated gum arabic.

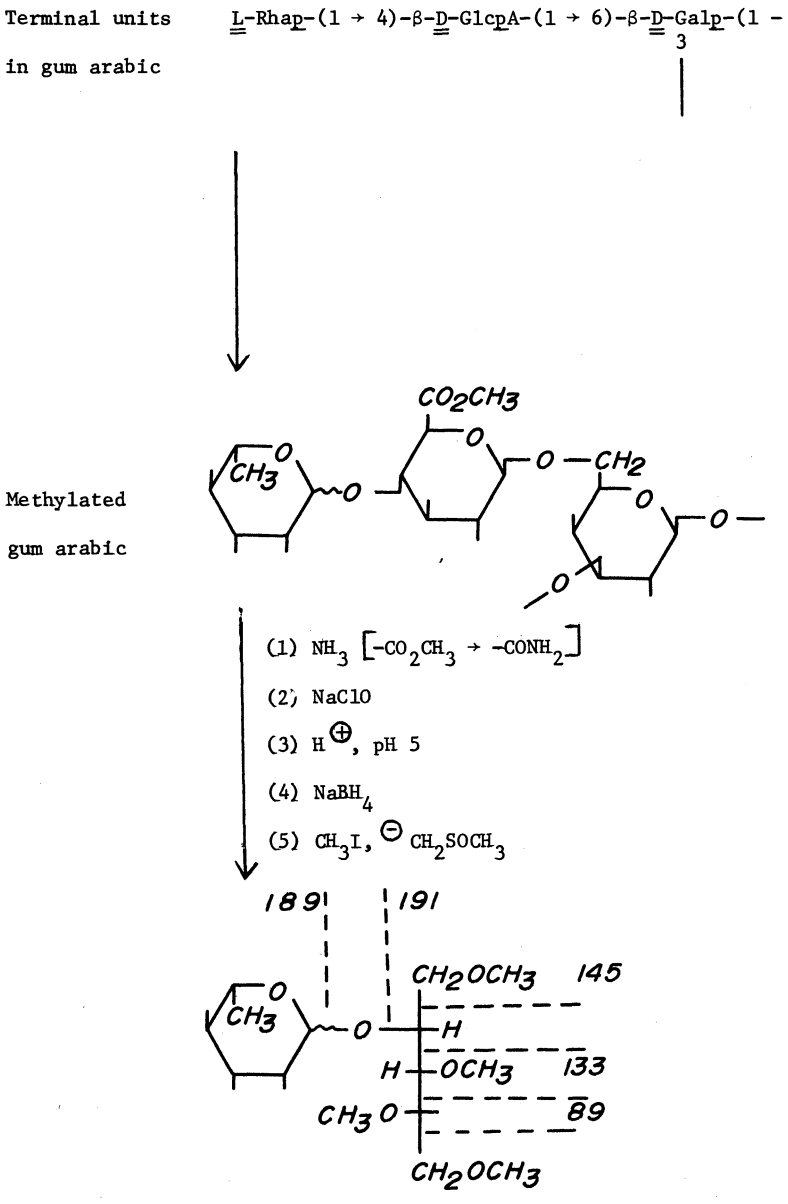


Fig. 31 Kochetkov-Hofmann glycosiduronamide degradation of methylated gum arabic [| = -OCH3]

Oligosaccharides formed in the deamination reaction are most conveniently characterized after reduction to give 2,5-anhydro-D-mannitol residues. The structural symmetry of such units means that methylation studies per se will not differentiate between 3-O- and 4-O-substitution. However, Professor Lindberg (personal communication) has indicated that the methylated derivatives 1,3,6- and 1,4,6-tri-O-methyl-2,5-anhydro-D-mannitol may be differentiated by mass spectrometry if C-1 is labelled, e.g. during reduction with sodium borodeuteride. We are presently studying the same problem in the characterization of 2,5-anhydro-D-mannitol-terminated oligosaccharides.

My last remarks on the deamination reaction relate to experiments still in progress, and therefore state a problem rather than report a solution. I have stressed earlier that, for other types of selective fragmentation, much more information is to be derived from the degradation of methylated than for unsubstituted polysaccharides. The most efficient procedure for O-methylation, that of Hakomori (3), also effects N-methylation of acetamido groups and thus rules out the possibility of deamination after N-deacylation. However, provided that the older and more laborious methylation techniques effect complete alkylation, and that the deamination reaction pathway is not significantly affected after O-methylation, the reaction sequence should yield partially methylated oligosaccharides, whose further alkylation would reveal the linkages split during their formation.

This review has directed attention to those approaches to the selective degradation of carbohydrate polymers which, when coupled with modern separation and identification techniques, provide the maximum structural information. For reasons of time a number of highly selective procedures, such as the Smith degradation and other glycol-cleaving reactions, have been omitted from the discussion. Likewise for reasons already mentioned enzymic methods have not been considered here. Looking back at the relatively underdeveloped state of polysaccharide chemistry when Whistler and Smart's book (61) appeared in the early 1950s, one cannot but be impressed by the progress achieved during the past 25 years. It is ironic that, as selective procedures are being developed to deal with many structural problems, for certain polysaccharides of regularly repeating structures degradative techniques may be becoming obsolete with the development of non-destructive methods for structure determination, such as ^{13}C n.m.r. spectroscopy (62). However, for the foreseeable future, selective fragmentations of increasing variety are likely to be required for irregular polysaccharides and glycoproteins if their complete structures are to be unravelled and, even more importantly, if the subtle changes in structure which form the basis of their biological functions are to be understood.

Acknowledgement - I express my gratitude to many collaborators for their skilled experimental work, especially that performed during the past 3 years by Drs. A.S. Chaudhari, T.N. Krishnamurthy, R.G.S. Ritchie and K.-G. Rosell. Thanks are also expressed to the National Research Council of Canada and the Atkinson Charitable Foundation for their generous financial assistance.

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