THERMAL DEPOLYMERIZATION ANALYSIS OF NEUTRAL POLYSACCHARIDES

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by

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Abstract

Polysaccharide analysis requires identification of monomeric units, sequence, linkage position and anomeric configuration. Conventional analysis is very tedious requiring release of monomeric units by chemical or enzymatic hydrolysis followed by derivatization to volatile species prior to gas chromatographic analysis. An alternative approach is thermal fragmentation which results in the formation of 1,6-anhydro-monosaccharides and 1,6-anhydro-oligosaccharides. The presence of the anhydro monomers relates information about the composition of monomeric isomers, the intact anhydro dimers provide information about linkage type and anomeric configuration. In this study, various neutral polysaccharides were analysed by off-line pyrolytic techniques, the pyrolysates were analysed by trimethylsilylation-gas chromatography and per-O-benzoylation high performance liquid chromatography. Pyrolysate identification was performed by comparison with retention times of standards as well as confirmation by mass spectrometry in both chemical ionization and electron impact modes.

Acknowledgements

I wish to express gratitude to my supervisor, Dr. Robert Helleur, for continued encouragement and advice throughout the last three years.

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I would also like to thank Miss Teresa Barker for patiently typing this thesis.

Glossary

amu	atomic mass units
B-I	The benzoylated derivative of compound I.
CI	chemical ionization
DCI	desorption chemical ionization
EI	electron impact ionization
GC	gas chromatography
GC-MS	gas chromatography - mass spectrometry
GC-(CI) MS	gas chromatography - chemical ionization mass spectrometry
GC-(EI) MS	gas chromatography - electron impact mass spectrometry
HPLC	high performance liquid chromatography
IS	internal standard
m/e	mass to charge ratio
M-I	the methylated derivative of compound I
MS	mass spectrometry
NMR	nuclear magnetic resonance spectroscopy
T-I	the trimethylsilyl derivative of compound I
TIC	total ion chromatogram
TMS	trimethylsilyl
t_R	retention time

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1. Introduction

1.1. Polysaccharide Structure and Analysis

1.1.1. Polysaccharide Structure

Polysaccharides are one of the most abundant and diverse classes of natural compounds whose functions include energy storage, structural morphology and protection. (1) Polysaccharides are biopolymers that consist of covalently bonded monosaccharide units usually joined by a glycosidic link between the anomeric carbon of one unit and a carbon of a neighbouring residue. The monosaccharide units are bound to a rigid configuration when linked to form a polymer, they exist as cyclic structures with one ring atom being an oxygen while the remaining are carbons. These may be either a 5-membered ring referred to as a furanose, or a 6-membered ring, a pyranose. (Fig. 1.1 a and b). (2)

Isomers are possible with various anomeric configurations at C-1. The configuration with the major substituent on the opposite side of the plane to the C-6 carbon is denoted as α and the configuration with the substituent on the same side, β. The structures of α-D-glucopyranose and β-D-glucopyranose are given in Figure 1.1 c and d, respectively.

Each series of monosaccharides consists of stereoisomers with identical formulas but different three-dimensional arrangements of atoms. Variation in configuration in any of the asymmetric carbons, with exception of the anomeric carbon, is denoted by the nomenclature scheme for monosaccharides. Example structures of α-D-galactopyranose, α-D-glucopyranose and α-D-mannopyranose are designated in Figure 1.1 e, f and g, respectively.

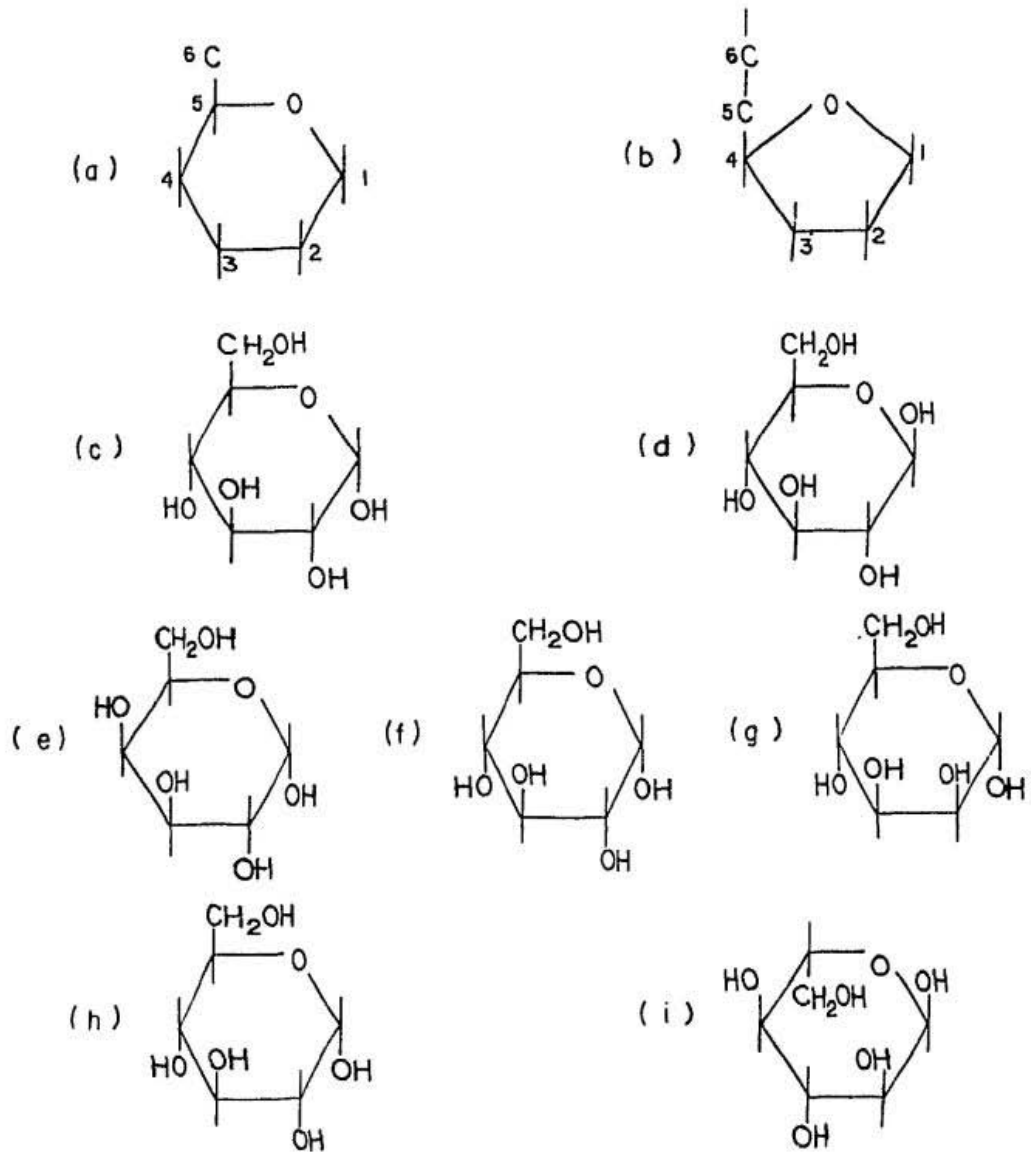


Figure 1.1. Monosaccharide structures

D and L nomenclature is also used to distinguish the configuration of monosaccharides. If the asymmetric carbon furthest from the aldehyde function is on the right of the Fischer projection it is denoted as a D sugar, if on the left, an L sugar. In nature, generally only one of these conformations is found for each monosaccharide. The structures of α-D-glucopyranose and α-L-glucopyranose are shown in in Figure 1.1 h and i respectively (1).

The nomenclature for polysaccharides must describe the monosaccharide units present, the enantiomeric form (D or L), the anomeric configuration (α or β), the ring size and the linkage type. The common polysaccharide, cellulose, is comprised of β-D-glucopyranose units linked from the anomeric carbon (C-1) of one unit to C-4 of the neighbouring unit and is designated as (1→4)-β-D-glucopyranan. Cellulose is an example of a homopolysaccharide, that is, it contains only one type of monosaccharide unit. Heteropolysaccharides are those polymers containing two or more types of monosaccharide units. For these polymers the sugar residues are listed as prefixes in alphabetical order with the exception of the unit that comprises the backbone, this is listed last. For example, a D-galacto-D-gluco-D-mannan would refer to a heteropolysaccharide with a D-mannose backbone with glucose and galactose residues.

All polysaccharides are not linear, structures may also include branched and cyclic forms. To better represent polysaccharide structures, shorthand nomenclature is used for regular repeating functions. These forms give monosaccharide units, ring size, enantiomeric and anomeric configurations and linkage positions. A D-galacto-D-mannan may be represented as in Figure 1.2. This denotes a branched polysaccharide with a β-D-mannopyranose backbone linked from the anomeric carbon to C-4 of a neighbour. Side chain residues of α-D-galactopyranose units are linked from the anomeric carbon of the galactose unit to C-6 of some mannose units. This nomenclature permits a more precise representation of the polysaccharide shape.(3)

$$\rightarrow 4)\text{-}\beta\text{-D-Manp-}(1 \rightarrow 4)\text{-}\beta\text{-D-Manp-}(1 \rightarrow 4)\text{-}\beta\text{-D-Manp-}(1\text{-}$$

$$6$$

$$\uparrow$$

$$\alpha\text{-D-Galp}$$

Figure 1.2. Short hand polysaccharide representation

1.1.2. Classical Methods for Polysaccharide Structure Determination

There are very few methods which permit analysis of intact polysaccharides. Some promising results have been obtained with NMR and mass spectrometry, however both require expensive instrumentation and there are limitations as to the complexity of polymer that can be analysed with these techniques. The determination of the structure of a polysaccharide requires identification of: (1) the types of monosaccharide residues (2) the D or L configuration (3) the degree of polymerization (4) the position of glycosidic linkages (5) monosaccharide ring size (6) configuration of the glycosidic linkage and (7) the sequencing of monosaccharide units (3,4).

To determine the monosaccharide components of polysaccharides these portions must initially be liberated from the polymer. This is most commonly performed by subjecting the polysaccharide to total or partial acid hydrolysis. The structural differences in the monosaccharide units and linkages present have varied optimum hydrolysis conditions reflecting glycosidic bond stability. The hydrolysis method used for any polysaccharide degradation must optimize glycosidic bond cleavage while minimizing degradation of the released monomeric units. These released monomeric or oligomeric fractions must then be separated prior to identification, usually by gas chromatographic analysis (6). Free sugars are not very volatile at normal GC conditions and often undergo degradation in heated injector ports. Therefore, prior to chromatographic analysis, derivatization into a more volatile analyte is

essential. In some cases, a reduction step is included, prior to derivatization, in order to lessen the number of isomeric products. Saccharide identification is achieved by comparison of GC retention times with those of known standards (5).

To determine the D or L configuration of a monosaccharide component the analyte must be isolated in pure form prior to analysis by circular dichroism absorptivity. Comparison with standards or literature values permits elucidation of configuration. An alternative method employs chromium(VI) oxide oxidation to distinguish D or L configuration (4,5).

The positions of glycosidic linkages are determined by a methylation technique. Polysaccharides are methylated prior to total acid hydrolysis. The partially methylated monosaccharides are then reduced and acetylated prior to gas chromatographic analysis. In addition to linkage position, this method allows distinction of monomeric ring size by positioning of methoxyl groups at C-4 or C-5 (4).

The ordering of monosaccharide components in a polysaccharide can be determined through chemical analysis that selectively cleaves glycosidic bonds. This may be implemented by sequentially cleaving terminal monosaccharide residues or by employing the variation in hydrolysis rate constants to selectively cleave specific glycosidic bonds.

To determine the anomeric configuration of a glycosidic linkage an enzymatic hydrolysis method may be used. Enzymes specific to a particular anomer are required for this determination. Nuclear magnetic resonance spectroscopy can also be used as different anomers experience different chemical shifts (4).

The complete elucidation of a polysaccharide structure, by these methods, is very time consuming and some of the procedures are difficult to adapt to micro scale analysis. A wide array of high purity chemicals and instrumentation is necessary for complete structural identification.

1.2. Analytical Pyrolysis

1.2.1. Introduction to Analytical Pyrolysis

Analytical pyrolysis is a technique that thermally degrades polymers into smaller fragments which are more suitable to chromatographic separation and analysis (7,8). Pyrolysis coupled with gas chromatography, gas chromatography-mass spectrometry or mass spectrometry permits rapid, direct analysis of polymeric materials.

Analytical pyrolysis has been shown to yield characteristic and reproducible fingerprints from the analysis of biomass. Numerous applications of analytical pyrolysis have been developed during the past few decades (7,10,11). Industrial studies of synthesized plastics provide both compositional and structural detail of these new polymers. Pyrolytic fingerprinting of paints and textiles has provided an effective technique for the field of forensic chemistry. The biological applications center around taxometric studies of fungi, yeasts and bacteria. Biochemical uses require the structural identification of complex compounds including carbohydrates, proteins and amino acids. Geochemical surveys include the use of analytical pyrolysis in composition studies of peats, coal and soils (10,11).

Early work in the field of analytical pyrolysis presented several problems due to lack of reproducibility and standardization of pyrolysis procedures. Improvements in instrumentation have permitted more accurate controls over some of the parameters that influence pyrolysate composition, enabling comparison of results (11).

Many variables influence the mechanistic routes of pyrolysis and the resulting composition of products. The sample size is one of these factors; as sample size increases, the probability of secondary reactions also increases. Additionally, with larger samples thermal gradients may exist and different portions of the sample are exposed to different temperatures leading to irreproducible results (10,11). Another variable, the matrix of the sample (*e.g.* the presence of neutral salts) can alter the pathway of the reaction and influence the composition

of the products (12).

The material nature of the pyrolyser may also vary pyrolysis composition *i.e.* metallic surfaces may catalyse various decomposition reactions. Pyrolysis chambers should be glass or quartz lined to prevent secondary reactions; this is especially crucial when analysing polar species such as biopolymers (8). The choice of inert atmosphere will change pyrolysis results as different gases exert varied cooling behaviour in the pyrolysis chamber, altering the range of observed products (11). A crucial parameter is stringent control of all temperatures and the sample exposure times to these temperatures. Ideal pyrolysis requires a rapid heating rate, precise temperature and time control during pyrolysis, efficient heat transfer to sample, rapid removal of the pyrolysis products from the thermal zone followed by rapid cooling. Minor variations in any of these parameters may have a drastic influence on the composition and nature of the pyrolytic products obtained (7,8,10,13).

Pyrolysis may also be performed off-line. For this technique, thermal degradation is performed separately from the chromatographic step. Pyrolysis is usually performed under vacuum conditions, ensuring rapid removal of primary products from the heated pyrolysis zone. Products are derivatized prior to chromatographic analysis. This technique permits analysis of larger fragments and therefore supplies more detailed structural information about the original polymer (14,15,16,17).

1.2.2. Analytical Pyrolysis of Carbohydrates

Interest has been shown in the pyrolysis of polysaccharides for fingerprinting structural features as well as studying the kinetics of thermal degradation. This latter interest has been enhanced by the attempt to understand the key influences affecting the usefulness of biomass as a fuel source. For this reason, much of the work in this field has centered around the most abundant natural polysaccharide, cellulose.

A review of the developments in analytical pyrolysis up to 1979 has been detailed by Irwin (10). Early pyrolytic studies of polysaccharides employed slow heating rates and low pyrolysis temperatures. Analysis of low molecular weight species by gas chromatography and mass spectrometry was used to differentiate amongst the various polysaccharide isomers (18,19,20). These studies fingerprint polysaccharide pyrolysates by comparing yields of alcohols, aldehydes, ketones, aromatic hydrocarbons and furans (10). These species are volatile enough to be readily analysed by gas chromatography.

Off-line analytical pyrolysis showed that 1,6-anhydro-hexoses were obtained under vacuum conditions (21). These species were later observed with direct pyrolysis-gas chromatography (22-24). The presence of anhydro monomers has been used for determination of the saccharide composition of oligosaccharides and polysaccharides.

Recently, anhydro oligomers have been detected in the pyrolysates of amylose and cellulose from off-line pyrolysis. These products have been proven to be primary products from pyrolytic bond cleavage of the polymers (14-17). These species are not volatile enough for direct chromatographic analysis, therefore derivatization is necessary to improve separation ability and detector response. These anhydro oligomers retain the intact glycosidic linkage, thus providing information about the structural nature of the original polysaccharide.

1.3. Pyrolysis Mechanisms of Carbohydrates

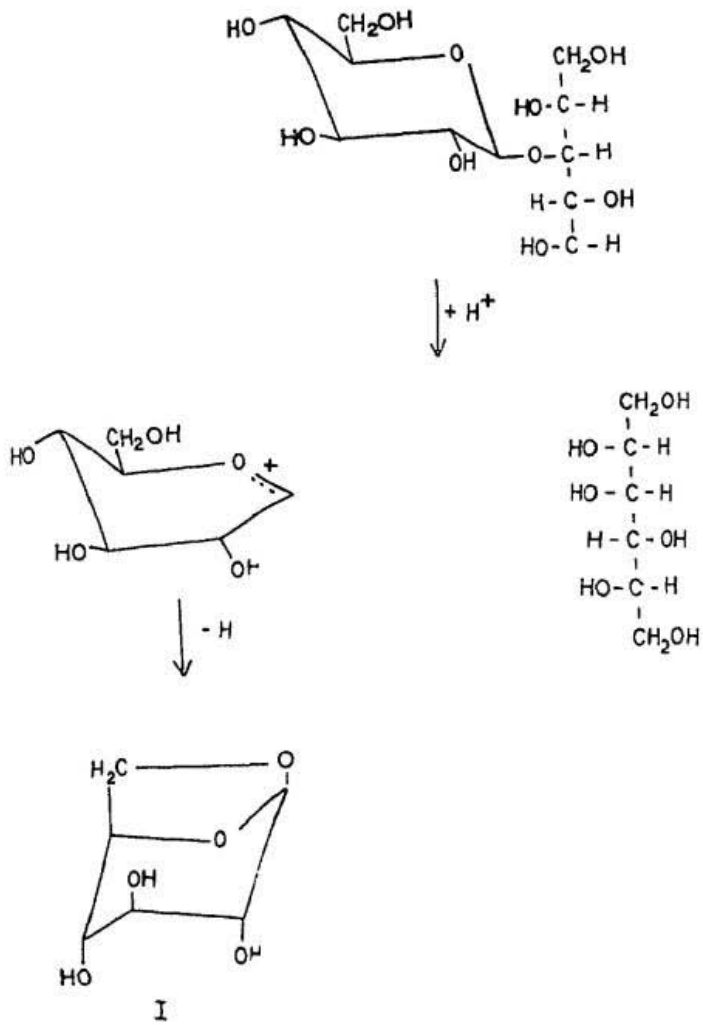
1.3.1. Mechanism for Formation of Anhydro Sugars

The pyrolytic pathways for the formation of anhydro monomers, and more recently anhydro oligomers, during polysaccharide pyrolysis have been studied using cellulose as a model. Early proposals for the mechanism cited a simple unzipping reaction as the pathway for depolymerization. However, the conclusive evidence for the presence of intact anhydro oligomers as primary products has altered that theory (15).

Recent theories propose the formation of an active cellulose as the initiation step in the thermal reaction. Three competing pathways then determine the evolution of the active material into (1) low molecular weight volatiles, (2) anhydro sugars or (3) gases and char (13).

To explain substantial amounts of anhydro oligomers a two step mechanism was formulated in which the initiation step is glycosidic bond cleavage followed by anhydro sugar formation (15). Studies using cellobiitol as a model for cellulose have suggested that pyrolysis induces a heterolytic scission of the glycosidic linkage resulting in a glycosyl cation and a glucitol anion (Fig 1.3). Intramolecular attack of 0-6 on the C-1 of the cation results in 1,6-anhydro-β-D-glucopyranose, commonly called levoglucosan[1] (25).

A homolytic mechanism with free radical formation has also been proposed (26). The pyrolysis of cellulose begins with an initial decrease in the degree of polymerization of the macromolecule and this is followed by a change in functional groups (Fig. 1.4).



I

Figure 1.3. Heterolytic Mechanism of Levoglucosan Formation (25)

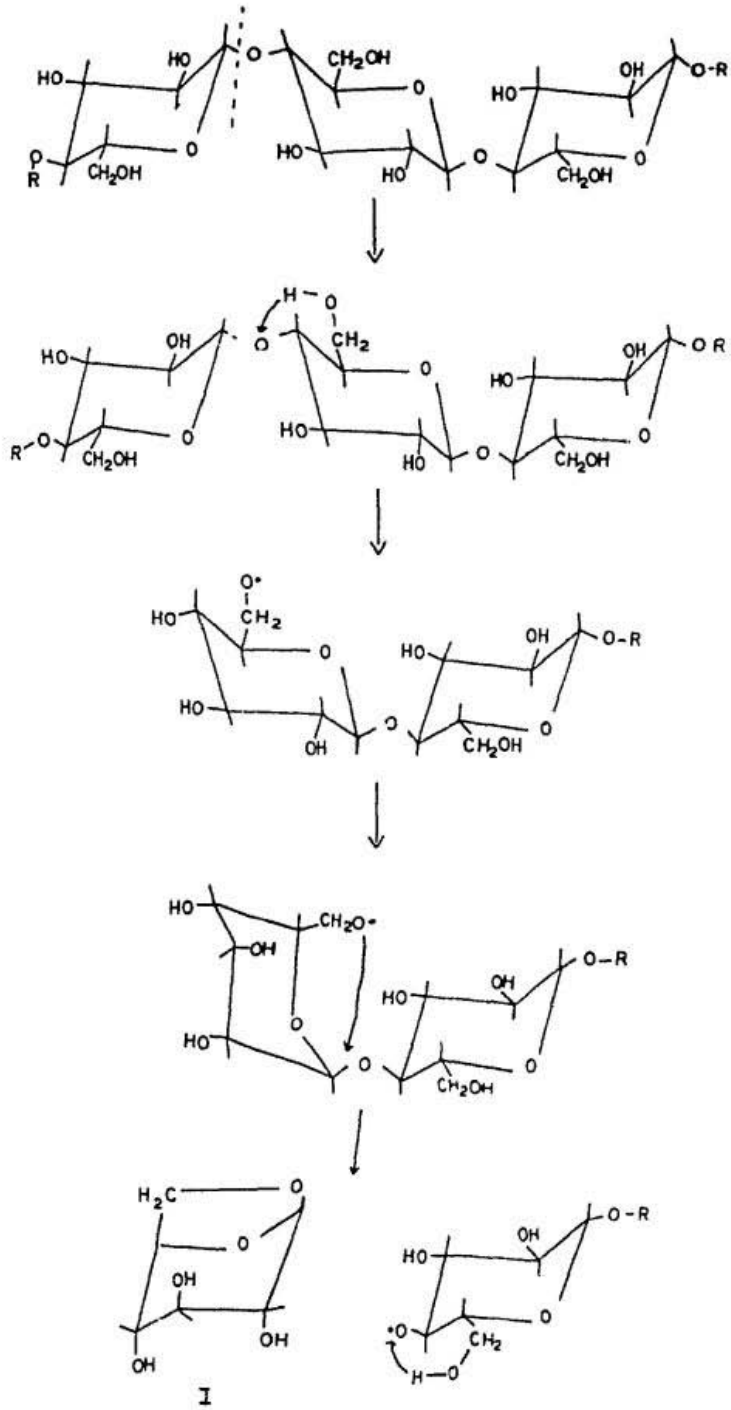
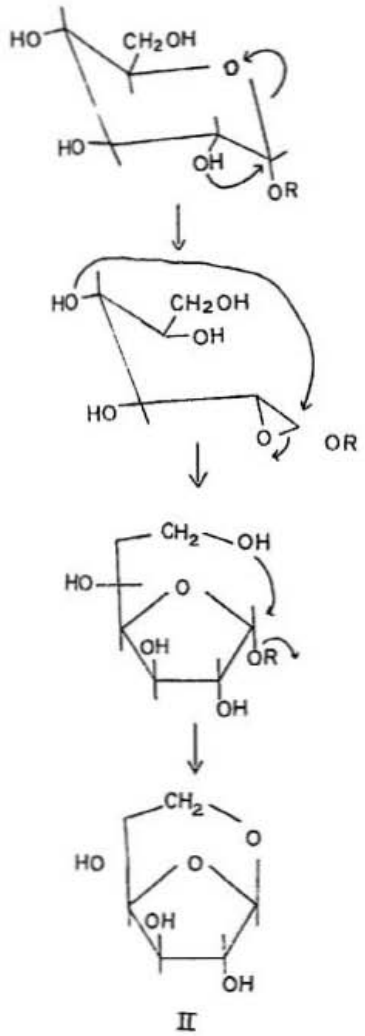


Figure 1.4. Homolytic Mechanism for Levoglucosan Formation (26)

In addition to 1,6-anhydrohexopyranoses, the corresponding furanose is often detected in the pyrolysis products of polysaccharides. The mechanism for the formation is illustrated in Figure 1.5 (27).



II

Figure 1.5. Formation of 1,6-anhydroglucofuranose (II) in polysaccharide pyrolysis (27)

1.3.2. Chemistry of Anhydro Sugars

Anhydro sugars are derivatives formed when dehydration of an aldose results in an intramolecular glycoside (28). These compounds may be prepared through alkaline degradation of aromatic glycosides or through treatment of an aldohexose with aqueous mineral acid. Anhydro sugars may also be isolated from the thermal depolymerization products of mono-, oligo- or polysaccharides. The major product from acid or thermal treatment of neutral sugars is a 1,6-anhydrohexopyranose, a minor product is the corresponding furanose.

Chemically, these derivatives resemble methyl glycosides: they do not have reducing properties, they are relatively stable in alkaline media and they are hydrolysed in acidic media to give free hexoses (28). These sugars are readily crystallized, are very soluble in water, pyridine and dimethyl sulphoxide and are slightly soluble in lower alcohols and acetone. Anhydro sugars are thermally stable at temperatures close to their boiling points, providing properties of a reasonable analyte for gas chromatographic analysis, *i.e.* on-line pyrolysis-gas chromatography.

Anhydro sugars may be polymerized either thermally (29) or by the use of an acidic catalyst (28). The products are oligo- or polysaccharides with various linkages, depending upon the conditions existing during the polymerization reaction. This reaction is initiated by the attack on C-1 by a free hydroxyl of another anhydro sugar. The result is the opening of the anhydro bridge and formation of various linked anhydro disaccharides. By using derivatized anhydro sugars and introducing catalysts this polymerization can be made highly selective (28). Potential repolymerization of anhydro hexose pyrolysis products during pyrolytic analysis of polysaccharide is a major concern when the goal is to isolate the original intact oligomeric anhydro sugar products (15,21).

1.3.3. Formation of Nonanhydro Sugar Products

In addition to anhydro sugars, many furan and pyran derivatives have been detected in polysaccharide pyrolysates. These result from dehydration of the primary pyrolysis products (Fig. 1.6). The major dehydration products consist of a 3-deoxy-D-erythro hexosulose (III), 5-hydroxymethyl-2-furaldehyde (IV), and levoglucosenone (V) while minor products include furan (VI) and furfural (VII) (13,30).

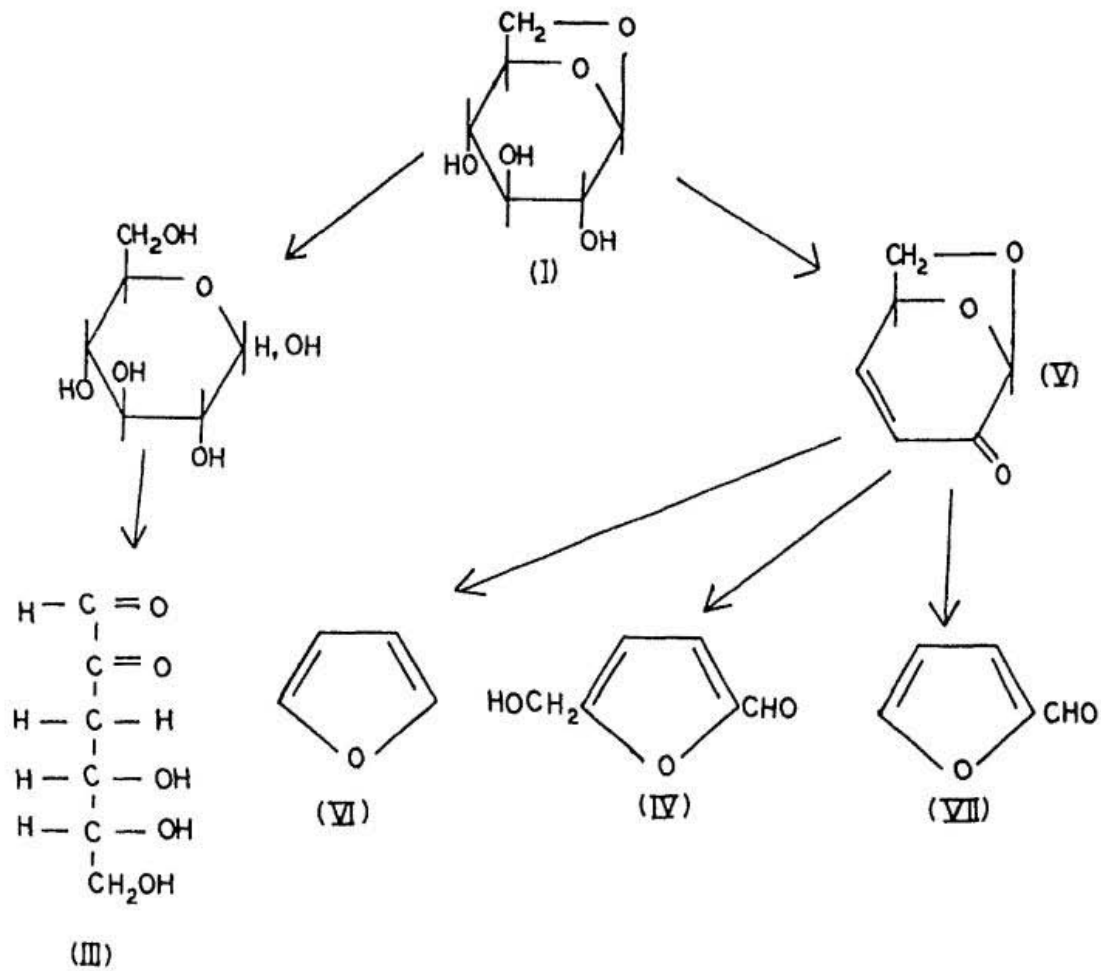


Figure 1.6. Decomposition of levoglucosan to furan and pyrans (13,30)

Carbonyl compounds have also been detected in the pyrolysates of polysaccharides. A proposed mechanism for their formation is described in Figure 1.7 (27).

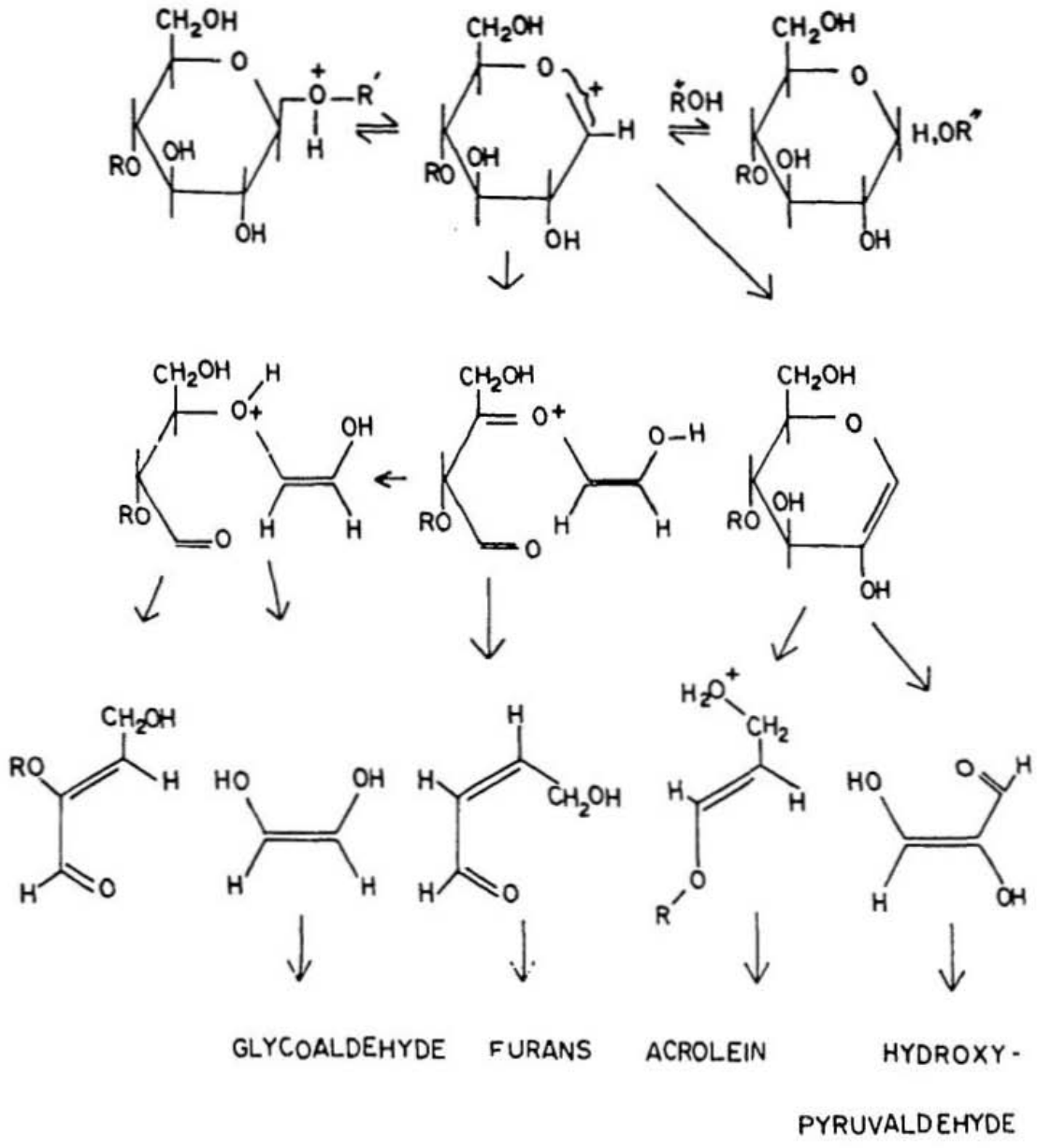


Figure 1.7. Formation of carbonyl compounds during polysaccharide pyrolysis (27)

1.4. Objectives and Order of Presentation

In recent years, the studies of cellulose pyrolysates have indicated the presence of anhydro oligomers (15-17). The objective of this study was to determine if anhydro oligomers are present in the pyrolysate tar of other neutral polysaccharides. These intact oligomers will retain some of the structural integrity of the original polymer and could provide structural identification of these compounds.

To analyse these oligomeric products, an off-line pyrolysis technique was adopted in which the isolated pyrolysates were further derivatized and analyzed by chromatographic techniques. Much of this study's emphasis was directed towards selection on the proper pyrolysis conditions and high molecular weight pyrolysate analysis. Mass spectrometry was used extensively for pyrolysate identification.

Off-line pyrolysis conditions require fast pyrolysis coupled with rapid removal of primary products from the heated zone, if reasonable quantities of high molecular weight species are to be obtained. The following parameters were optimized to achieve reasonable yields of both anhydro monomers and dimers: type of pyrolysis apparatus, pyrolysis temperature and time, carrier gas flow and vacuum. In addition, derivatization and chromatographic procedures were determined to provide reasonable separation of isomers and to maximize sensitivity. Both liquid and gas chromatography were employed; where various derivatives, analytical columns and separation conditions were tested.

When optimium conditions were developed, using amylose and cellulose as standards, a variety of polysaccharides were examined to determine the scope of off-line pyrolysis. Polysaccharides containing different monosaccharide isomers, linkage positions and anomeric configurations were analysed.

Substantial use of mass spectrometry and gas chromatography coupled with mass spectrometry was adopted in this study for identification of the oligomeric species in the pyrolysate

products. Electron impact, chemical and direct chemical ionization modes were used. Mass spectral fragmentation pathways of a number of derivatized carbohydrate pyrolysates are presented to support structural elucidations.

2. Experimental

2.1. Materials

2.1.1. Solvents and Reagents

All solvents and reagents were used as supplied. HPLC Spectro-grade acetonitrile was purchased from Canlab (Mississauga, Ont.). Sep-Pak C-18 cartridges were obtained from Waters (Milford, M.A.). Sephadex LH-20 was obtained from Pharmacia Fine Chemicals (Montreal, Quebec). Silyl-grade pyridine and Tri Sil-Z were from Pierce Chemicals (Rockford, Il.). Sylon TP was from Supelco (Oakville, Ont.). Hexane was purchased from BDH (Toronto, Ont.). ACS grade chloroform was from Fisher Scientific (Halifax, N.S.). DMSO, benzoic anhydride, 4-dimethylaminopyridine, sodium hydride and methyl iodide were obtained from Sigma Chemicals (St. Louis, Mo.).

2.1.2. Carbohydrate Standards

1,6-Anhydro-β-D-glucopyranose, 5-hydroxymethyl-2-furaldehyde, amylose, cellulose, laminarin, dextran, nigeran, pullulan, lichenan, locust bean gum, amylopectin, β-cyclodextrin, methyl-6-deoxy-α-L-galactopyranose were from Sigma Chemicals (St. Louis, Mo).

Larch gum and ivory nut mannan were a gift from Dr. G. Hay, Queen's University. 1,6-Anhydroglucofuranose, 1,6-anhydrogalactopyranose and 1,6-anhydromannopyranose were a gift from Dr. A.S. Perlin, McGill University. Samples of 4-O-β-D-glucopyranosyl-1,6-anhydro-β-D-glucopyranose, 4-O-α-D-glucopyranosyl-1,6-anhydro-β-D-glucopyranose and 4-O-(6-O-β-D-glucopyranosyl-β-D-galactopyranosyl)-1,6-anhydro-β-D-glucopyranose were kindly donated by Dr. Taku Chiba of Nagoya City University.

2.2. Derivatization Procedures

2.2.1. Permethylation

Dried pyrolysate oils were permethylated according to Hakomori's procedure (31). Samples were dissolved in 0.2 mL of dimethylsulfoxide in 5 mL serum vials containing a stirrer bar and fitted with a teflon-faced silicone septum. The vial was flushed with nitrogen prior to the addition of 0.4 mL of methylsulfonyl anion (0.4 mL of the sodium salt). The reaction was stirred for one hour. Cold methyl iodide (1 mL) was added and the reaction stirred for an additional four hours at room temperature. The methylated carbohydrates were purified by passage through a small column of Sephadex LH-20 suspended in chloroform. The methylated derivatives were eluted from the column with chloroform. Fractions (1 ml) were analysed by thin layer chromatography and the appropriate portions were combined.

The methylsulfonyl anion containing solution was prepared by adding 50 mg of washed sodium hydride (3 x 1 mL dry benzene) to a serum vial with 1 ml of dimethylsulfoxide. The vial was fitted with a teflon-faced septum and flushed with nitrogen. The solution was stirred at 50-60°C for one hour.

2.2.2. Trimethylsilylation

Trimethylsilyl (TMS) derivatives were prepared using Sylon TP or Tri Sil-Z (32). These commercially prepared reagents are a mixture of trimethylsilyl-imidazole in dry pyridine. Pyrolysate oils were transferred from the off-line pyrolysis apparatus to 0.5 mL reacti-vials using small washes of pyridine. For quantitative measurement, an internal standard, methyl-6-deoxy-α-L-galactopyranose in pyridine was added. The derivatizing reagent (150 μL) was added and samples were heated at 90°C for twenty minutes. The TMS derivatives were extracted from excess reagent by the addition of 30 μL of hexane and 200 μL of water. An aliquot of this hexane extract was analysed by gas chromatography.

2.2.3. Per-O-Benzoylation

Perbenzoylated derivatives of pyrolysates were prepared by a procedure similar to that of Daniel (33). Pyridine (0.5 mL) containing 10% (w/v) benzoic anhydride and 5% (w/v) 4-dimethylaminopyridine (an acylation catalyst) was added to the pyrolysate oils in a 5 mL reacti-vial equipped with a teflon-lined septum. Derivatization was achieved by heating at 37°C for four hours. Excess anhydride was destroyed by addition of 4.5 milliliters of water (complete in thirty minutes). Excess benzoylating reagent was removed using a C-18 solid phase extraction cartridge. Samples were applied to the cartridge, washed with four milliliters of water and eluted with two milliliters of acetonitrile. The derivatives were evaporated to dryness under nitrogen and reconstituted in acetonitrile (~ 150 mL) prior to HPLC analysis. For quantitative measurements an aliquot of methyl-6-deoxy-α-L-galactopyranose in pyridine, an internal standard, was added to the pyrolysate oil prior to the derivatization.

2.3. Off-line Pyrolysis

2.3.1. Furnace Pyrolysis

Polysaccharides were pyrolysed on a semi-preparative scale using a Hamilton multi-purpose sampling system (Reno, Nevada). The furnace was fitted with a 25 centimeter quartz pyrolysis chamber having an indentation about 10 centimeters from one end (Figure 2.1). The sample (100-200 μg) was transferred to a 2.5 centimeter quartz tube containing a small plug of silanized quartz wool; an additional piece of wool was placed on top of the sample to keep the polymer in position. The quartz tube was placed at the upper end of the pyrolysis chamber. A vacuum of 2 mm Hg was applied while a nitrogen flow of 6 ml/min flushed the pyrolysis chamber. The apparatus was tilted to drop the tube down the chamber until it was stopped by the indentation, about 2 cm from the end of the 450°C furnace. The pyrolysates condensed on the acetone cooled walls of the chamber beyond the furnace and were transferred to reaction

vials using a small quantity of the solvent used in the derivatization procedure.

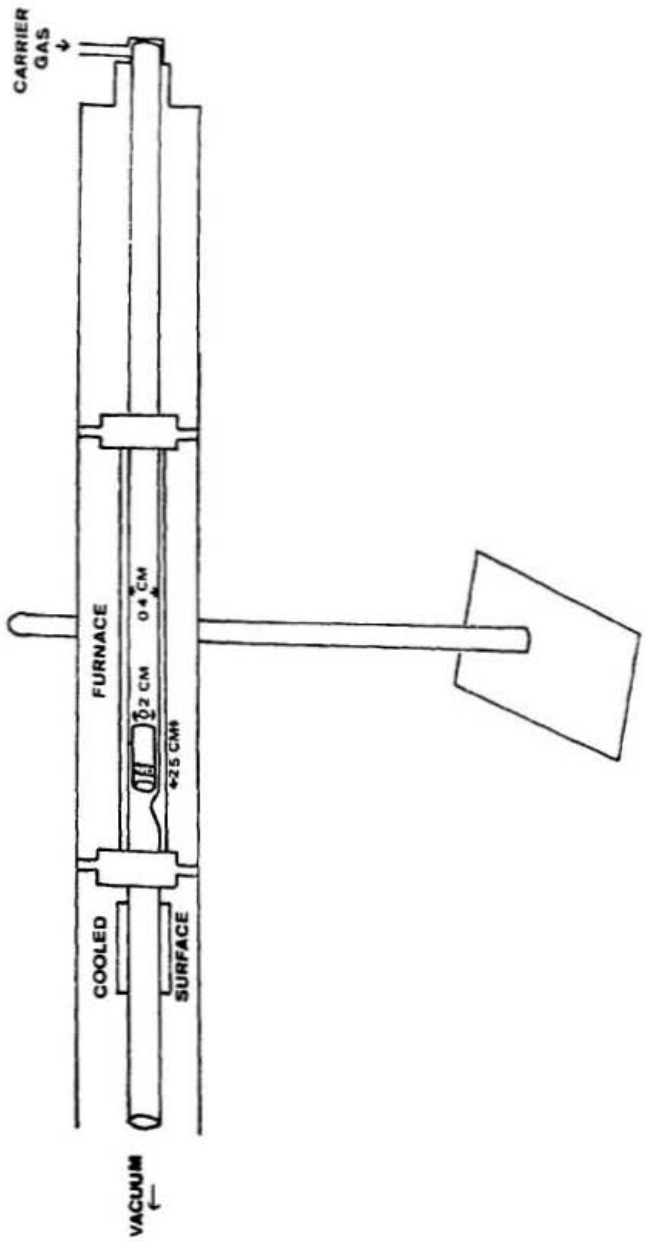


Figure 2.1. Furnace Pyrolysis Apparatus

2.3.2. Off-line Pyrolysis Pyroprobe Coil with Quartz Tube

The off-line pyrolysis apparatus equipped with the Pyroprobe [R] platinum coil/quartz tube pyrolyser is illustrated in Figure 2.2. The apparatus was constructed from the condenser of a microsublimator (Kontes Glassware $24/25 joint, #306500-000) and an Ace-Thred thermometer adapter (Aldrich Chemicals $24/40 joint #217, 501.3). The adapter was modified by the addition of a gas-purge side arm. The end of the sublimator was drawn out to a fine tip so as to fit just inside the quartz pyrolysis tube.

The polysaccharide sample (50-200 μg) was added to a quartz pyrolysis tube by positioning between two pieces of silanized quartz wool. The tube was placed inside the coil of a Pyroprobe [R] one-quarter inch probe (Chemical Data Systems, Oxford, Pennsylvania) so that the sample was about 0.25 cm from the tip of the sublimator. The water coolant was turned on, a nitrogen flow of 6 ml/min and a vacuum of 2 mm Hg were applied during pyrolysis. Pyrolysis was performed at 450°C for 10 seconds. The condensed pyrolysate oils were removed from the sublimator by washing with a small amount of derivatizing solvent.

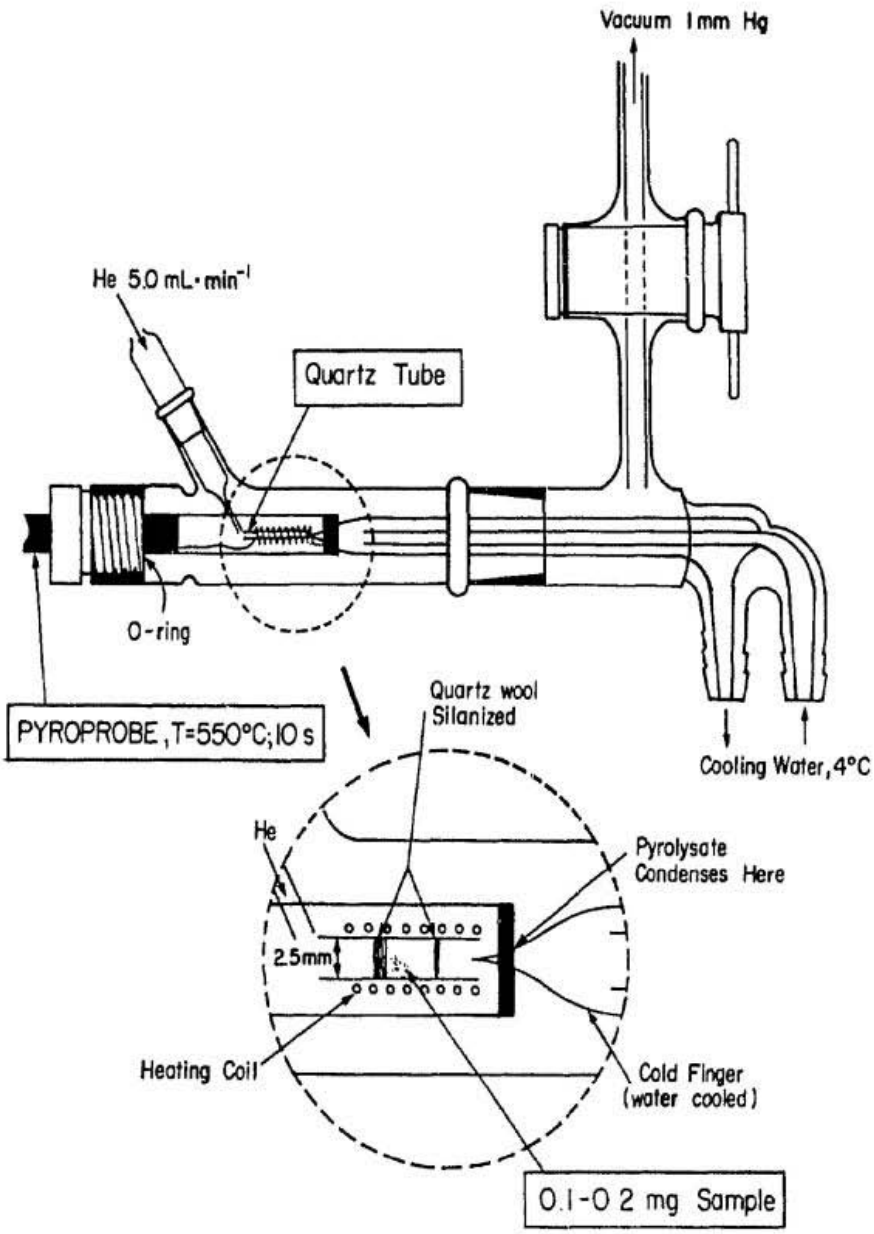


Figure 2.2. Off-line Pyroprobe Platinum Coil/Quartz Tube Pyrolysis Apparatus

2.3.3. Off-line Pyroprobe Ribbon Pyrolysis Apparatus

The off-line pyrolysis apparatus shown in Fig 2.3 was constructed from a "home-made" micro condenser equipped with a thermometer adapter (Aldrich, Milwaukee, Wisconsin). The condenser was modified by the addition of a gas flow purge arm and a vacuum adapter.

The polysaccharide sample (~ 200-600 μg) was adhered to the ribbon of a pyroprobe platinum ribbon probe (Chemical Data System, Oxford, Pennsylvania) with a small quantity of methanol. The solvent was removed by preheating the filament at 150°C for 10 seconds. The probe was positioned in the pyrolysis apparatus so that the ribbon was surrounded by the water coolant jacket. The system was evacuated, then purged with nitrogen prior to pyrolysis. Pyrolysis was carried out at 450°C for 10 seconds. The condensed pyrolysates were washed from the condenser wall with a small quantity of derivatizing solvent.

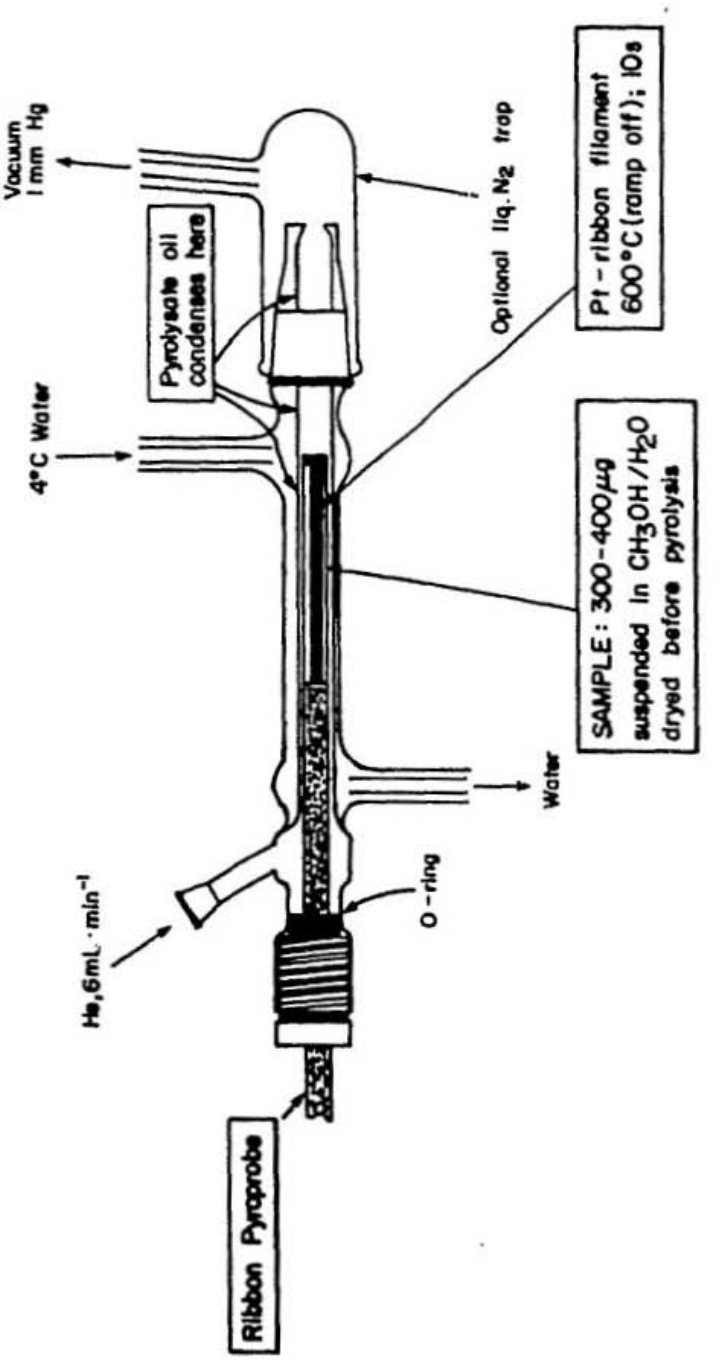


Figure 2.3. Off-line Pyroprobe Platinum Ribbon Pyrolysis Apparatus

2.3.4. Curie-point Pyrolysis Apparatus

Off-line Curie point pyrolysis (Fig 2.4) was performed using a Packard Model 891 Curie Point Pyrolyser (Downers Grove, Illinois). Sample was applied to 480°C Curie point wires by adhering with acetonitrile and vacuum drying to remove the solvent. The wire was positioned inside the radio frequency coil. A vacuum of 2 mm Hg was applied and a 60 second nitrogen purge was used to flush oxygen from the pyrolysis chamber. Samples were pyrolysed for two seconds. The pyrolysate oil was washed from the quartz liner with a small quantity of the solvent used in the derivatization step.

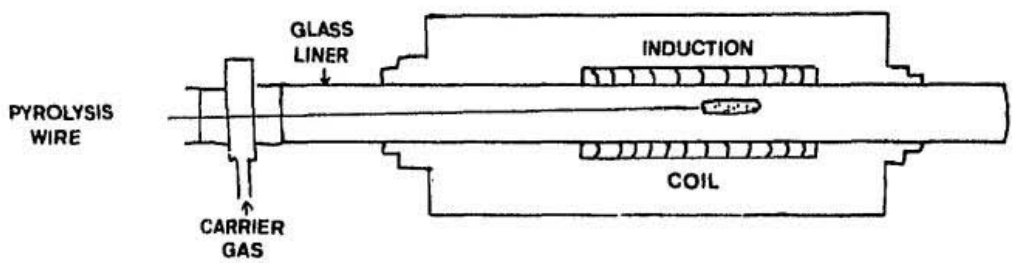


Figure 2.4. Curie Point Pyrolysis Apparatus

2.4. Chromatographic Methods

2.4.1. Gas chromatography

Gas chromatography was performed on a Varian 3700 gas chromatograph (Palo Alto, California) with a flame ionization detector. Data was collected on a Spectra-Physics SP4290 Integrator and a Tandy 1200 HD personal computer equipped with a Spectra-Physics Winner Data System (San Jose, California). A non-polar fused silica capillary column (J & W Scientific, DB-5, 1.0 μm thickness, 30 m x 0.322 mm i.d.) operated at a column flow rate of 2.5 mL/min helium and a split ratio of 10:1 was used for high resolution separation of trimethylsilyl derivatives. Temperature program was: 120°C, hold 2 minutes, increase 5°C/min until 270°C, hold for 15 minutes. The injection port and detector were heated at 280°C. Trimethylsilyl derivatives were also analysed using an on-column injector (J & W Scientific, Folsor, California) and a non-polar capillary column (J & W Scientific, DB-5, 1.0 μm thickness, 10 m x 0.25 mm i.d.) with a column flow rate of 2 mL/min. Temperature program was: 80°C, hold 2 mins, increase 8°C/min until 340°C, hold 10 mins. The detector was heated at 350°C.

2.4.2. High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) of per-O-benzoylated carbohydrates were analysed using a Varian 5000 HPLC (Palo Alto, California) equipped with a Varian Model UV-10, variable wavelength detector. Derivatives were monitored by measuring absorbance at 230 nm and signals were recorded on a Varian Model 9176 chart recorder. Reverse phase chromatography was performed on a 5 μm Spherisorb ODS-2, 250 nm x 4.6 mm analytical column (Mandel Scientific, Quelph, Ontario). A ten microliter sample loop was used. Gradient elution was as follows: 60% CH_3CN / 40% H_2O to 90% CH_3CN / 10% H_2O in twenty minutes; these conditions were held for 10 minutes; then a linear gradient to 100% CH_3CN over the subsequent ten minutes. A flow rate of one milliliter per minute was maintained throughout.

2.5. Mass Spectrometry

2.5.1. Desorption Ammonia Chemical Ionization

Mass spectra were acquired with a VG analytical ZAB-EQ hybrid instrument equipped with a VG 11-250J data system. Samples were applied onto a DCI platinum filament probe and inserted into a combination EI-CI source in the CI mode. The source was operated at 160°C. Ammonia gas was admitted into the source until the pressure over the diffusion pump was 7×10^{-5} mbar. Electrons with 100 eV energy were used to ionize the ammonia reagent gas. A reasonably high ammonia gas pressure was obtained in this source where the ion abundance ratios of the ammonia adducts m/z 18, 35 and 52 were measured at 2:8:5. The samples were desorbed by ramping the platinum filament temperature for approximately 120 s, to a final desorption temperature close to 350°C. The mass spectrum was scanned at 2s per decade and the mass spectra were time-integrated.

2.5.2. Gas Chromatography-Chemical Ionization Mass Spectrometry

The conditions of chromatography were identical to those used for high resolution separation (see section 2.4.1), *ie.*, 30 m column but with a reduced column flow rate. The column outlet was led directly into the ion source of a VG 7070HS double focusing mass spectrometer equipped with a DS 2035 data system. Mass spectrometric detection was carried out under chemical ionization conditions using ammonia as the reagent gas and with a combination EI-CI source in the CI mode. The ammonia gas was admitted into the ion source until the pressure above the diffusion pump was 6×10^{-5} mbar. The electron impact ionization energy was set at 100 eV, the source temperature at 140°C and the column inlet temperature at 270°C. The resulting ion abundance ratio of adducts of ammonia m/z 18 and 35 were observed to be approximately 5 to 1. The mass spectrum was scanned at 1s per decade.

2.5.3. Gas Chromatography-Electron Impact Mass Spectrometry

Chromatography was performed on a Hewlet Packard Model 5980/A GC/MS equipped with a 5934A data system. The mass spectrometer was a low resolution quadrupole instrument with a mass range of 50 to 650 amu. An ionizing voltage of 70 eV was used for the electron impact mass spectra. The gas chromatograph was equipped with a CPSil 5 fused silica column (0.33 mm x 30 m, 0.2 μm film thickness). (Chrompack, Blenheim, Ontario). The initial column temperature was 100°C which was held for 5 minutes and a linear ramp of 5°C/min raised the oven to the final temperature of 290°C which was held for 5 minutes.

3. Optimization of Off-line Pyrolysis Techniques

3.1. Pyrolysis Instrumentation

Analytical pyrolysis involves the thermal fragmentation of polymers into smaller, characteristic fragments which are more readily analysed by chromatographic or mass spectrometric methods. Many factors influence the pathways of this degradation and instruments must be designed so as to control these parameters and ensure reproducible pyrolysis.

Pyrolysis reactions are a series of temperature dependent, competing reactions; at low temperatures incomplete pyrolysis may occur while at higher temperatures small, less characteristic fragments may result (7). Decomposition may begin before the final temperature is reached. To obtain pyrolysis over the same temperature range a reproducible, rapid heating rate is necessary. Efficient heat transfer to the sample is required to avoid temperature gradients across the sample and thus prevent the production of secondary products. The use of small sample sizes has been found to markedly reduce the number and quantity of secondary products. Once pyrolysis is initiated, rapid removal of the primary products from the heated area is necessary to ensure primary products are collected (off-line pyrolysis) or transferred to the analytical device (on-line pyrolysis) (34).

Amylose was used as a standard for these studies to compare product distribution and yields obtained with various pyrolytic apparatus. The instruments used include: furnace pyrolyser, Curie-point pyrolyser and resistively heated pyrolyser with both platinum coil and ribbon pyrolysis probes.

3.1.1. Furnace Pyrolyser

A furnace pyrolyser is comprised of a long quartz tube positioned inside a continuously heated furnace. The sample is dropped into the heated zone, the temperature of the sample begins to rise and eventually, pyrolysis occurs. The temperature at which the sample is

pyrolysed is generally lower than the furnace temperature and is not precisely known (34). The sample travels through approximately ten centimeters of heated furnace before being retarded by the indentation in the pyrolysis chamber. This long, variable residence time in the pyrolysis zone presents the possibility of additional fragmentation of the primary pyrolysis products. Large sample sizes, up to milligram quantities, are often used permitting temperature gradients across the sample that may result in secondary recombination products (34).

Amylose, a linear α-D-(1→4) linked glucan, was pyrolysed in a furnace at 450°C. The trimethylsilyl derivative of the pyrolysate oil was analysed by on-column gas chromatography. The two major products, peaks T-I and T-II of Figure 3.1, were identified by comparison of retention times with standards and confirmed by mass spectrometry as described in Section 4.2. The first peak (T-I) corresponds to the trimethylsilyl derivative of 1,6-anhydro-β-D-glucopyranose (I) while the second peak (T-II) is the analogous furanose, 1,6-anhydro-β-D-glucofuranose (II). The yield of products was determined by the addition of an internal standard, methyl-6-deoxy-α-L-galactopyranose (peak IS), prior to the derivatization step. The yield of anhydro sugars was found to be approximately 28%.

No anhydro oligomers were observed in the pyrolysis product, these have retention times of thirty to forty minutes. Long residence times in the hot furnace would most likely decompose any thermally labile anhydro oligosaccharides should they have been formed in the primary pyrolysis step.

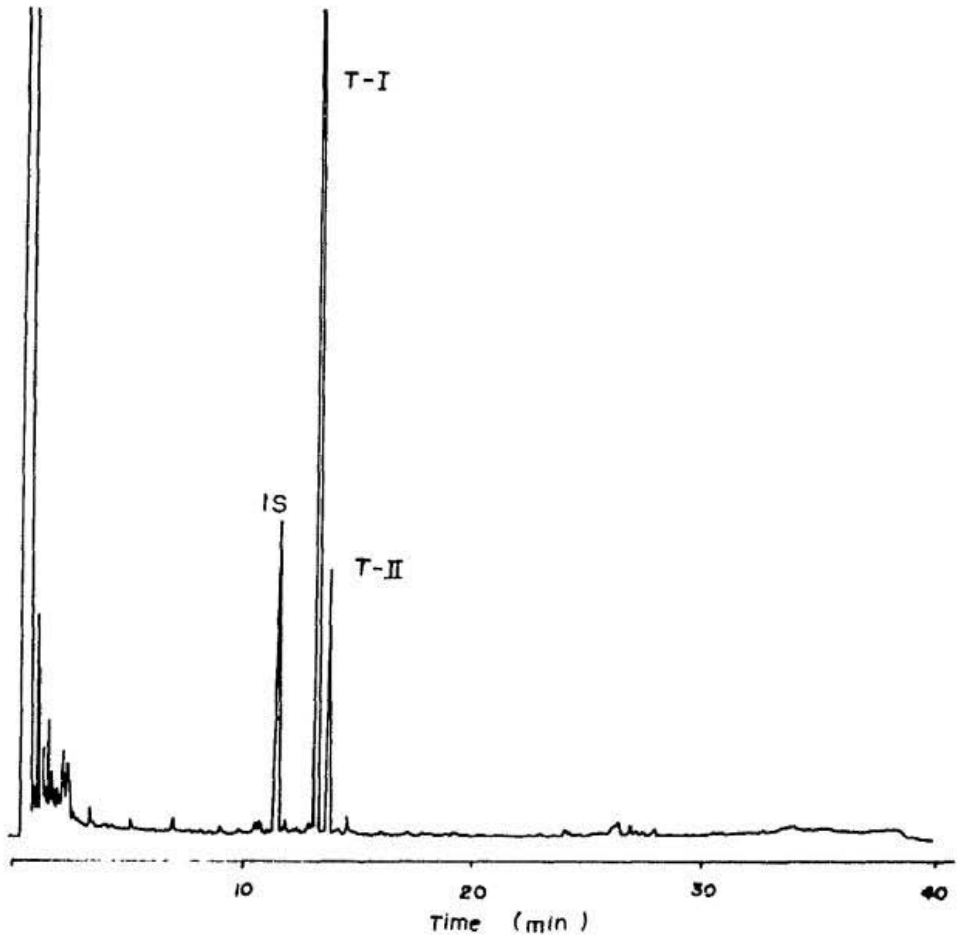


Figure 3.1. On-column Gas Chromatogram of Trimethylsilyl Derivatives of Amylose Pyrolysate from Furnace Pyrolyser.

3.1.2. Curie Point Pyrolysis

The Curie-point pyrolyser employs inductively heated ferromagnetic alloys. Samples are applied to a Curie-point wire. When a radio frequency is applied the alloy absorbs energy until the Curie-point, specific for each alloy, is reached (11). The temperature stabilizes at this range. This technique offers rapid temperature risetimes to a reproducible final temperature; a typical risetime of 10 to 100 milliseconds is attainable. Since the pyrolysis temperature is determined by the alloys, only discrete pyrolysis temperatures are possible. A thin layer of sample is applied to the wire so as to reduce temperature gradients. However, the potential catalytic effect during pyrolysis of the metals from which the alloys are made, i.e. iron, cobalt and nickel, cannot be ignored.

Pyrolysis of amylose at 480°C using the off-line Curie-point apparatus gives a product yield of anhydro sugars of 42% (Figure 3.2). The anhydro monomers, 1,6-anhydro-β-D-glucopyranose (peak T-I) and 1,6-anhydro-β-D-glucofuranose (peak T-II) are the major peaks. The later eluting peak (T-VIII) is the intact anhydro dimer, 4-O-α-D-glucopyranosyl-1,6-anhydro-β-D-glucopyranose (VIII), the yield being 8%. The anhydro dimer was identified by comparing retention time with the standard and by mass spectral data as described in Section 4.2. The unidentified small peaks eluting after peak T-II are most likely products comprised of glucose with additional ring fragments attached.

The rapid temperature risetime obtained in this pyrolysis technique permits the recovery of intact oligomeric units. Reasonable yields of anhydro sugar products are obtained, however, small samples ($\sim$ 100 μg) must be applied to the wire in order to obtain reproducible results. The product distribution allows interpretation of the original polysaccharide structure, the presence of anhydro monomers relates information about the monosaccharide isomers while the anhydro oligomers provide structural information about linkage positions and configurations.

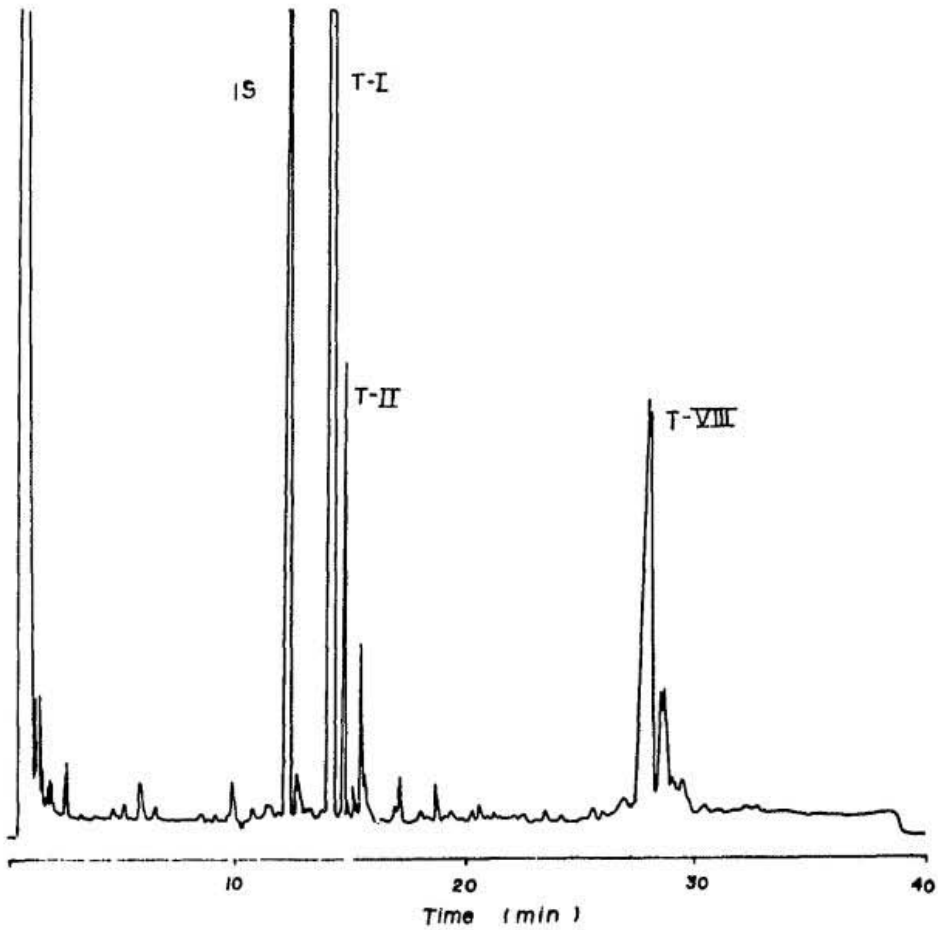


Figure 3.2. On-column Gas Chromatogram of the Trimethylsilyl Derivative of Amylose Pyrolysate from Curie-Point Pyrolyser.

3.1.3. Resistively Heated Pyrolysers

Electric current passes through a resistive filament and transfer of power results in temperature increase (34). The Pyroprobe system allows for variation in pyrolytic surface, where the sample may be applied directly onto a platinum ribbon or placed in a quartz tube and positioned within a platinum coil. With a platinum ribbon pyrolyser the temperature risetime is very rapid, however, as with Curie-point possible interferences from a catalytic surface may occur. With the platinum coil a delay of several hundred milliseconds is required for transfer of heat from the coil to the sample in the quartz tube. This technique may also present problems due to temperature gradients across the sample. The Pyroprobe system permits programmed pyrolysis with a linear temperature ramp from one to six-hundred degrees Celsius per second. Stepped pyrolysis may also be performed where samples are repetitively pyrolysed at higher temperatures (11).

Amylose was pyrolysed at 450°C on a platinum ribbon pyrolyser and the TMS derivatives were analysed by GC (Figure 3.3). It is difficult to accurately transfer a weighed solid sample to the ribbon, therefore approximate yields are calculated. The total yield of anhydro monomers (T-I & T-II) and dimer (T-VIII) is only about 7% with roughly equal proportions of each. Several additional peaks with retention times less than the internal standard (IS) are observed. These are levoglucosan (I) degradation products such as 5-hydroxymethyl-2-furaldehyde (T-IV).

Conditions were optimized to obtain maximum yields of anhydro monomers and dimers with the off-line platinum coil/quartz tube pyrolysis apparatus. It was determined that location of the cold finger affected product distribution; if the cold finger was too close to the pyrolysis zone inhibition of gas flows occurred, if too distant primary pyrolysis products were not obtained. The vacuum was maintained at 2 mm Hg; with high vacuum heat transfer from pyrolysis coil to sample tube was retarded and incomplete pyrolysis occurred, at low vacuum

products were not effectively removed from the pyrolysis zone and secondary products were obtained.

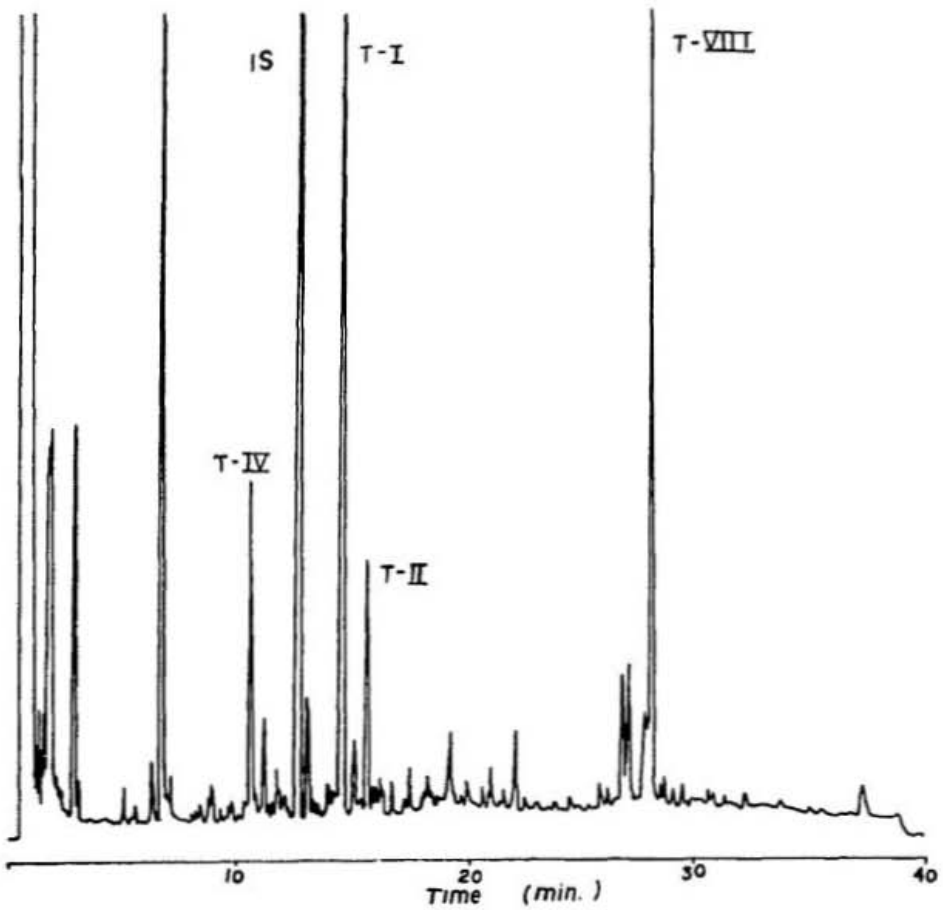


Figure 3.3. On-column Gas Chromatogram of Trimethylsilyl Derivative of Amylose Pyrolysate Pyroprobe Ribbon Pyrolyser.

With the platinum coil pyrolyser, a good ratio of dimeric to monomeric product was obtained when amylose was pyrolysed at 450°C, total yields of anhydro sugars of 4% were obtained (Figure 3.4). There is a lower abundance of secondary pyrolysis products (such as product T-II). This technique was more easily adapted to quantitative analysis due to the ease of sample handling. Rapid temperature risetime coupled with effective transfer of primary pyrolysis products assist in the collection of reasonable quantities of oligomeric species.

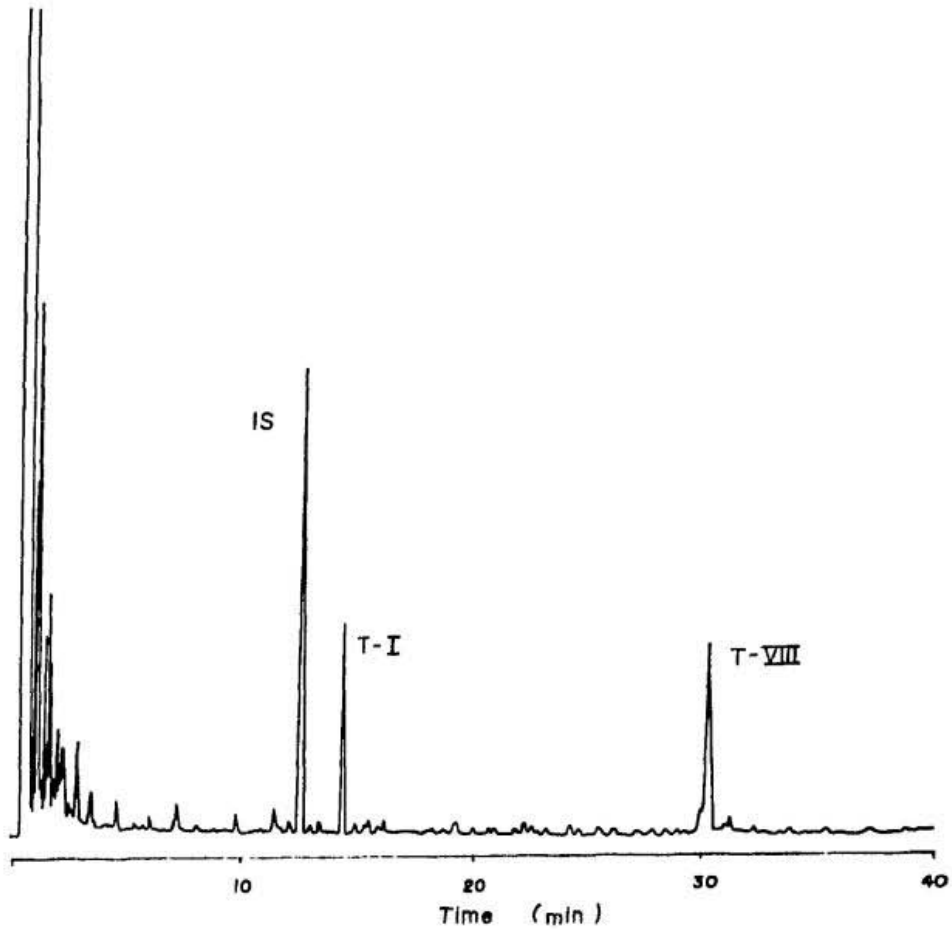


Figure 3.4. On-Column Gas Chromatogram of Trimethylsilyl Derivative of Amylose Pyrolysate from Pyroprobe Platinum Coil/Quartz Tube Pyrolyser.

3.1.4. Comparison of Instrumentation

In summary, both Curie-point and resistively heated pyrolysers gave reasonable quantities of oligomeric anhydro sugars. The yields and ratios of momeric to dimeric species obtained when amylose was pyrolysed with the various pyrolysis methods is summarized in Table 3.1.

Table 3.1. Yields of Anhydro Sugars for Curie-point, Furnace and Resistively Heated Pyrolysis of Amylose

	Curie Point	Furnace	Resistively heated	
			Ribbon	Coil
Total Yield	42%	28%	7%	4%
Ratio Monomer to Dimer	4:1	no dimer	1:1	2:1

These results can be attributed to the important design features of each pyrolysis unit. A comparison of these parameters is reviewed in Table 3.2 (11,34).

To achieve reasonable yields of oligomeric anhydro sugars either Curie point or resistively heated pyrolysers should be employed. The Curie point pyrolyser has rapid, reproducible pyrolysis temperatures, however, only discrete final temperatures are possible, preventing small variations in the analytical technique. The application of the sample to the small wire is difficult for non-soluble polymers. On the other hand, the resistively heated pyrolyser provides versatility in pyrolysis conditions, where minor alterations in these conditions can provide dramatic influences in pyrolysate composition. The use of the platinum coil provides easily manipulated sample handling that is appropriate for all types of biopolymers. By keeping the sample size small, deliterious effects of thermal gradients and secondary products are eliminated. A disadvantage of the resistively-heated pyrolyser is the much lower yields of anhydro sugar products (as shown in Table 3.1). This factor must be considered when only small quantities of sample are available.

Table 3.2. Comparison of Pyrolysis Instrumentation

	Curie Point	Furnace	Resistively heated	
			Ribbon	Coil
Temperature Rise time	Rapid (10 ms)	Slow (unmeasurable)	Rapid (10 ms)	Rapid (200 ms)
Temperature range	Discrete	Continuous	Continuous	Continuous
Catalytic Surface	Yes	No	Yes	No
Secondary Reactions	No	Yes	No	No
Sample Size	100 μg	mg	100 μg - 1 mg	100 μg
Thermal Gradients	No	Yes	No	Yes

3.2. Factors Influencing Pyrolysis

3.2.1. Effects of Salts

When a sample of amylose was pyrolysed using the platinum coil/quartz tube pyrolysis apparatus a 4.5% yield of anhydro sugars was obtained with 3.9% being contributed by levoglucosan (I) while the remaining 0.6% was maltosan (VIII). When this sample was washed with acid to remove the presence of trace salts, pyrolysis under identical conditions more than doubled the yield to 9.9% with 6.3% being levoglucosan (I) and 3.6% the anhydro dimer, maltosan (VIII).

It has been reported that the presence of neutral salts (such as sodium chloride) in a carbohydrate sample will drastically influence the composition of the pyrolysis products

(12,27,35,36). As little as 0.01% salts was found to vary the product distribution in favor of low molecular weight compounds and significantly increase the yields of char (35). These products are less characteristic of the original biopolymer and lend very little insight into composition or structure analysis.

The pyrolysis of polysaccharides proceeds by a series of competing pathways: one leads to the formation of char and gaseous products; the second, low molecular weight volatiles and the third, the formation of anhydro sugars (13). It has been proposed that anhydro sugar formation proceeds by the unzipping of the free chain ends of the polysaccharide, the presence of trace salts could result in the inhibition of this initiation step (36). Such a mechanism would explain why the presence of minute quantities of salt can affect pyrolysis. At 0.01% sodium chloride there is only one mole of salt for every 3000 moles of glucose molecules in cellulose. Essig has proposed that the salt varies the dielectric constant of the solid polysaccharide influencing the homolytic formation of anhydro sugars (35). Salts may also act as a catalyst for the decomposition of glucosyl units to lower molecular weight species (27,35,36). Such inhibition of depolymerization will result in the favoring of the competing ring scission pathways.

3.2.2. Sample Size

The amount of sample pyrolysed has been shown to affect the product distribution during analytical pyrolysis (17). With large sample sizes temperature gradients may occur across the sample affecting the nature of the pyrolysis reactions. As sample size increases the likelihood of obtaining greater quantities of secondary recombination products also increases resulting in reduced yields of primary products.

A large sample, approximately 200 μg, of amylose was pyrolysed using a platinum coil pyroprobe unit. The recovery of anhydroglucose was approximately 3% while that of the anhydro dimer was 2%. By reducing the sample weight to approximately 50 μg, recoveries of

7% anhydroglucose and 2% anhydro dimer were achieved. With the larger sample more peaks occur in the front of the chromatogram, these peaks result from the breakdown of levoglucosan molecules.

3.2.3. Pyrolysis Temperature

As previously noted, pyrolysis of polysaccharides proceeds by a series of competing pathways. These pathways are strongly temperature dependent with high temperature pyrolysis leading to low molecular weight fragments. Pyrolysis temperature in this study was optimized to give good yields of both monomeric and dimeric anhydro sugars. Table 3.3 shows the pyrolysis temperature and the resultant ratio of anhydro dimer to anhydro monomer for a sample of amylose pyrolysed with the Pyroprobe platinum coil/quartz tube pyrolysis apparatus.

Table 3.3. Comparison of Pyrolysis Temperature to Product Distribution.

Pyrolysis Temperature	Ratio dimer/monomer
400°C	4
500°C	0.6
600°C	0.2
800°C	0.1

The temperature chosen for optimum pyrolysis conditions was 450°C; this results in significant amounts of both monomer and dimer. Lower temperatures give reasonable yields of dimer but at the expense of reduced monomeric yields.

3.3. Chromatographic Analysis

3.3.1. Gas Chromatography of Trimethylsilyl Derivatives of Pyrolysates

Trimethylsilyl derivatives of polysaccharide pyrolysates were analysed by gas chromatography. Trimethylsilyl derivatives were chosen due to the ease of derivatization and the enhanced response to the flame ionization detector. Several problems were initially encountered with the analysis of these derivatives. When the derivatization mixture was analysed directly, large peaks occurred at the front of the chromatogram. These peaks were due to excess silylating reagent, this excess reagent could be removed by extraction into hexane without any loss of the silylated carbohydrates. Any residual water in the hexane extract would also result in a single broad peak near the beginning of the chromatogram.

These derivatives are not stable with time and should be analysed soon after derivatization. In addition, the reagents are not stable over long periods of time at room temperatures. Two different commercial reagents Sylon TP (Supelco) and Tri-Sil-Z (Pierce) were used throughout these studies. It was found that Sylon TP proved to be most suitable for these studies.

Amylose pyrolysate obtained from the Pyroprobe platinum ribbon pyrolyser was derivatized using Sylon TP then analysed by gas chromatography using an on-column injector (see Fig. 3.3). This technique avoids injecting thermally labile samples into a heated injection port. The trimethylsilyl derivative of an anhydro trisaccharide standard, 4-O-(6-O-β-D-glucopyranosyl-β-D-galactopyranosyl)-1,6-anhydro-β-D-glucopyranose, can be eluted from the analytical column under these conditions, however no anhydro trimer was observed in the amylose pyrolysate. All anhydro mono- and disaccharides studied gave unique retention times under these conditions. As well, this chromatographic technique permits resolution of the small molecular weight fragments that occur near the front of the chromatogram.

Chromatographic analysis using a thirty meter column with a hot split injection gave similar results (Fig. 3.5). Higher temperature programming must be used to elute oligosaccharides from this longer column. It should be noted that if oligomers larger than dimers are present there is a possibility that degradation may occur in the injection port and these species would remain undetected.

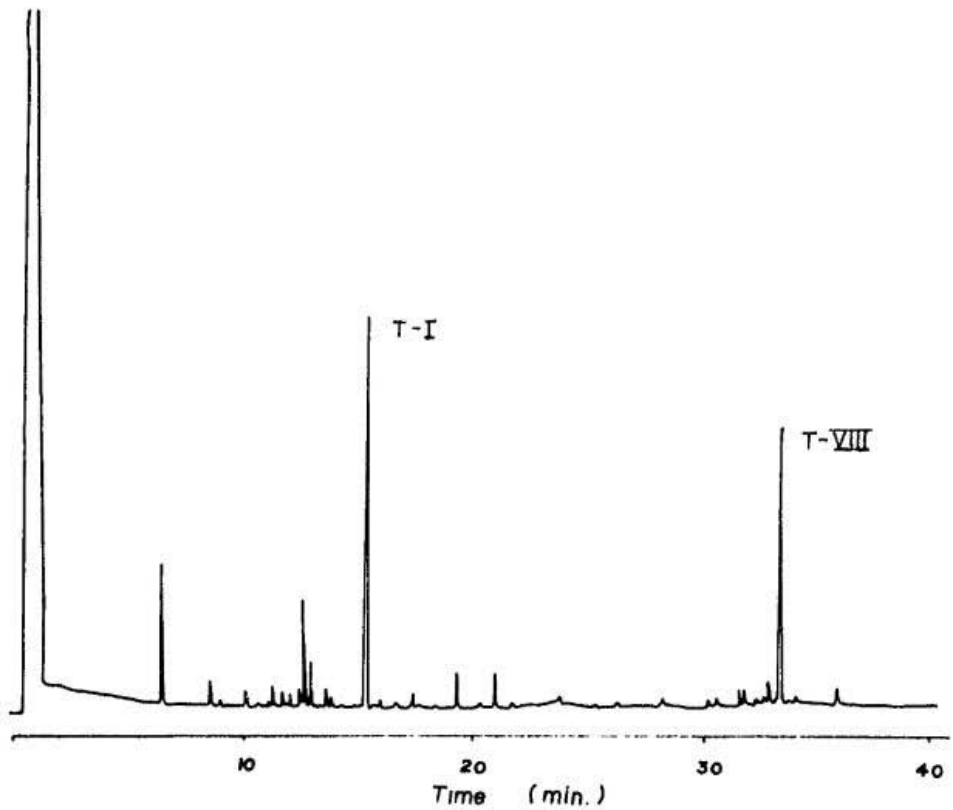


Figure 3.5. Hot Split Injection Gas Chromatogram of Amylose Pyrolysate from Pyroprobe Ribbon Pyrolyser.

3.3.2. High Performance Liquid Chromatography of Per-O-benzoylated Pyrolysates

Analysis of amylose pyrolysate by per-O-benzoylation followed by high performance liquid chromatographic (HPLC) analysis was also performed. In this analysis ultra violet absorption by the benzoyl esters provides a very sensitive mode of detection. This method permits detection of small quantities of products not recorded in the gas chromatogram of trimethylsilyl derivatives. Both derivatization and analysis occur at low temperatures thus avoiding degradation of thermally labile species such as anhydro sugars. Gradient elution is performed to enable separation of isomers within an oligomer series. Oligosaccharides containing up to nine monosaccharide units have been analysed using a similar technique (33).

The HPLC chromatogram of a per-O-benzoylated derivative of amylose pyrolysate from pyroprobe ribbon pyrolyser is shown in Figure 3.6. The major peaks are the benzoyl esters of 1,6-anhydro-β-D-glucopyranose (peak B-I), 1,6-anhydro-β-D-glucofuranose (peak B-II) and 4-O-α-D-glucopyranosyl-1,6-anhydro-β-D-glucopyranose (peak B-VIII) and the internal standard, methyl-6-deoxy-α-L-galactopyranose (peak IS). These per-o-benzoylated pyrolysates were identified by comparing the retention times with authentic reference standards.

It is believed that if a 1,6-anhydro trimer product was present, its per-o-benzoylated derivative would have been observed with a retention time of about thirty-five minutes. An authentic 1,6-anhydro trimer reference compound was used to determine its characteristic retention time.

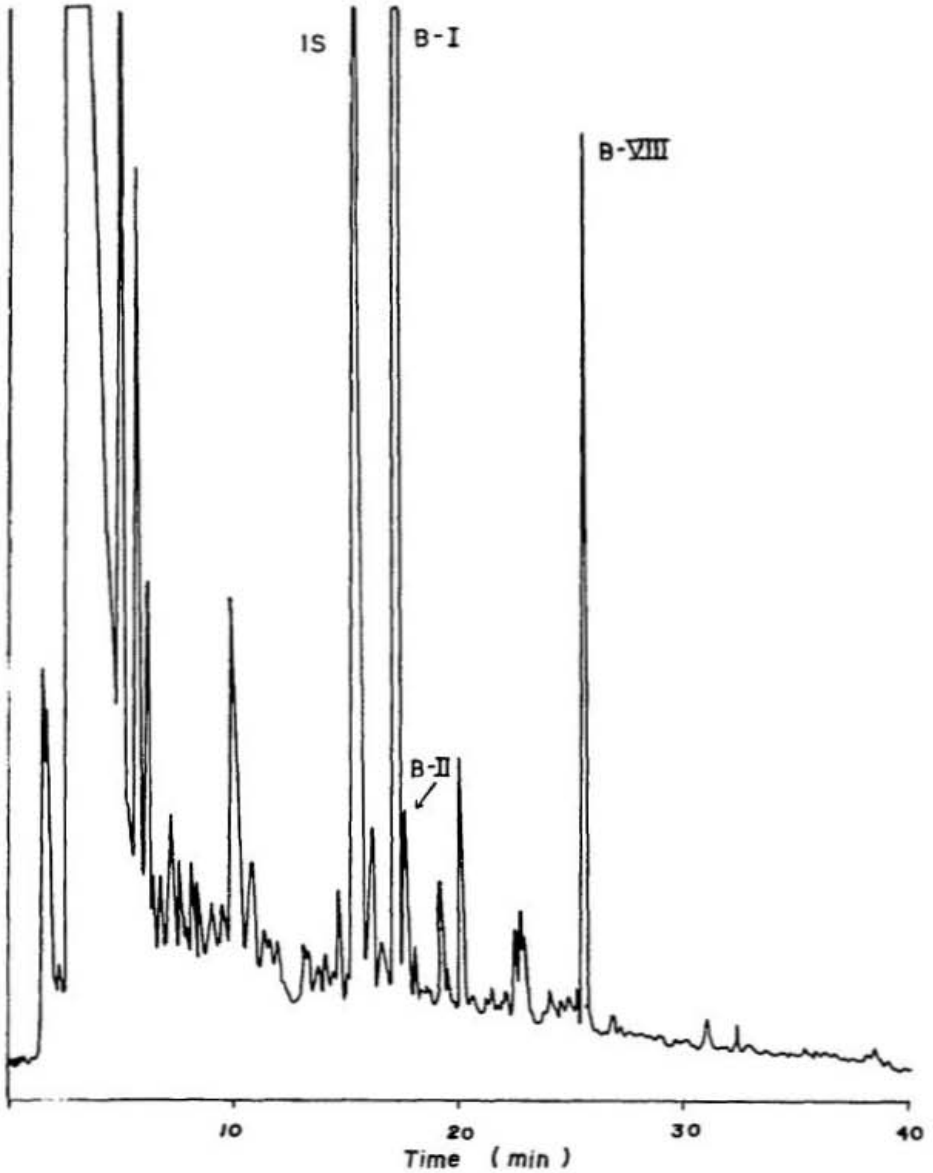


Figure 3.6. Liquid Chromatogram of Per-O-benzoylated Amylose Pyrolysate from Pyroprobe Ribbon Pyrolyser.

3.3.3. Gas Chromatography of Methylated Pyrolysates

Several pyrolysates and carbohydrate standards were derivatized by a methylation technique for gas chromatographic analysis and for the purpose of performing mass spectrometry experiments for structure identification. The derivatization procedure is very lengthy, and in many cases, the background contamination is found to be quite large. When a standard mixture, containing 1,6-anhydro-β-D-glucopyranose (I) and 4-O-α-D-glucopyranosyl-1,6-anhydro-β-D-glucopyranose (VIII), was analysed by this procedure, several small unidentified peaks in addition to the standard peaks were evident in the chromatogram. A cellulose pyrolysate collected from the off-line pyroprobe platinum coil pyrolyser was analysed by methylation gas chromatography (Fig. 3.7). In addition to the two major pyrolysis products, 1,6-anhydro-β-D-glucopyranose (M-I) and 4-O-β-D-glucopyranosyl-1,6-anhydro-β-D-glucopyranose (M-IX), a background of multiple peaks of low intensity were observed. Pre-reduction is normally performed on oligosaccharides to limit degradation involving free carbonyl functions during methylation. The absence of this step may also contribute to the high background (17).

3.3.4. Comparison of Chromatographic Techniques

All chromatographic techniques permitted resolution of the various anhydro sugars encountered in this study. Major differences occur in sensitivity, ability to separate high molecular weight species and in the complexity of derivatization steps.

The methylation analysis was a time-consuming procedure that resulted in high background in many of the samples. There is much mass spectral data available for the methylated derivatives of carbohydrates which makes this method useful for structural analysis. However this would not be the method of choice for routine, rapid analysis.

Trimethylsilylation transformed the carbohydrates into species volatile enough for gas

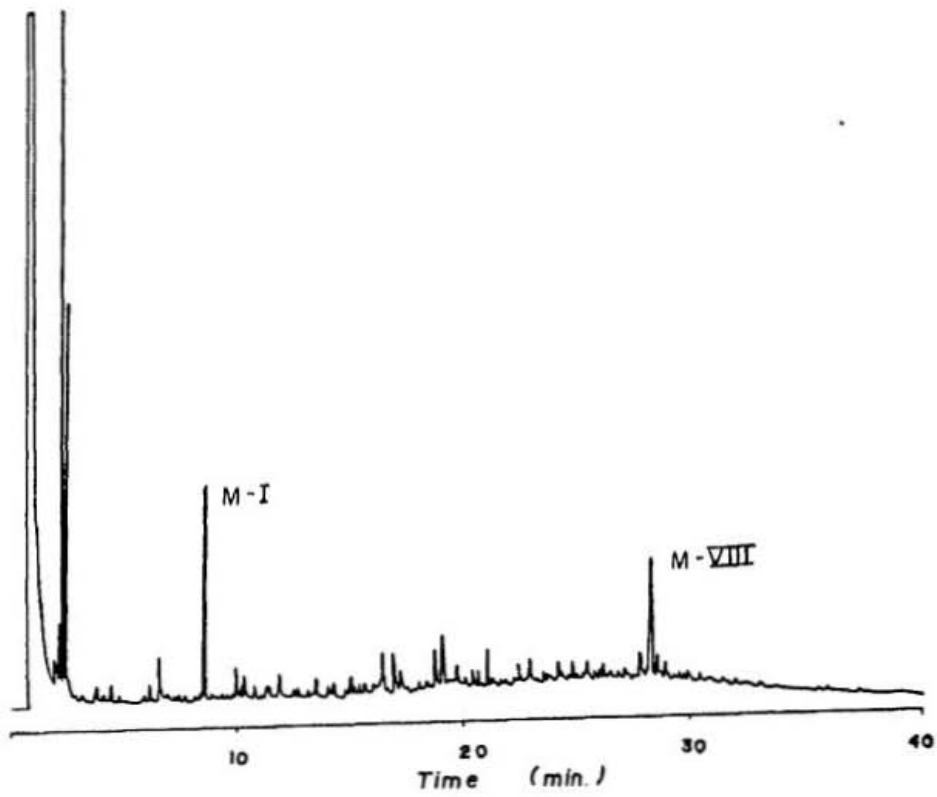


Figure 3.7 Gas Chromatogram of Methylated Derivative of Cellulose Pyrolysate from Pyroprobe Platinum Coil Pyrolyser.

chromatographic analysis. Extraction of the reaction mixture with hexane provided a clean sample with few background peaks. The derivatized carbohydrates are not stable over an extended period of time and should be analysed shortly after preparation. Analysis of these derivatives with a short GC column and cool on-column injection avoids possible breakdown of labile species and permits analysis of anhydro sugars in the monomer to trimer range.

Benzoylation followed by ultra violet detection of the liquid chromatograph eluant pro-

vided a rapid, sensitive analysis. Derivatization occurs under mild conditions and concentration of the sample through solvent evaporation is possible without any loss of products. Derivatives are stable for extended periods of time. The high molecular weights of these derivatives does not easily avail them to mass spectral analysis, an important consideration for confirmation of the identity of pyrolysate products.

The combination of on-column GC analysis of trimethylsilyl derivatives and HPLC analysis of benzoylated derivatives permits a complete overview of pyrolysate composition. The GC analysis can be a rapid screening technique that is readily adapted to mass spectral analysis. Benzoylation-HPLC is a slightly longer procedure, however, it is extremely sensitive and provides a better analysis of higher oligosaccharides if they are present.

4. Mass Spectrometry of Carbohydrates

4.1. Mass Spectrometry of Carbohydrate Derivatives

The interpretation of the structural detail of polysaccharides and oligosaccharides requires elucidation of monosaccharide components as well as linkage position and configuration. Early mass spectral studies of carbohydrates were not encouraging but recent developments in mass spectrometers have facilitated the analysis of these biologically important compounds and the applications of mass spectral analysis of carbohydrates have increased rapidly in the past decades (37). Gas chromatography coupled with mass spectrometry (GC-MS) has become irreplaceable for the analysis of complex mixtures of carbohydrate compounds. GC-MS provides both retention time and mass spectra data to aid in identification (4).

The necessary qualification for mass spectrometric and gas chromatography-mass spectrometric analysis is the volatility of the analyte. As most carbohydrates are not highly volatile, these polar species must be derivatized. Various derivatives have been used for carbohydrate analysis, these include: trimethylsilyl ethers, methyl ethers, alditol acetates and trifluoroacetates. Factors influencing the choice of derivative include ease of preparation, suitability for gas chromatography and mass spectrometry and the complexity of the mass spectra of the derivative (37). Direct derivatization often results in a mixture of anomers which require fractionation prior to mass spectral analysis.

Reduction to non-cyclic species, prior to derivatization, results in a single peak for each monomer. Stereoisomers give very similar electron impact mass spectra and small differences in relative peak ratios often do not permit distinct indentification. For instance, α and β- hexopyranosides give virtually identical mass spectra (38).

Mass spectrometry permits selection of various ionization techniques enabling tailoring

of the analysis to fulfill the experimenter's needs. Conventional electron impact ionization does not produce molecular ions from derivatized sugars and extensive fragmentation often occurs. Soft ionization techniques (chemical ionization, field ionization and field desorption) give a large abundance of molecular ions but fragmentation indicative of fine structural detail is absent (38). The combination of electron impact with a soft ionization technique provides both molecular weight and structural information necessary for identification.

4.1.1. Permethylated Carbohydrate Derivatives

The preparation of permethylated glycosides is very time consuming and harsh conditions are required. The technique is difficult to adapt for micro-scale analysis. However, the mass spectra of these derivatives have been extensively studied and some fragmentation pathways have been determined by the use of deuterium labelled compounds.

The major fragmentation pathways of permethylated glycosides are indicated in Figure 4.1. The molecular ion is not detected in electron impact mass spectrometry. The fragmentation routes are represented by upper case letters and subscript numbers according to the nomenclature of Kochetkov and Chizhov (37,38). The A series of fragments result from consecutive losses of methanol starting with an initial loss of methoxy radical to form A_1. Loss of the C-5 and C-6 portion of the molecule gives rise to the B fragment. Elimination of C-1 gives the C_1 ion which is unstable and loses a methoxyl radical. Rearrangement with transfer of methoxyl from C-3 to C-1 carbon results in the D fragments, further degradation gives the prominent J fragment. The E_1 fragment is formed when the C-6 portion is removed from the molecular ion (37,38).

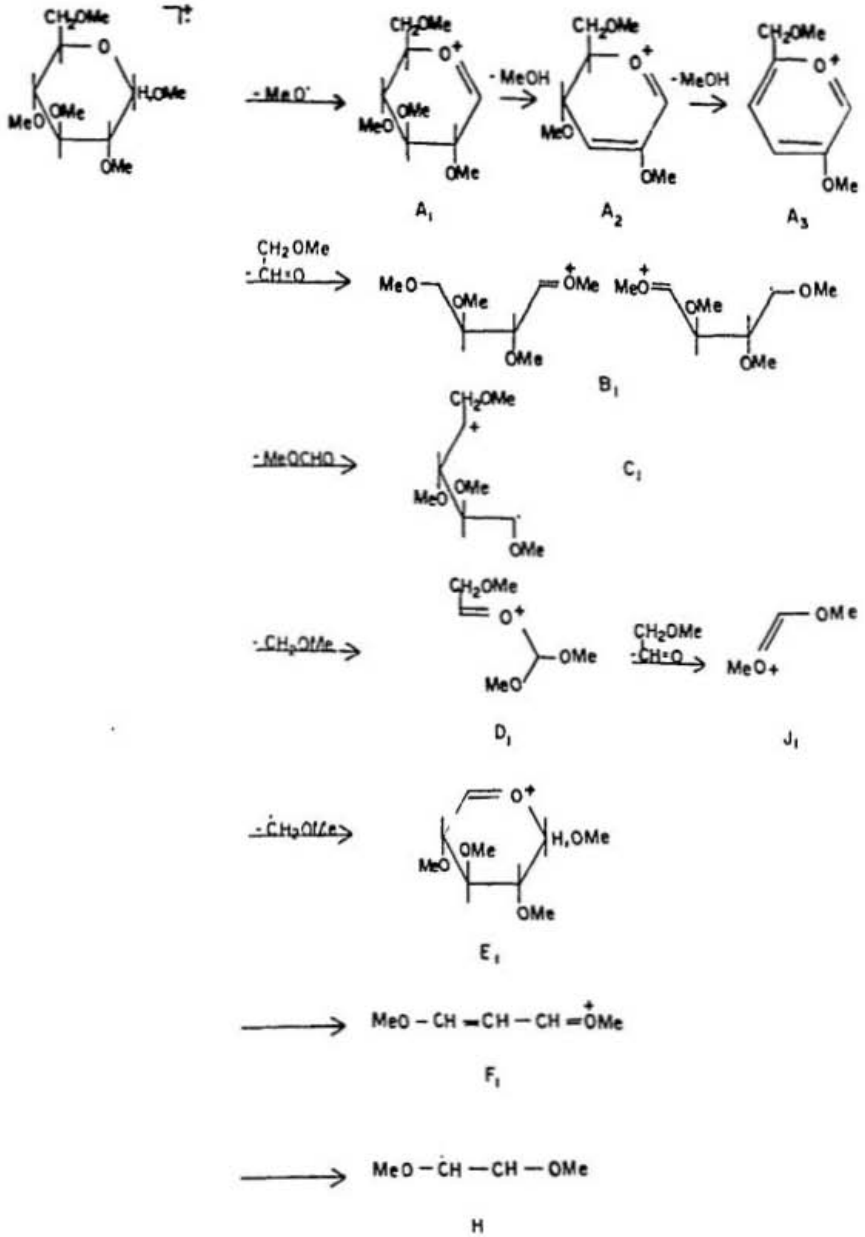


Figure 4.1. Mass Spectral Fragmentation Pathways of Methylated Glucopyranose (37,38).

Mass spectrometry has been used to study the various isomeric species possible. The spectra of α and β isomers are virtually identical. The only variations recorded are the relative intensities of the A_2 and B_1 fragments. The α isomers exhibit a more intense A_2 fragment since the C-1 methoxyl group is in the axial position promoting elimination (37). Similarly the various momeric isomers within a series give very similar electron impact mass spectra.

Ring size may also be determined from the electron impact mass spectra. The furanose structure has unique fragments resulting from the cleavage of the C-4 to C-5 bond giving intense peaks at m/e 161 and 89 (37). In addition to these unique fragments, furanose rings can be identified by relative intensities of F and H fragments. Furanose compounds give a base peak corresponding to the F fragment and a very weak H fragment. In pyranose carbohydrates the H series results in the base peak and F fragment occurs at lower intensity.

Mass spectrometry can aid in the determination of the size and position of the ether ring of anhydro sugars, since this influences the fragmentation pathways. The methylated derivative of a 1,6-anhydro-hexopyranose forms major fragment ions via the C_1, C_2, A and E_1 pathways (37). Loss of formate ion gives the C_1 ion at m/e 159, additional loss of methanol from this ion reduces the fragment to m/e 127 (Fig. 4.2). A_1 elimination gives an ion at 173, successive loss of -CH_2O via the E_1 pathway produces a fragment at m/e 143.

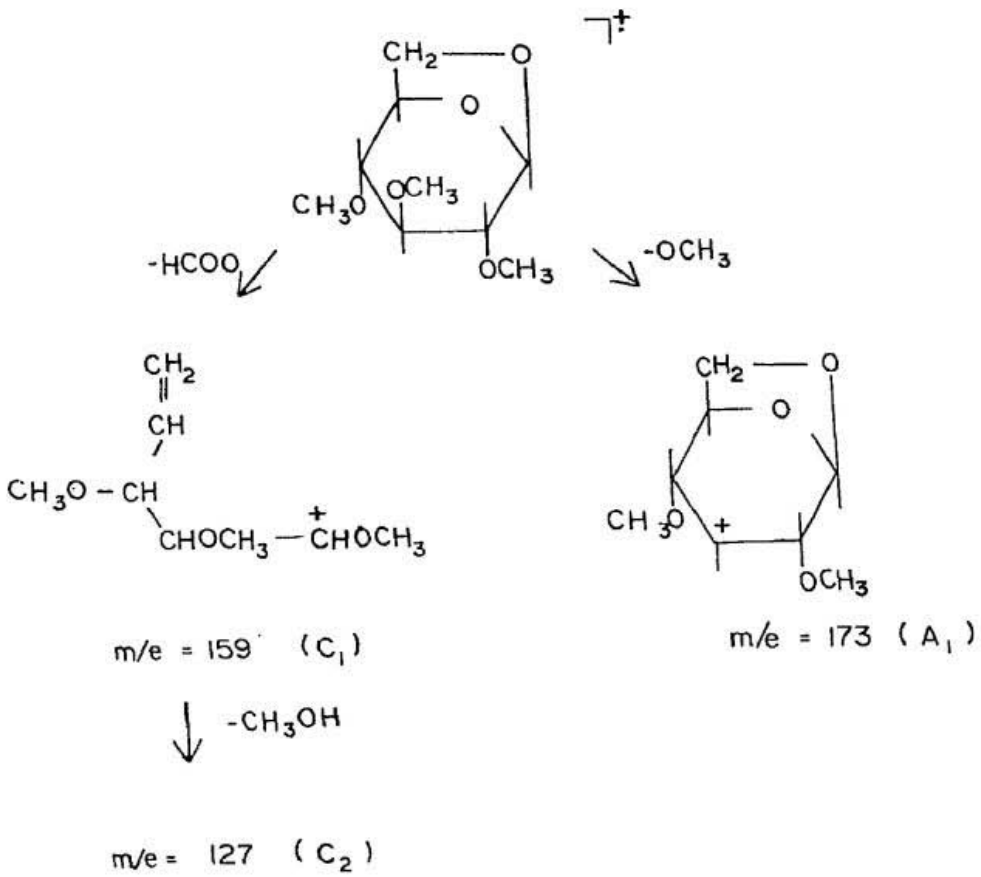


Figure 4.2. Mass Spectral Fragmentation Pathways of Methylated Anhydro Hexopyranoses (37).

The derivative of a 1,6-anhydro-hexofuranose gives unique fragments reflecting the ring structure (Fig. 4.3). The C_1 ion occurs at m/e 145 and loss of methoxy radical then gives a fragment at m/e 114. A low molecular weight fragment at m/e 58 arises from the C_4 to C_6 portion of this anhydro furanose molecule. These three unique fragments permit differentiation of anhydro furanose and pyranose molecules (37).

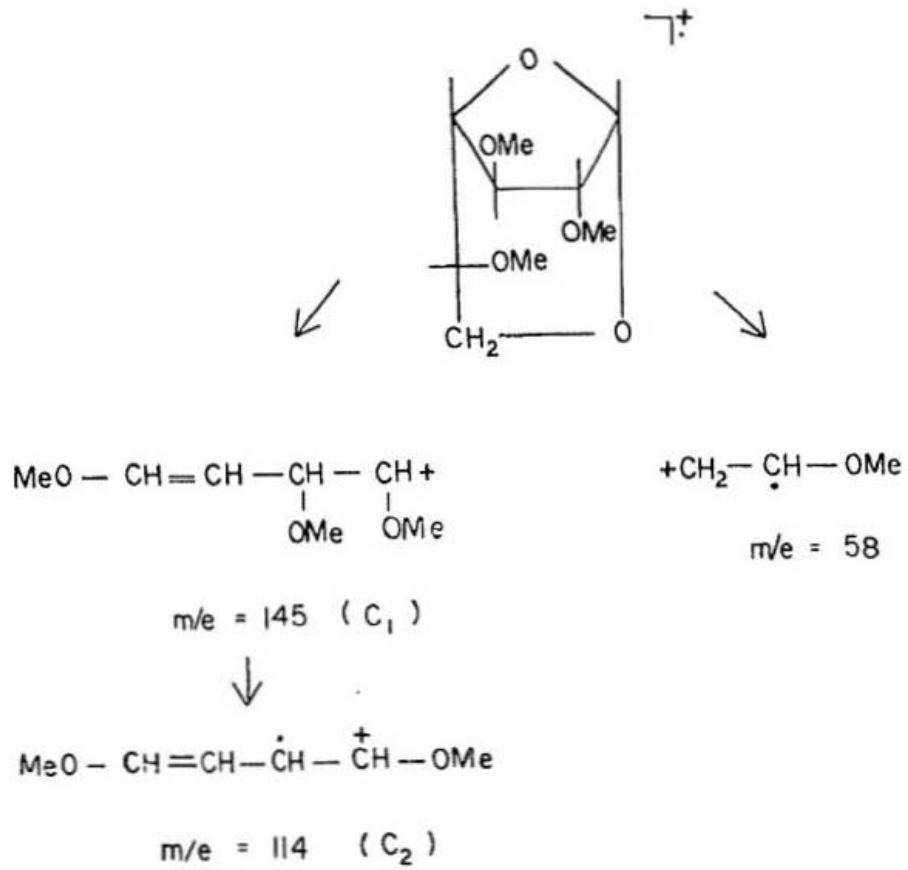
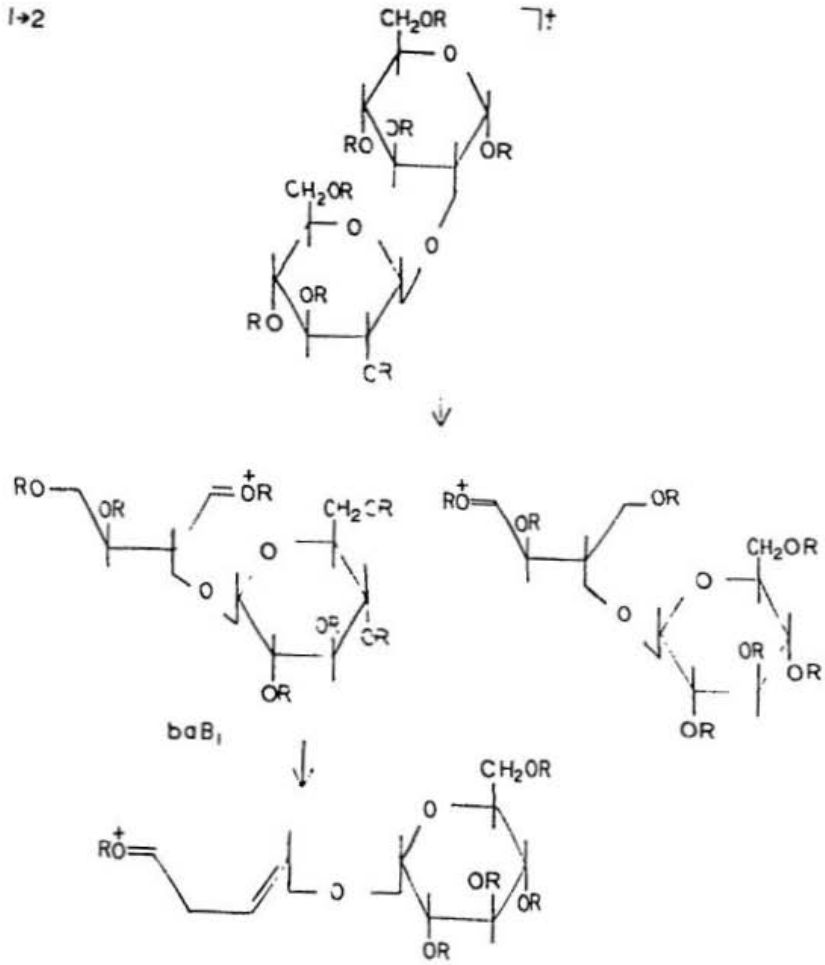


Figure 4.3. Mass Spectral Fragmentation Pathways of Methylated Anhydro Hexofuranoses (37).

Position of the ether ring may also be determined (37). For example, a 3,6-anhydro hexopyranoside would have unique fragments at m/e 71, 113 and 144 with 71 being the base peak. The corresponding hexofuranoside would exhibit many of the same peaks however, the base peak would occur at m/e 58.

The methylated derivatives of higher saccharides may also be analysed by GC-MS to obtain linkage position and configuration information (38). The nomenclature for EI oligosaccharide fragments is denoted by lower case letters to refer to the rings or ring fragments present in the ion as well as an upper case letter and a number to refer to the fragmentation route (39). The first lower case letter denotes the ring from which the fragment arises and the second, the intact ring. For example, abE_1 would refer to an ion that arises from the fragmentation of the a ring by the E_1 pathway with the b ring remaining intact.

The A- series of fragments can be used to deduce the molecular weight of the sugar residues while the B- series provides information about the linkage between sugar residues (38). The (1→4) linked disaccharides give baB_1, bB_3 and baF_1^2 fragments; (1→2) linked disaccharides provide baB_1 and baF_1^2 fragments while the (1→6) linked disaccharides give D-fragments (Fig. 4.4).


1→2
CH2OR
O
RO
OR
OR
CH2OR
O
O
RO
OR
CR
RO
OR
OR
CH2CR
O
O
OR
CR
OR
baB1
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OR

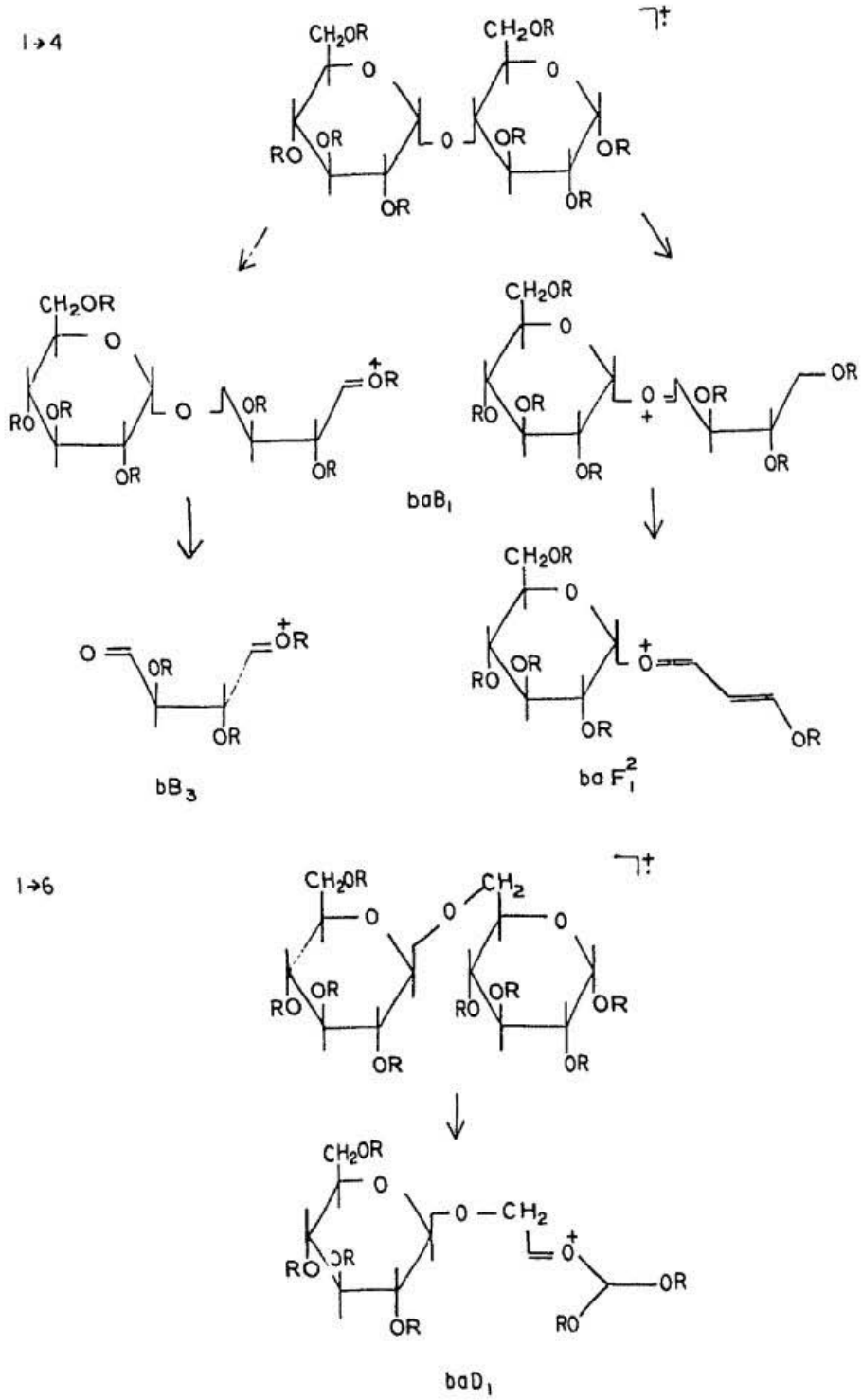


Figure 4.4. Mass Spectral Fragmentation Pathways of Methylated Disaccharides (38).

4.1.2. Trimethylsilyl Ether Derivatives

Trimethylsilyl derivatives of carbohydrates form fragments by many of the pathways seen for the methylated derivatives (section 4.1.1) (37). In addition, TMS derivatives readily undergo rearrangements and these extra peaks make structural identification more difficult. Molecular ions are often absent, however the molecular ion minus a methyl fragment (M-CH_3), M-CH_3-TMSOH and M-CH_3-2TMSOH ions are usually intense and from these, molecular weights may be deduced (40).

Several rearrangement ions are noted for TMS ethers of hexoses. The J_1 fragment at m/e 191 arises from rearrangement from a C-3 to a C-1 configuration analogous to that of methyl ethers (40). A fragment at m/e 147 also arises from rearrangement and can be attributed to the TMSi-O-$Si^+(CH_3)_2$ structure. Higher molecular weight fragments at m/e 379, 279 and 265 also arise from rearrangement reactions and have been identified as the structures shown in Fig. 4.5.

The mass spectra of trimethylsilyl ethers of monosaccharides enable differentiation between pyranose and furanose ring structures. In methyl ethers unique fragmentation pathways enable this distinction, however, with TMS derivatives variation of peak intensities must be employed. The relative intensities of m/e 204 and 217 distinguish trimethylsilyl derivatives of monosaccharide pyranose and furanoses (32). The m/e 204 ion results from fragmentation via the H pathway; it contains a two carbon chain with either C-2 to C-3 or C-3 to C-4. The fragment at m/e 217 is the F fragment containing a three carbon chain with C-2 to C-4 intact. The pyranose ring structure exhibits a very large m/e 204 fragment and a smaller m/e 217. With the furanose isomer, a very large m/e 217 is observed and m/e 204 is almost absent.

As with methyl ethers, fragmentation of disaccharide trimethysilyl ethers can provide structural information. Fragmentation of the a ring provides molecular weight information while fragments of the b ring can be used to deduce linkage configuration. Disaccharides with

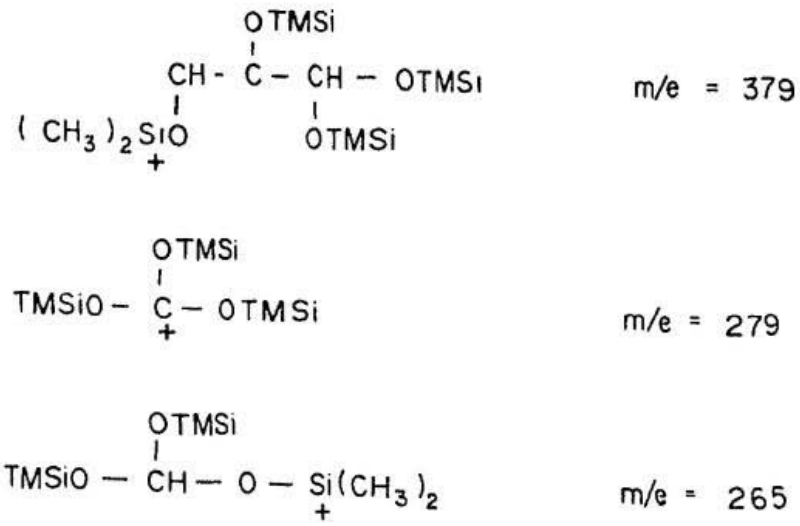
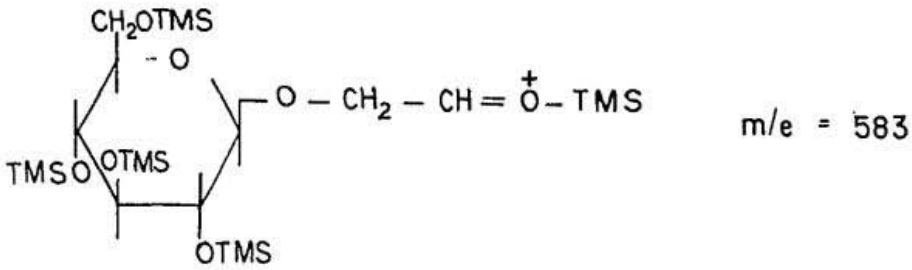
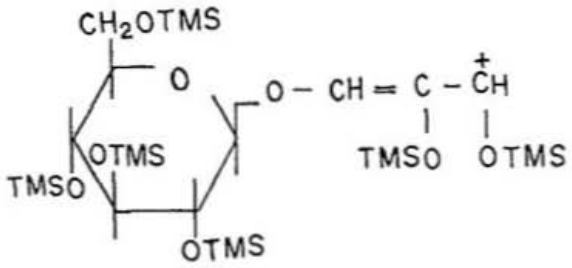


Figure 4.5. Rearrangement Fragments from TMS Derivatives of Monosaccharides (40).

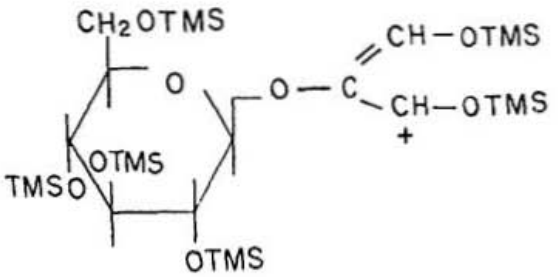
a (1→6) linkage provide an abundant fragment at m/e 583 that distinguishes this from (1→2), (1→3) and (1→4) linkage configurations. This fragment shown below contains an intact a ring and the C-5 to C-6 portion of the b ring (41).



The (1→2), (1→3) and (1→4) linked disaccharides give a B_2 fragment of m/e 683 with structures as illustrated below.



m/e = 683 from 1→2 and 1→4



m/e = 683 from 1→3

Additional peaks at m/e 668 and 578 are present in (1→2) and (1→4) linked species differentiating these from (1→3) compounds. The intensity of fragment ions enable differentiation of (1→2) from (1→4) linked oligomers.

4.2. Mass Spectra of Carbohydrate Pyrolysis Products

4.2.1. Desorption Ammonia Chemical Ionization Mass Spectrometry

To confirm the presence of anhydro oligomeric species in the products of polysaccharide pyrolysis, several pyrolysate oils were analysed by desorption ammonia chemical ionization mass spectrometry. In addition, the methylated derivatives of some pyrolysate oils were analysed under similar conditions.

The mass spectra of an underivatized cellulose pyrolysate from the Pyroprobe platinum coil system (Fig. 4.6) indicated the presence of ions with molecular weights of 100 to 504 amu. The major ions present correspond to a series of anhydro oligomers with quasimolecular ions of 162n + 18, where n is the number of monosaccharide units in the oligomer. In the cellu-

pyrolysate anhydro monomer, dimer and trimer products were detected (MNH_4^+ = 180, 342, and 504). The small molecular weight ions are glucose decomposition products present in the pyrolysate oil, the major ones being 1,4-dideoxy-D-glycero-hex-1-enopyranose-3-ulose (X) (MNH_4^+ = 162) and levoglucosenone (V) or 5-hydroxymethyl-2-furaldehyde (IV), both of which have a molecular weight of 126 and could therefore be responsible for the quasimolecular ion at m/e 144. The ion at 18 mass units higher than levoglucosan *i.e.* m/e 198, can be explained by the presence of glucose. The additional ions are most likely ring fragmentation products with an intact hexose unit that contains a portion of the neighbouring unit. These results are similar to these obtained by Pouwels for off-line Curie point pyrolysis of cellulose (16).

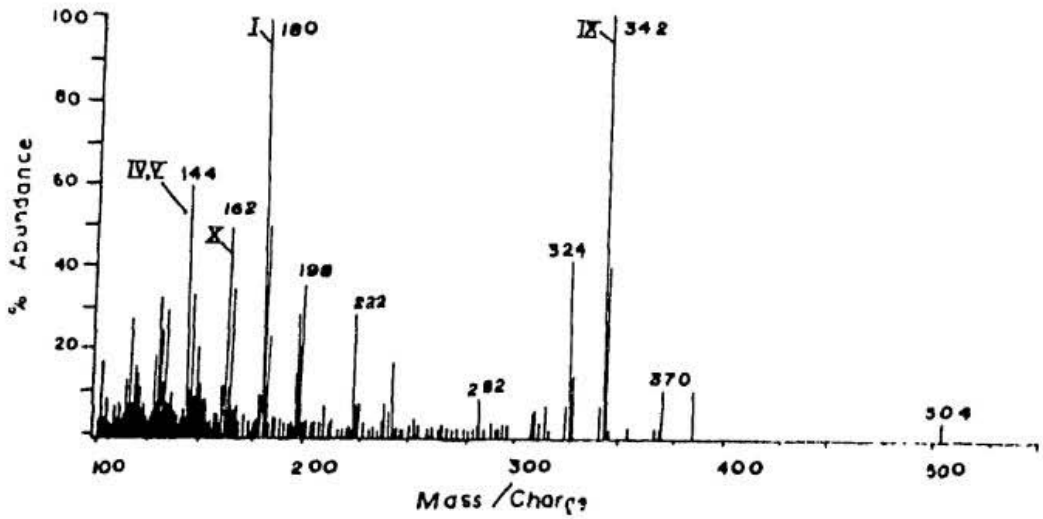


Figure 4.6. Desorption Ammonia Chemical Ionization Mass Spectra of Cellulose Pyrolysate.

The DCI mass spectra of the methylated cellulose pyrolysate (Fig. 4.7) showed the corresponding anhydro oligomers with the dimer at m/e 426, trimer at m/e 630 and tetramer at m/e 835. Major differences between the underivatized and methylated pyrolysate occur in the low mass range as the anhydro monomer peak is very small in the spectrum of the methylated sample but is the major peak in the spectrum of the underivatized material.

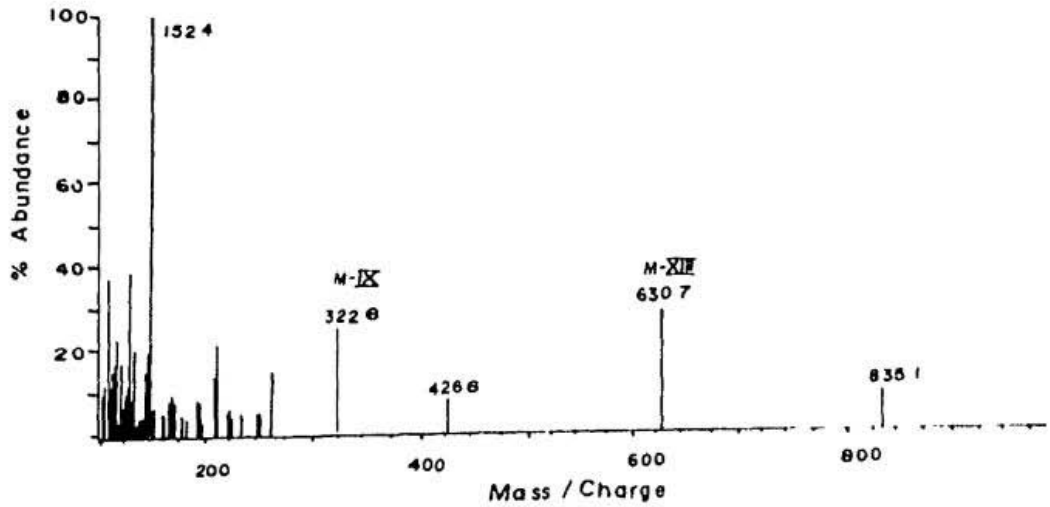


Figure 4.7. Desorption Ammonia Chemical Ionization Mass Spectra of Methylated Cellulose Pyrolysate.

Similar results are obtained from the analysis of pyrolysate oil from amylose. The underivatized product indicated three major ions at m/e 180, 342 and 504 corresponding to the anhydro series of monomer to trimer. For the methylated sample quasi- molecular ions indicating anhydro monomer to tetramer are indicated (m/e = 222, 426, 630 and 834). In the amylose pyrolysate, a large anhydro monomeric peak was observed in the derivatized pyro-

lysate, differentiating this from the cellulose. However, more fragmentation ions are indicated in the spectrum of the methylated derivative in comparison to that of the underivatized analyte.

4.2.2. Gas Chromatography - Mass Spectrometry of Methylated Derivatives of Carbohydrate Pyrolysates

The methylated derivative of cellulose pyrolysate was analysed by gas chromatography-mass spectrometry in the electron impact ionization mode. The total ion chromatogram (Figure 4.8) indicates a large unresolved background resulting from bleed from the analytical column. The major peaks have been identified as 1,6-anhydro-2,3,4-tri-O-methyl-β-D-glucopyranose (M-I) and 4-O-2,3,4,6-tetra-O-methyl-β-D-glucopyranosyl-1,6-anhydro-2,3-di-O-methyl-β-D-glucopyranose (M-IX).

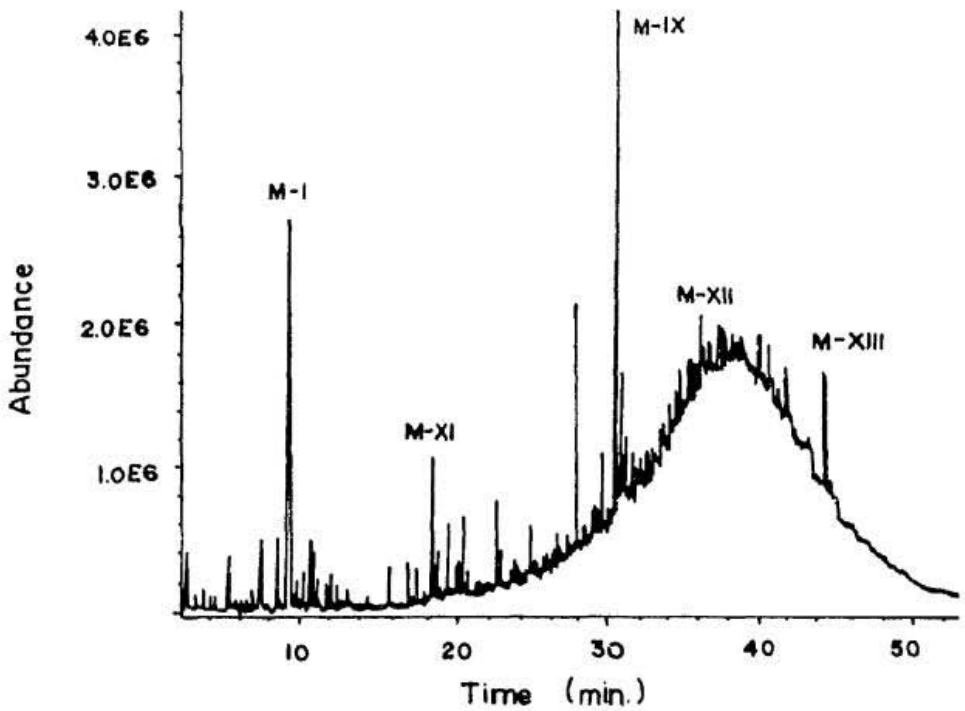


Figure 4.8. Total Ion Chromatogram of Methylated Cellulose Pyrolysate in Electron Impact Mode.

The electron impact fragmentation pattern of the anhydro-glucose derivative (Figure 4.9) is comparable to that quoted in literature (37). The fragmentation pathways are indicated in Figure 4.10. The loss of formate ion (HCOO) results in a fragment at m/e 159 (C_1), subsequent loss of methanol leads to the C_2 fragment at m/e 127. Breakdown of the hemiacetal ring function leads to the ion at m/e 143. The fragments at m/e 101 and 88 are common to many carbohydrate functionalities.

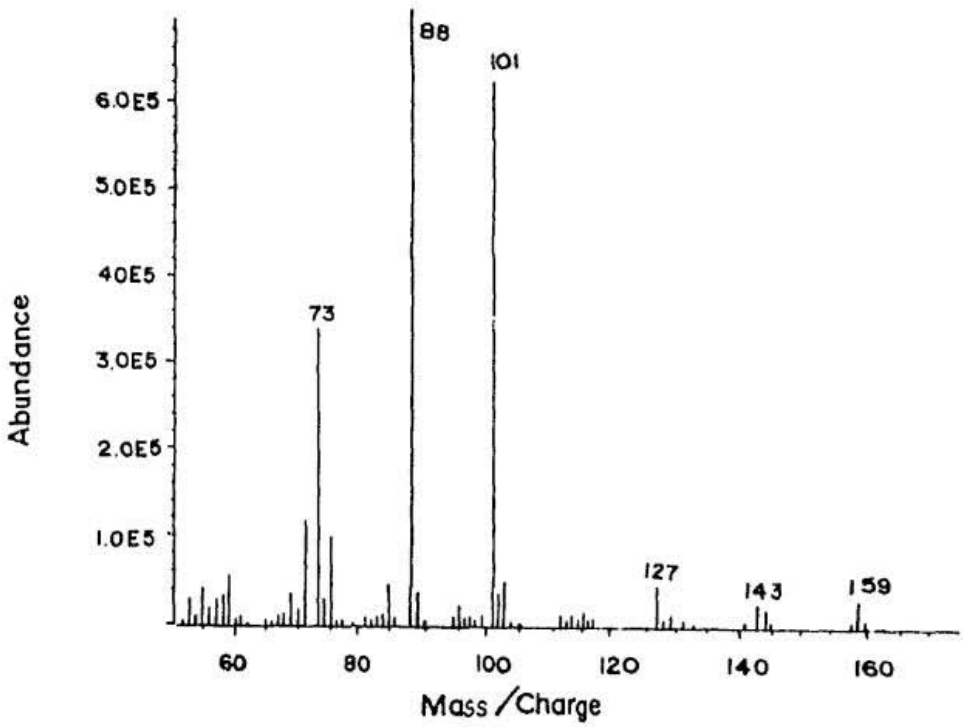


Figure 4.9. The Electron Impact Mass Spectra of Methylated Levoglucosan (M-I).

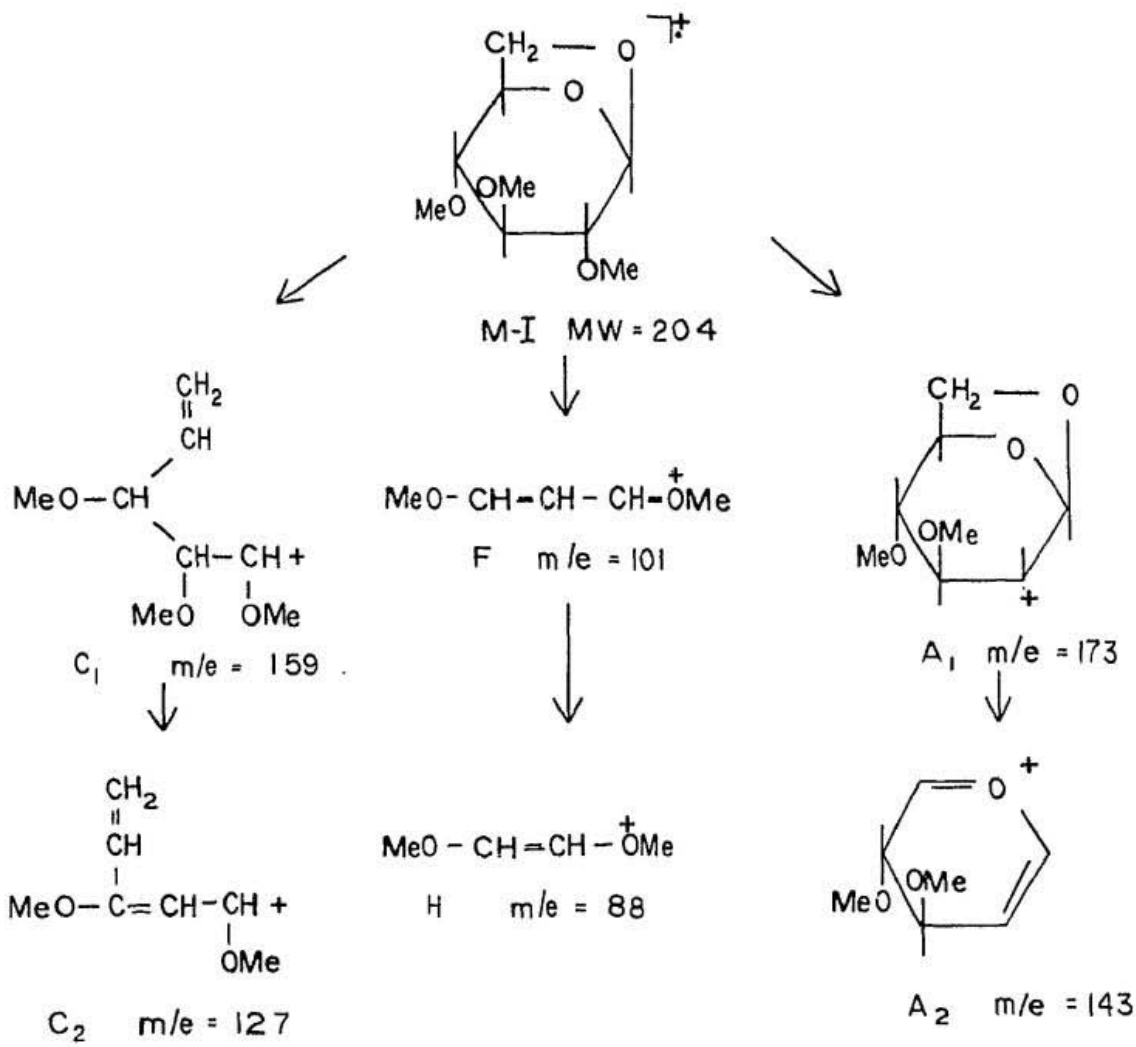


Figure 4.10. The Fragmentation Pathways of 1,6-anhydro-2,3,4-tri-O-methyl-β-D-glucopyranose (M-I).

The anhydro dimer has a fragmentation pathway similar to that of the monomer with additional ions due to ring fragments attached to one of the intact monomeric units. The electron impact mass spectra of 4-O-2,3,4,6-tetra-O-methyl-β-D-glucopyranosyl 1,6-anhydro-2,3-

di-O-methyl-β-D-glucopyranose is represented in Figure 4.11 and the fragmentation pathways of this derivative is illustrated in Figure 4.12. The fragment at m/e 305 is comprised of the glucose unit with a partial anhydro glucose unit combined, loss of methanol from this fragment results in an ion at m/e 223. The ion at 233 arises from an intact anhydroglucose unit with a glucose ring fragment attached. Breakdown of the glucose ring gives the fragment at m/e 187. The additional ions at m/e 88, 127 and 173 are the same as those described for the anhydro monomer (see Figure 4.10).

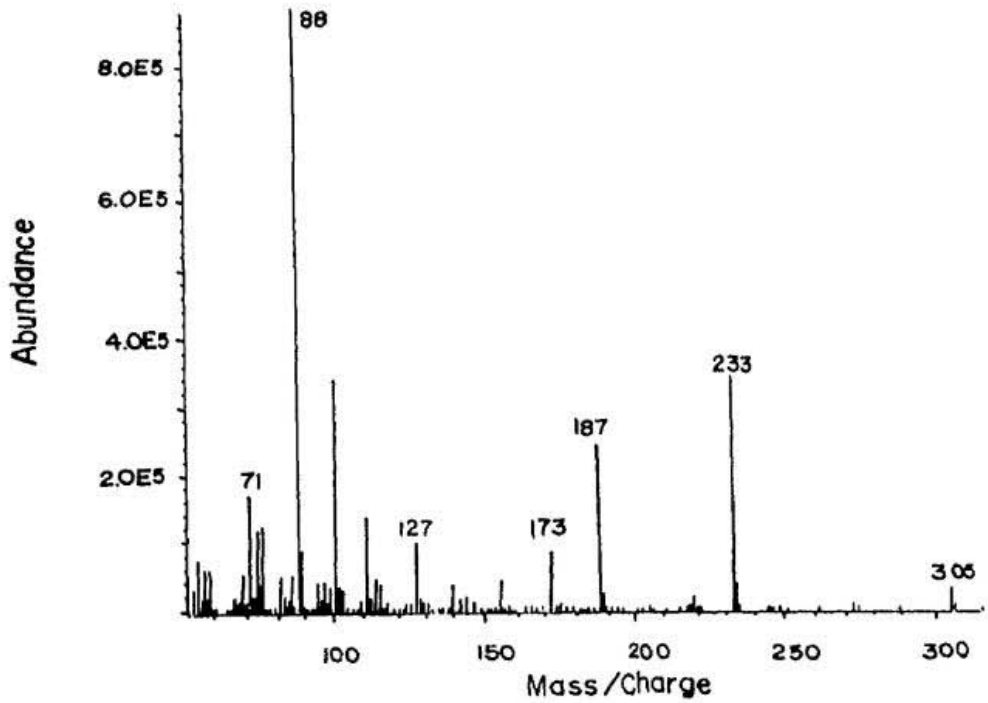


Figure 4.11. Electron Impact Mass Spectra of 4-O-2,3,4,6-tetra-O-methyl-β-D-glucopyranosyl 1,6-anhydro-2,3-di-O-methyl-β-D-glucopyranose (M-IX).

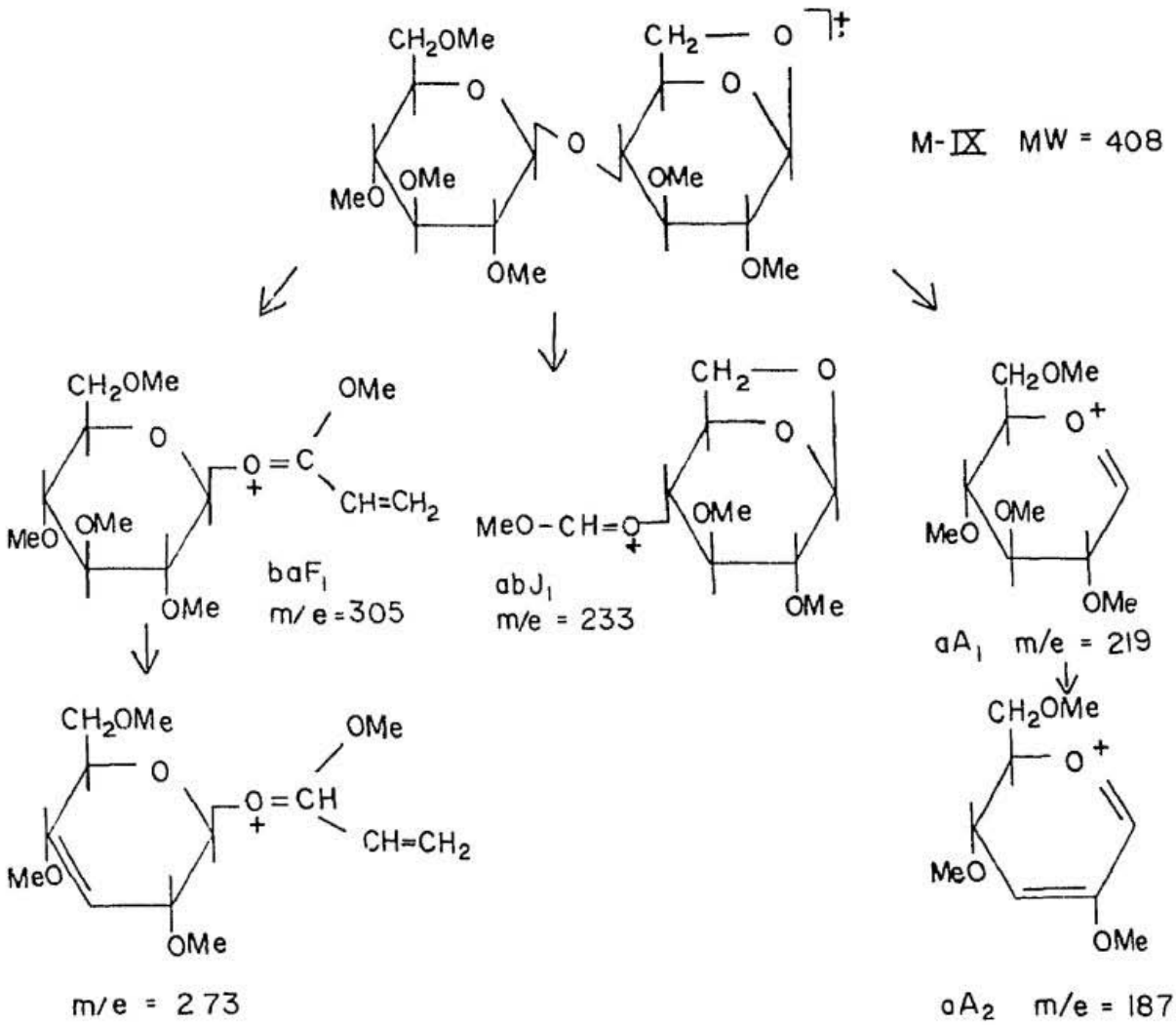


Figure 4.12. Fragmentation Pathways of 4-O-2,3,4,6-tetra-O-methyl-β-D-glucopyranosyl 1,6-anhydro-2,3-di-O-methyl-β-D-glucopyranose (M-IX).

Glucose is also identified in the pyrolysate (t_r = 10.776). The electron impact mass spectra of methylated glucose is given in Figure 4.13. The fragmentation routes for this derivative have already been described (Figure 4.1). The largest fragment detected, m/e 176, results from the loss of the C-6 substituent from the original molecule. The D_1 fragment, m/e 149 results when the C-2 to C-4 fragment is removed. A fragment containing carbons two to four is represented by the ion at m/e 131. A larger portion containing C-2 to C-6 is responsible for the ion at m/e 159. Loss of methanol from this fragment gives the ion at m/e 127.

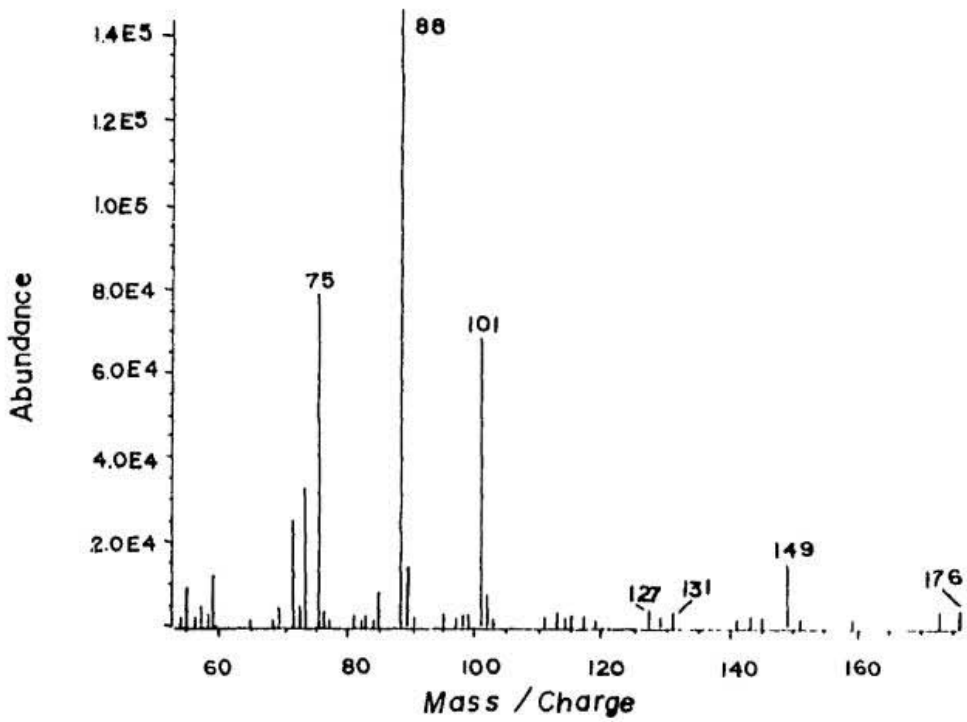


Figure 4.13. The Electron Impact Mass Spectra of Methylated Glucopyranose.

A number of polysaccharide pyrolysates have been found to contain intact monomeric units with additional ring fragments attached at the linkage position (14). The peak with retention time of 18.26 minutes (Figure 4.8), can be attributed to a methylated glucose unit with an acetaldehyde function attached at carbon one (M-XI). The electron impact mass spectra of peak M-XI is shown in Figure 4.14. The molecular weight of the methylated derivative is 278 and loss of methanol provides a fragment at m/e 246 (Figure 4.15). Loss of the C-6 functionality from the parent compound leaves a fragment at m/e 233 (E_1); additional loss of methanol is responsible for the E_2 fragment at m/e 201. Loss of the ring fragment at C-1 provides a small fragment at m/e 187, a fragment common to many glucose derivatives. Fragmentation with loss of the ring oxygen as well as C-5 and C-6 leaves a fragment ion with m/e 204; loss of C-1 from this gives a smaller fragment at m/e 145. Loss of C-1 and the ring oxygen provides a fragment at m/e = 159, the C_2 fragment.

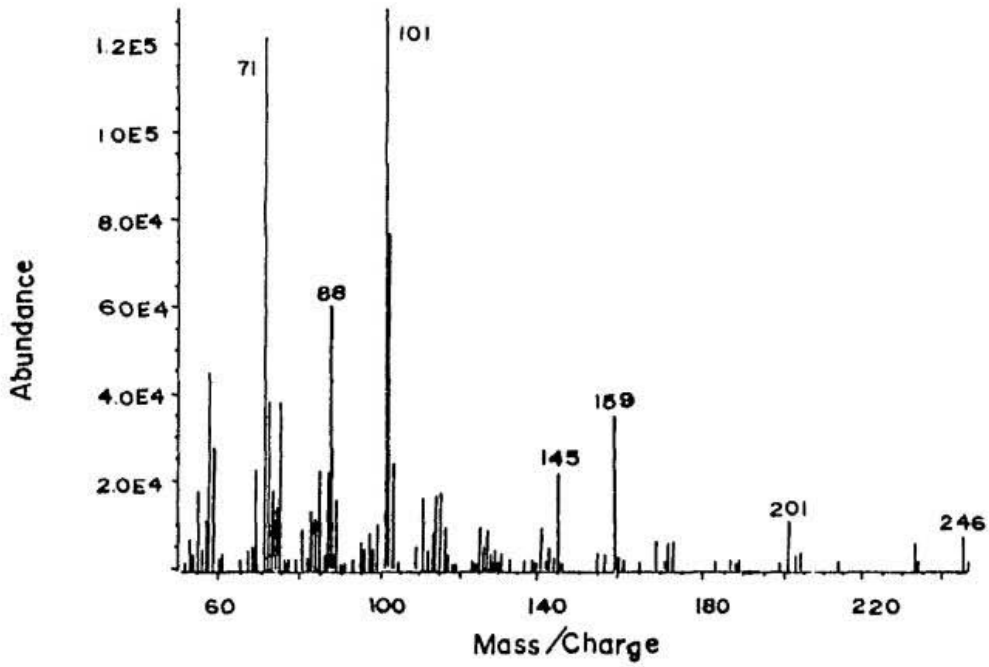


Figure 4.14. The Electron Impact Mass Spectra of Methylated Glucose Acetaldehyde (M-XI).

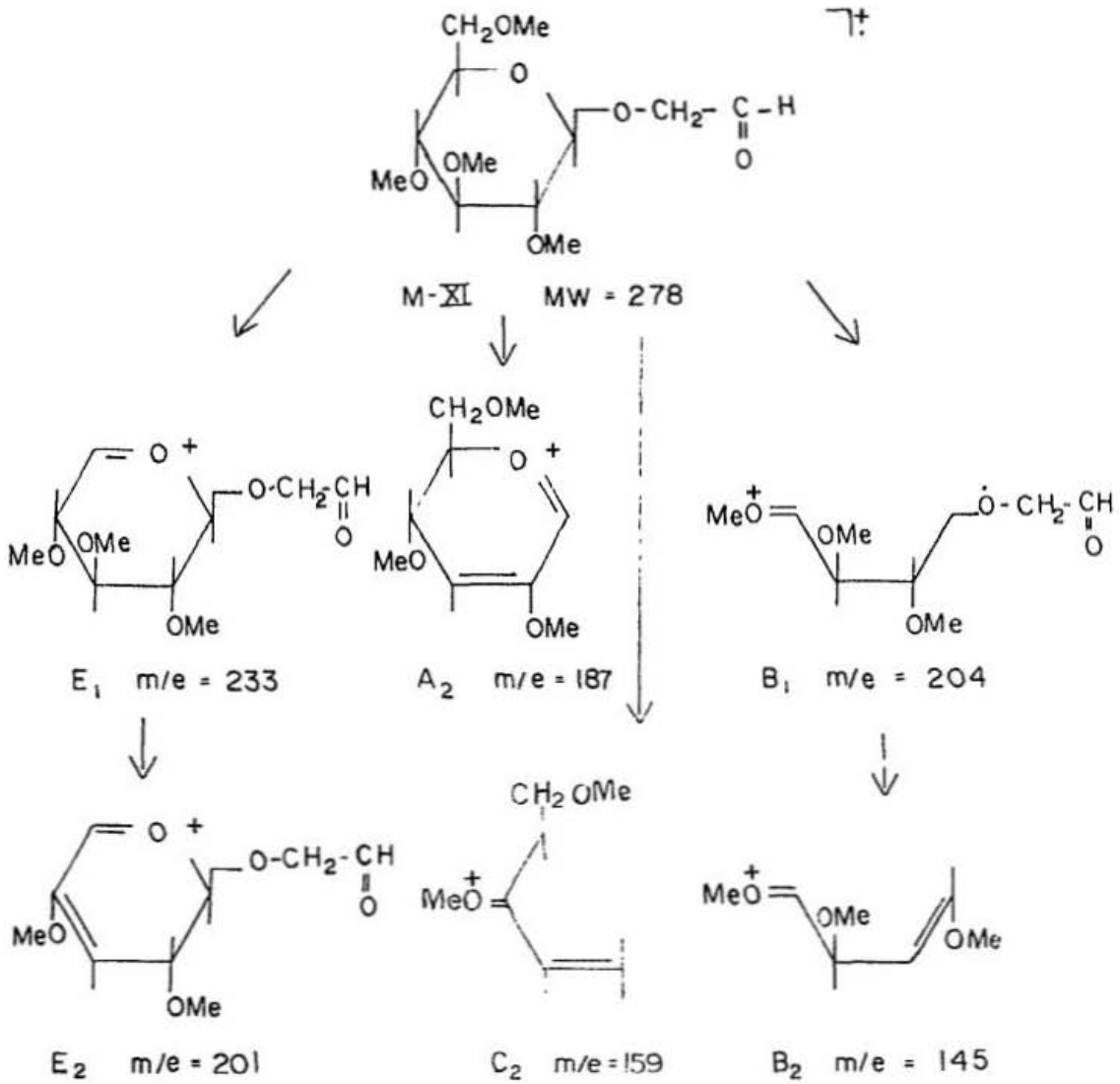


Figure 4.15. Proposed Fragmentation Pathways of Methylated Glucose Acetaldehyde (M-XI).

The gas chromatography peak at 36.11 minutes (Figure 4.8) has been tentatively assigned to a methylated cellobiose unit with an acetaldehyde ring fragment attached (M-XII). The electron impact mass spectra of methylated cellobiose acetaldehyde is given in Figure 4.16. The large fragment at m/e 307 would be the abJ_1 fragment (Figure 4.17). The fragment at m/e 215 can be assigned to the bA_2 fragment. Loss of 74 mass units from the abJ_1 fragment provides a smaller fragment at m/e 233.

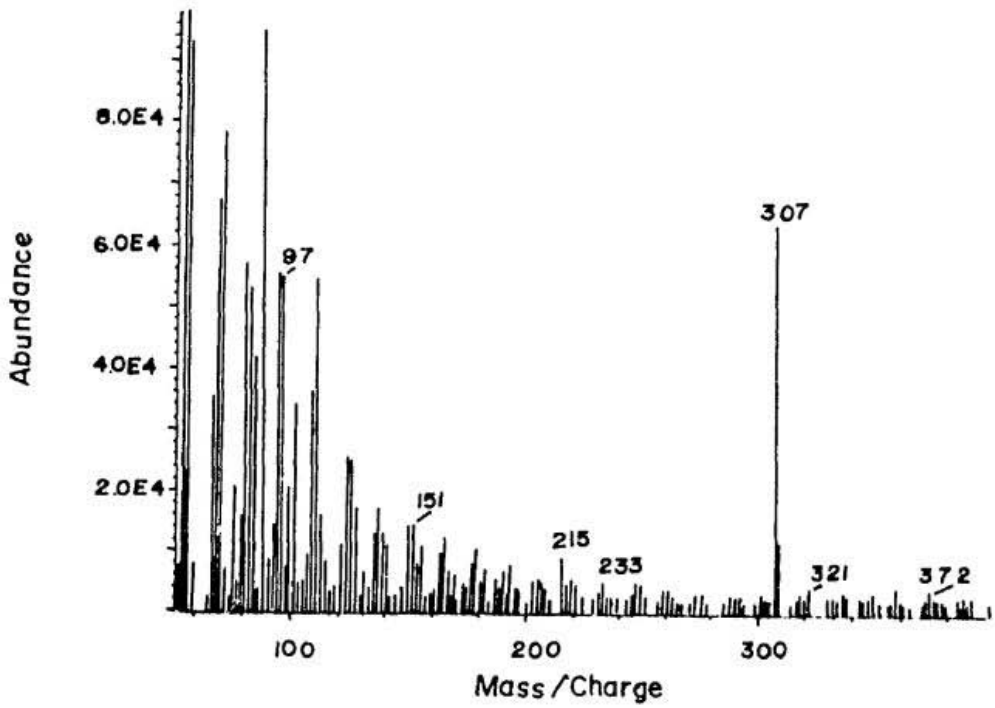


Figure 4.16. The Electron Impact Mass Spectra of Methylated Cellobiose Acetaldehyde (M-XII).

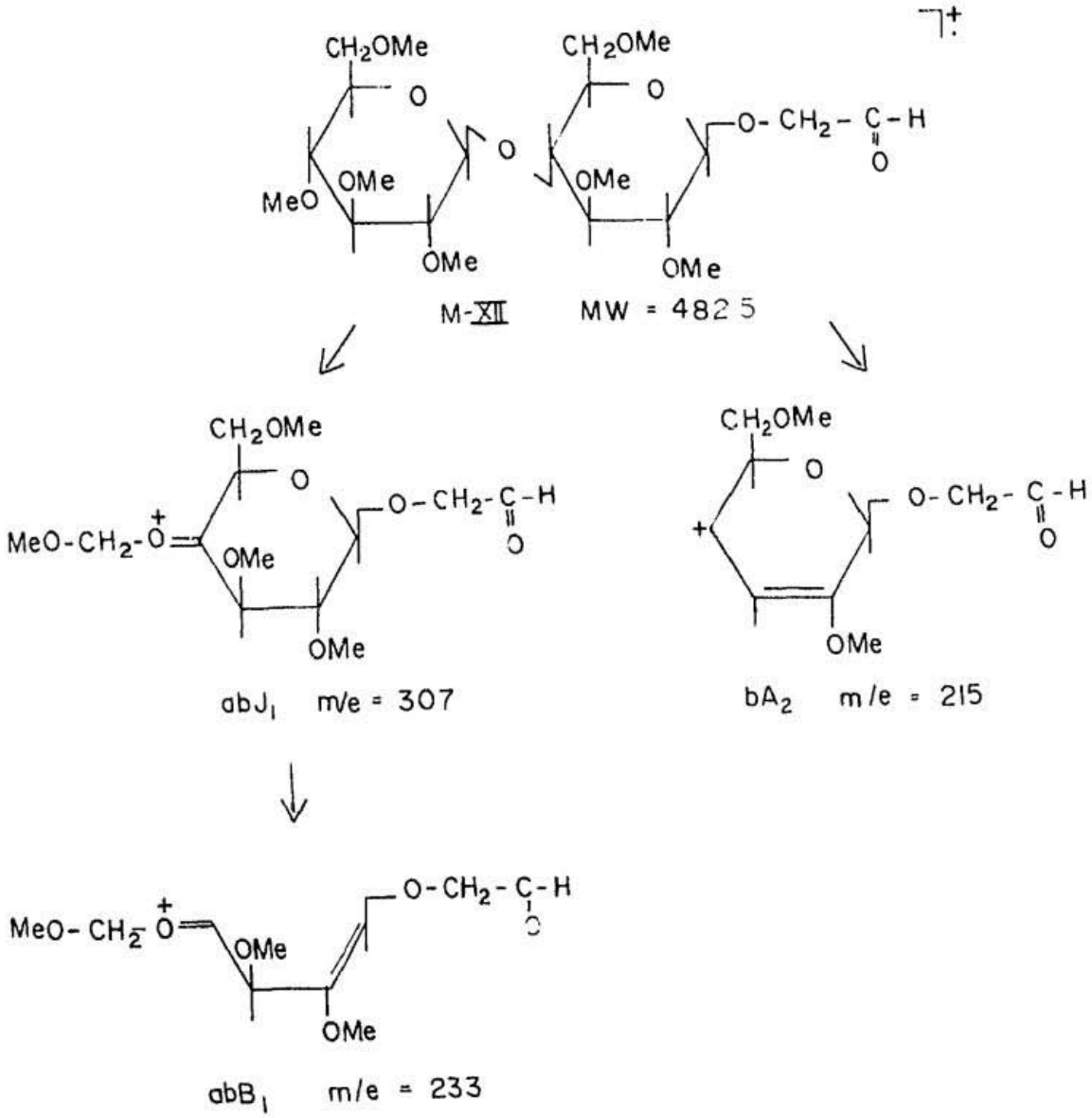


Figure 4.17. The Proposed Fragmentation Pathway of Methylated Cellobiose Acetaldehyde (M-XII).

The mass spectra of the peak with retention time 44.51 minutes (Figure 4.18) is similar to that of the methylated anhydro cellobiose (M-IX) (Fig. 4.11). This peak has been assigned to the anhydro trimer, *i.e.*, 4-O-(2,3,4,6-tetra-O-methyl-β-D-glucopyranosyl)-4-O-(2,3,6-tri-O-methyl-β-D-glucopyranosyl)-1,6-anhydro-2,3-di-O-methyl-β-D-glucopyranose (M-XIII). The largest fragment in the electron impact mass spectrum (Figure 4.18), m/e 377, results from cleavage of the glycosidic linkage between rings a and b. The fragments at m/e = 305 and 233 are the same as those observed for the methylated anhydro dimers. Fragment ions at m/e 187 and 155 result from the A fragmentation routes of methylated glucose units.

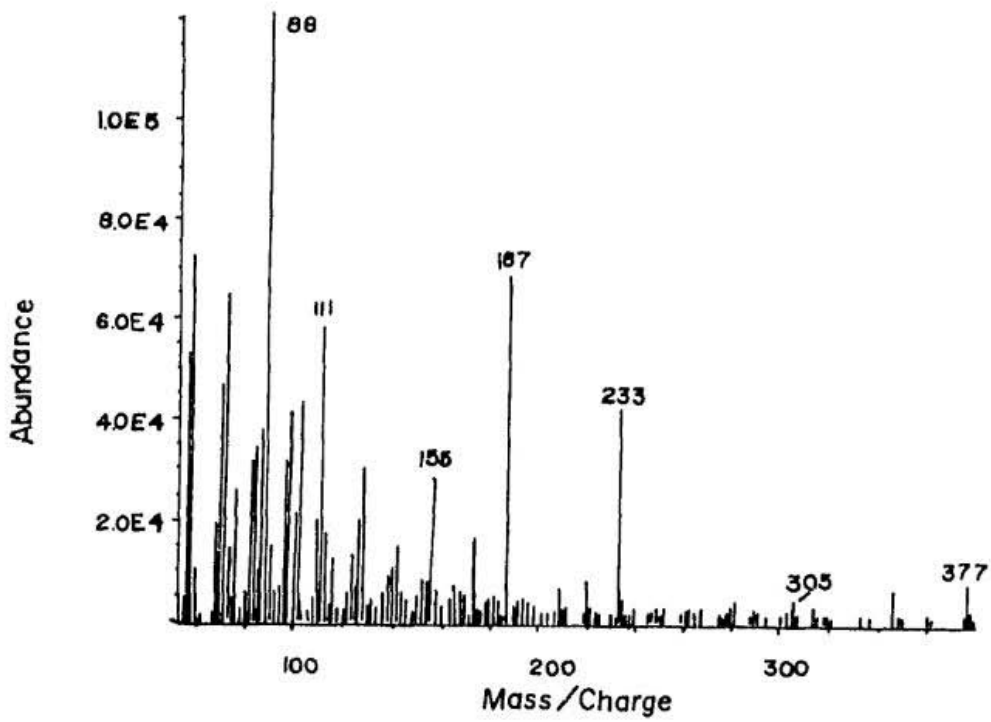


Figure 4.18. The Electron Impact Mass Spectra of Methylated Anhydro Cellotriose (M-XIII).

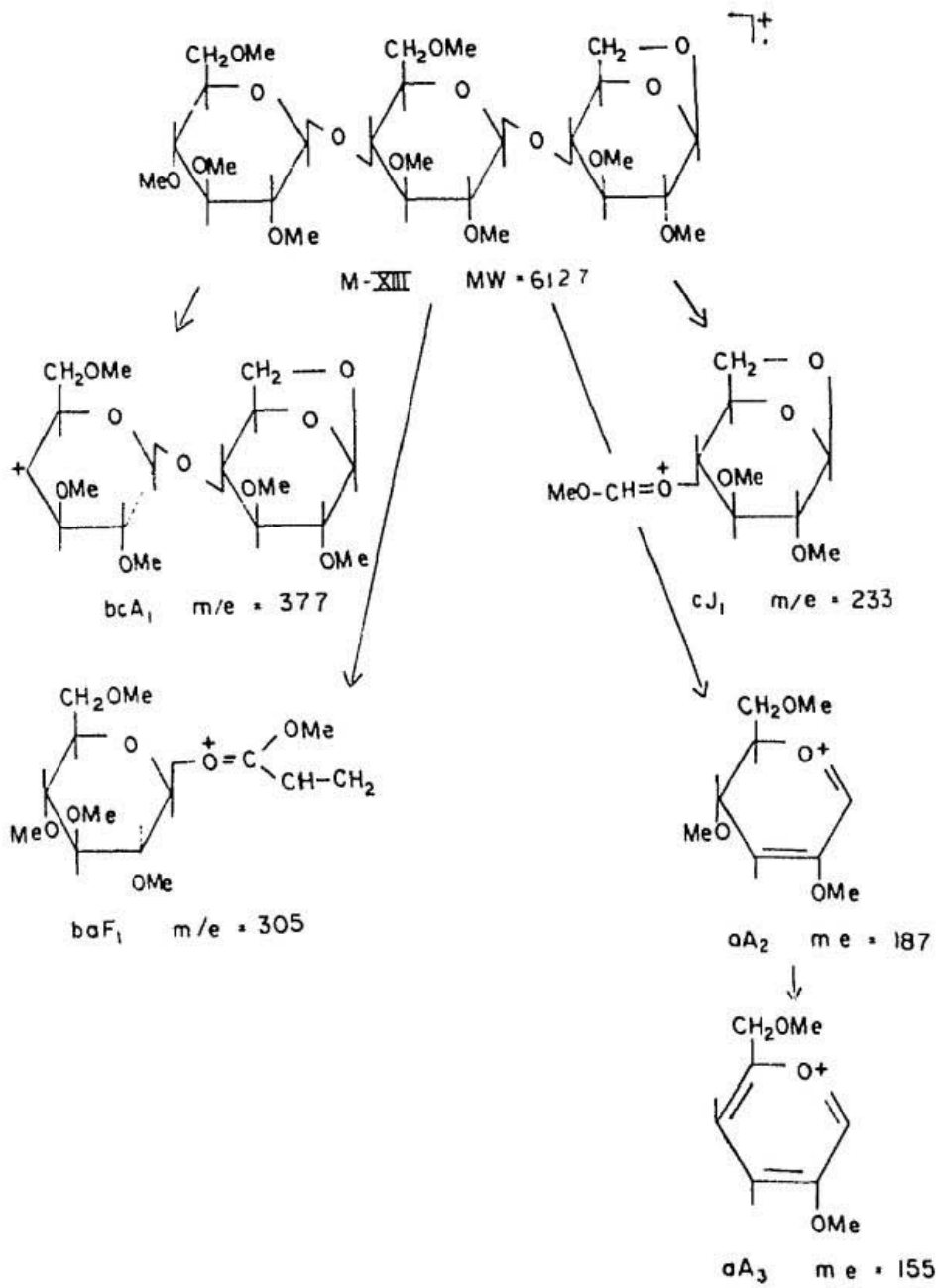


Figure 4.19. Proposed Fragmentation Pathways of Methylated Anhydro Cellotriose (M-XIII).

The methylated derivative of cellulose pyrolysate was also analysed by gas chromatography-mass spectrometry in the ammonia chemical ionization mode. The total ion chromatograph is given in Figure 4.20. The CI-MS enabled determination of the molecular weights of the pyrolysate components. Quasimolecular ions, $(M + NH_4)^+$, of the methylated anhydro saccharides, 1,6-anhydro-2,3,4-tri-O-methyl-β-D-glucopyranose (M-I), 4-O-2,3,4,6-tetra-O-methyl-β-D-glucopyranosyl1,6-anhydro-2,3-di-O-methyl-β-D-glucopyranose (M-IX) and 4-O-(2,3,4,6-tetra-O-methyl-β-D-glucopyranosyl)-4-O-(2,3,6-tri-O-methyl-β-D-glucopyranosyl)-1,6-anhydro-2,3-di-O-methyl-β-D-glucopyranose (M-XIII), are observed with m/e 222, 426 and 631.7, respectively. Methylated glucose is also identified in the methylated pyrolysate by the presence of the quasimolecular ion at m/e 268. The ring fragment product, methylated glucose acetaldehyde (M-XI) is recorded as having odd number molecular weight ion (MH^+) at 279. It is believed that the aldehyde group is preferentially protonated. A representative ammonia chemical ionization mass spectra, namely the mass spectra of 2,3,4-tri-O-methyl-1,6-anhydro-β-D-glucopyranose (M-I) is given in Figure 4.21.

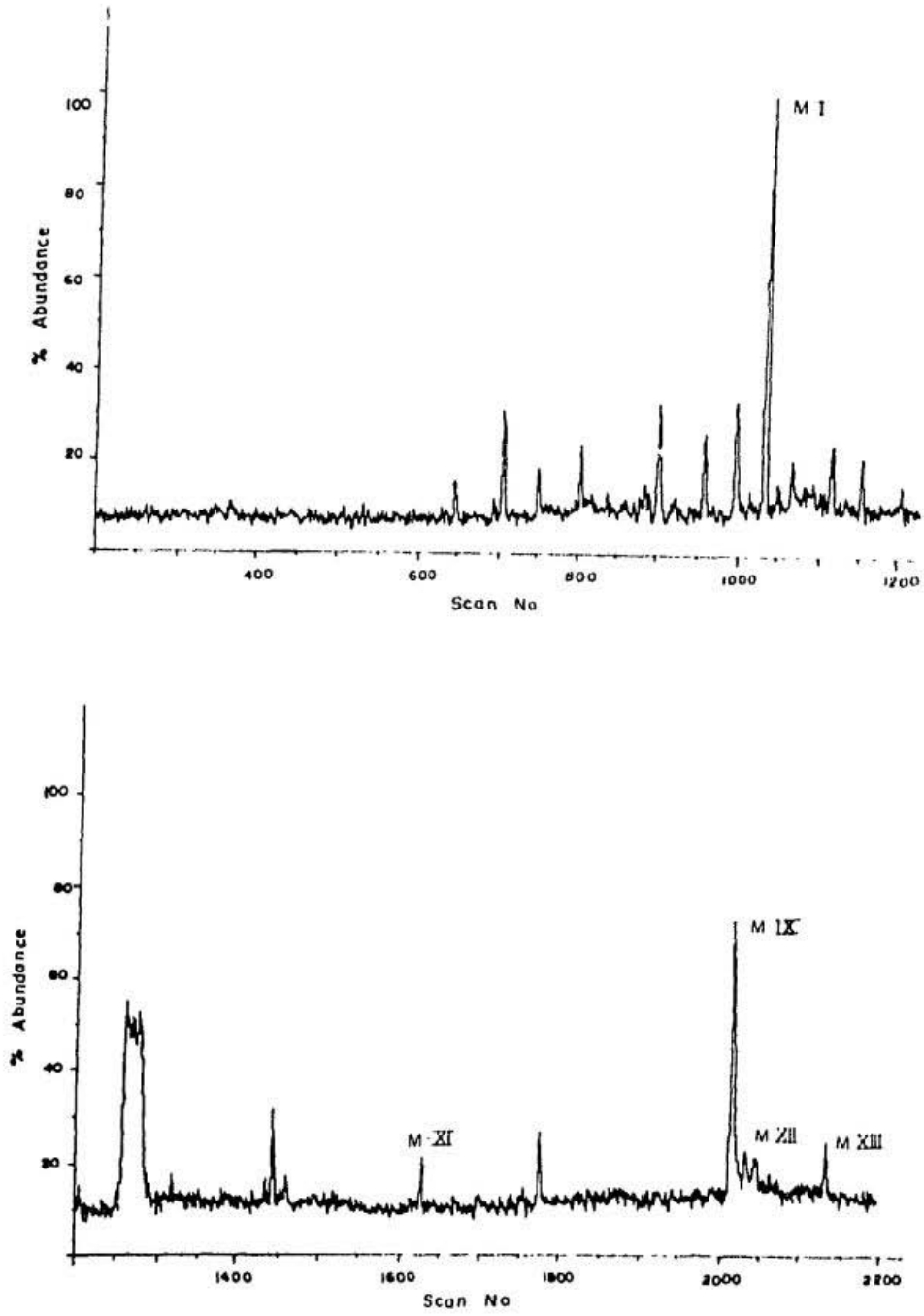


Figure 4.20. The Total Ion Chromatogram of Methylated Cellulose Pyrolysate in Ammonia Chemical Ionization Mode.

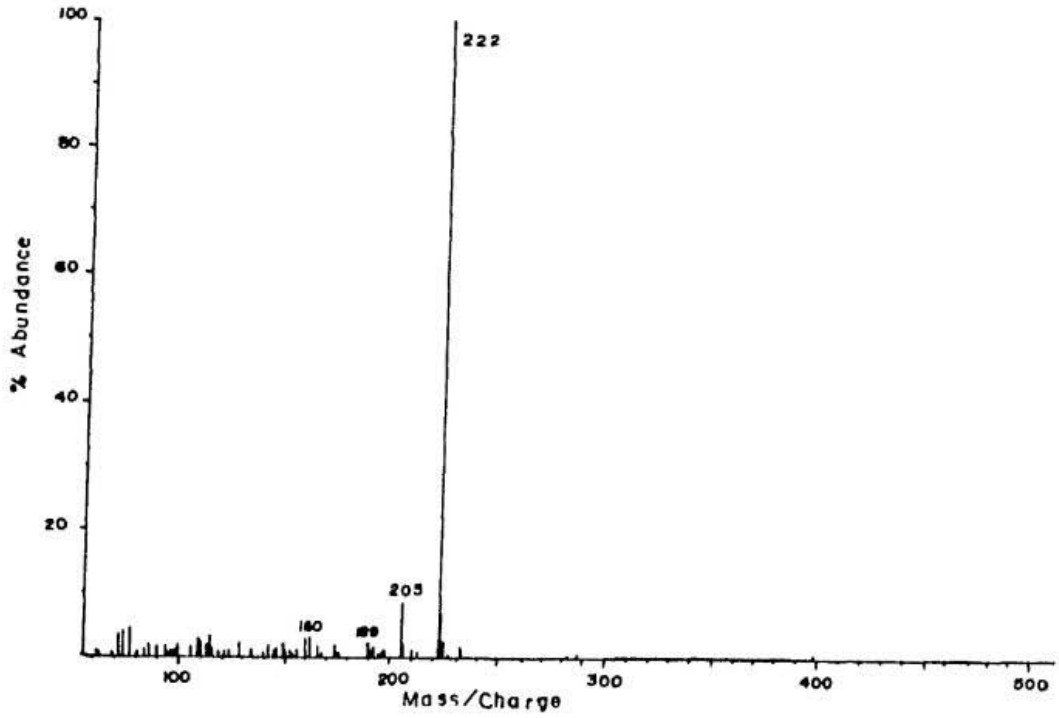


Figure 4.21. Ammonia Chemical Ionization Mass Spectra of 1,6-anhydro-2,3,4-tri-O-methyl-β-D-glucopyranose (M-I).

Similar analysis of amylose pyrolysate yielded major peaks of 1,6-anhydro-β-D-glucopyranose (I) and 4-O-α-D-glucopyranosyl-1,6-anhydro-β-D-glucopyranose (VIII). The glucose ring fragment molecules observed in cellulose pyrolysate were not evident in this pyrolysate product. The electron impact mass spectra of 4-O-(2,3,4,6-tetra-O-methyl-α-D-glucopyranosyl)-1,6-anhydro-2,3-di-O-methyl-β-D-glucopyranose (M-VIII) (Figure 4.22) and 4-O-(2,3,4,6-tetra-O-methyl-β-D-glucopyranosyl)-1,6-anhydro-2,3-di-O-methyl-β-D- glucopyranose (M-IX) (Figure 4.11) are almost identical. The only differences noted are variations in the ratios of abundance of fragment ions at m/e 187 and 233. The mass fragment at m/e 187 results from the aA_2 fragmentation scheme; this ion is more abundant in the α-anomer than in the β-anomer. This fragment is the result of the loss of the C-1 functionality. The axial position of this C-1 function in α-anomers is energetically less favoured and thus promotes elimination.

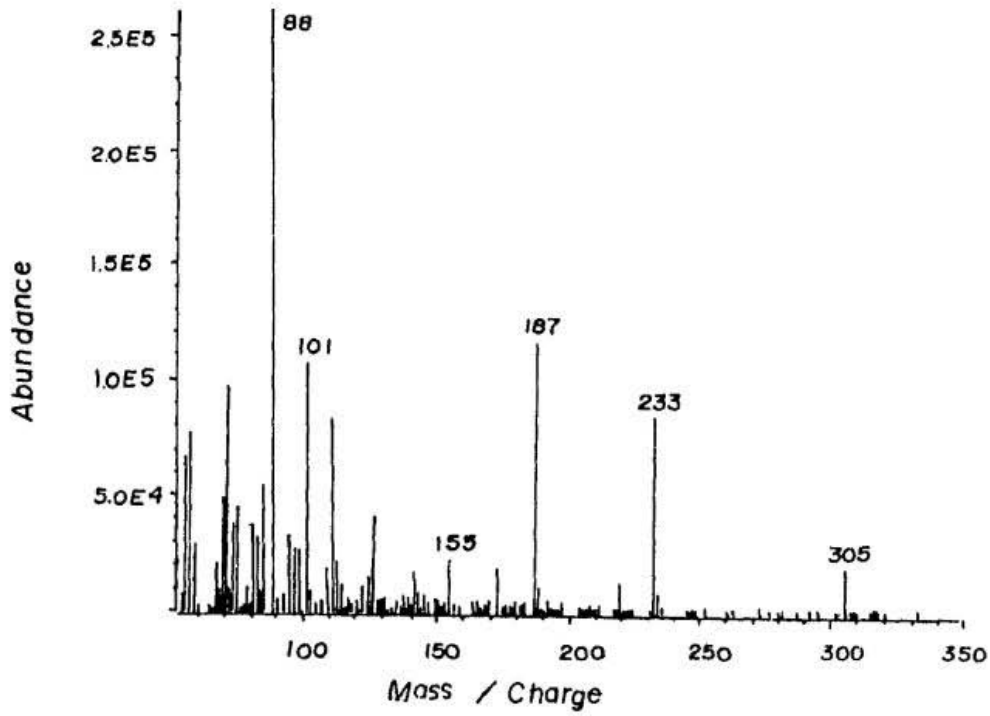


Figure 4.22. The Electron Impact Mass Spectra of 4-O-(2,3,4,6-tetra-O-methyl-α-D-glucopyranosyl)-1,6-anhydro-2,3-di-O-methyl-β-D-glucopyranose (M-VIII).

4.2.3. Gas Chromatography - Chemical Ionization - Mass Spectrometry of Trimethylsilyl Derivatives of Pyrolysates.

The polysaccharides from larch gum, nigeran and locust bean gum were analysed by trimethylsilylation followed by gas chromatography-mass spectrometry in chemical ionization mode. The quasimolecular ions of TMS pyrolysate derivatives were smaller and more fragmentation was evident in comparison to similar analysis of the methylated pyrolysate derivatives. Fragmentation results from the more labile trimethylsilyl groups as well as within the carbohydrate ring structure. Samples of trimethylsilyl derivatives of an anhydro monomer and

dimer, namely 1,6-anhydro-2,3,4-tri-O-trimethylsilyl-β-D-glucopyranose (T-I) and 4-O-(2,3,4,6-tetra-O-trimethylsilyl-α-D-glucopyranosyl)-1,6-anhydro-2,3-di-O-trimethylsilyl-β-D-glucopyranose (T-VIII) were prepared and used as standards. The anhydro monomer (T-I) was observed to have a quasimolecular ion $(MNH_4)^+$ at m/e 396. The protonated adduct $(MH)^+$ is observed at m/e 379. Major fragment ions result from losses of 15, 30, 73 and 90 daltons which correspond to CH_3, CH_2O, $(CH_3)_3Si$ and $(CH_3)_3SiOH$ fragments, respectively.

The chemical ionization mass spectra of the anhydro dimer standard indicates a large quasimolecular ion at m/e 774 (Fig. 4.23). Major fragments present result from the breaking of the glycosidic bond leaving two monomeric units.

Larch gum

The total ion chromatogram from the GC-(CI)MS analysis of the trimethylsilyl derivative of larch gum pyrolysate, a plant arabinogalactan, indicates several peaks eluting in the TMS-monomeric region and one major peak in the region of TMS-dimer elution (see figure 5.26). The first major peak has been identified as 2,3,4-tri-O-trimethylsilyl-1,6-anhydro-β-D-galactopyranose (T-XIV) giving a quasimolecular ion of m/e 396.

Three small peaks were observed to elute after this anhydro monomer. These appear to be isomers of galactose where the quasimolecular ion (MNH_4^+) of the trimethylsilyl derivative was m/e 540. In this mass spectra a large fragment ion occurred at m/e 468 which corresponds to MNH_4^+-$(CH_3)_3SiOH$ of the free sugar. Another peak was observed between the monomeric and dimeric regions and is believed to be an acetaldehyde ring moiety attached to a galactose unit (T-XV). The trimethylsilyl derivative (T-XV) has a molecular weight of 510 giving rise to a quasimolecular ion of m/e 528 in the ammonia chemical ionization mass spectra. The anhydro dimer, 3-O-2,3,4,6-tetra-O-trimethylsilyl-β-D-galactopyranosyl-1,6-anhydro-2,4-di-O-trimethylsilyl-β-D- galactopyranose (T-XVI) gives a quasimolecular ion peak at m/e 774.

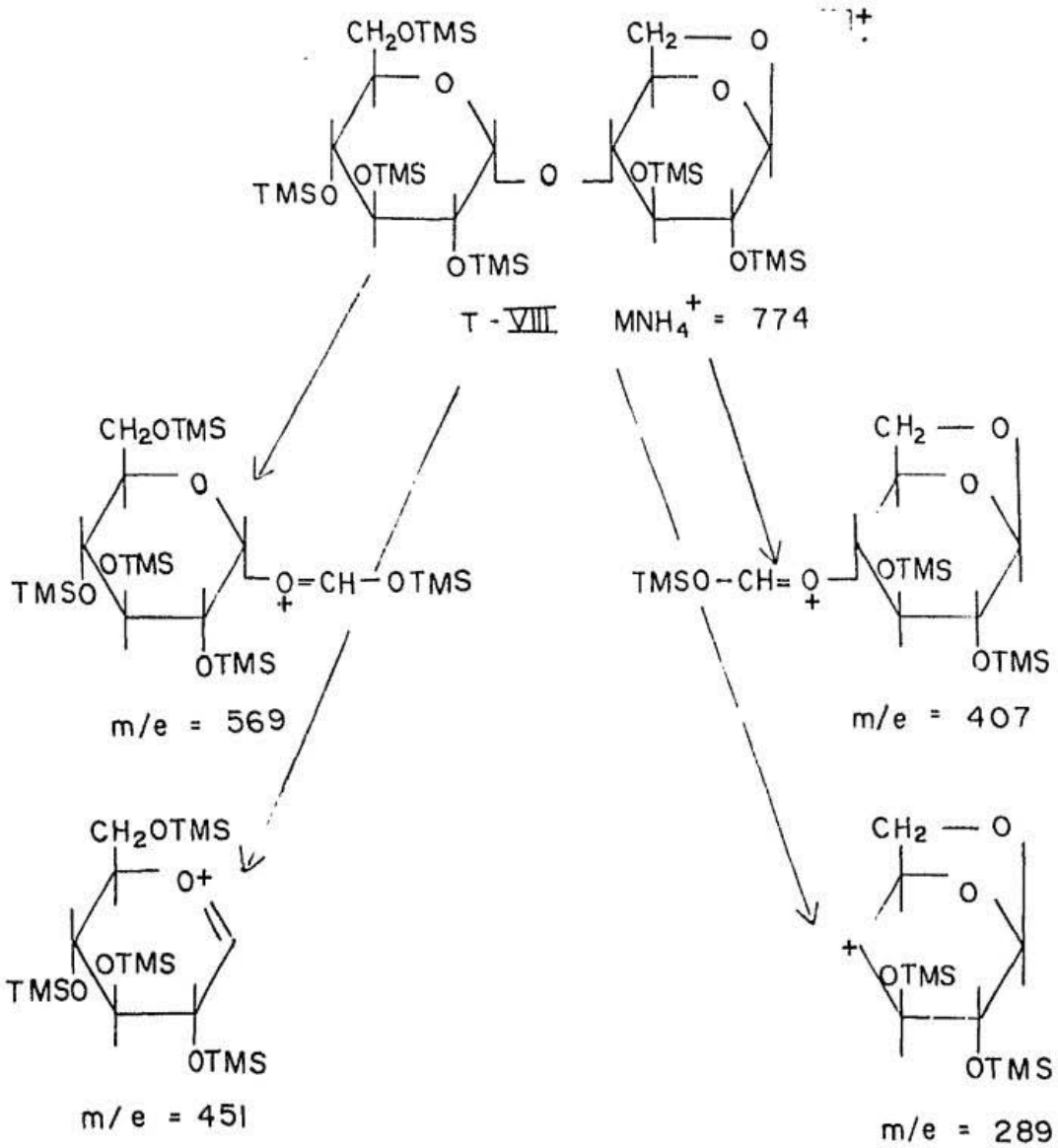


Figure 4.23. Fragment Ions from the Trimethylsilyl Derivative of an Anhydro Disaccharide (T-VIII).

Nigeran

Nigeran, a (1→3), (1→4)-α-D-glucan, resulted in a less complicated chemical ionization total ion chromatogram for the TMS derivative of the pyrolysate with only six major peaks (see figure 5.18). The first peak was found to have a small molecular ion peak (MNH_4^+) at m/e 380 in the chemical ionization mass spectrum. This pyrolysis product is most likely a dehydration product of levoglucosan, namely 1,4-dideoxy-2,4,6-tri-O-trimethylsilyl-α-D-glucos-2-en-pyranose (T-X), a structure with a molecular weight of 362.

The next peak was found to be the anhydro monomer, 1,6-anhydro-2,3,4-tri-O-trimethylsilyl-β-D-glucopyranose (T-I) indicated by the quasimolecular ion at m/e 396. Two additional monomers were observed, namely, glucose isomers. The last two peaks are the TMS derivatives of anhydro glucan dimers, one with a (1→3) and the other a (1→4) glycosidic linkage. The major differences in the chemical ionization mass spectra are due to variation in ion intensities, where the (1→3) linked dimer has a smaller quasimolecular ion and more extensive fragmentation while the (1→4) linked dimer spectrum is dominated by the molecular ion and the monomeric fragments.

Locust Bean Gum

The plant galactomannan, locust bean gum, gave several monosaccharide and one disaccharide peak in the chemical ionization TIC of the trimethylsilyl derivative of the pyrolysate product (see figure 5.24). The three TMS anhydrohexose units gave quasimolecular ions at m/e 396 in their mass spectrum. From comparison with retention times of trimethylsilyl derivatives of anhydro sugar standards, these products were identified as 1,6-anhydro-2,3,4-tri-O-trimethylsilyl-β-D-galactopyranose (T-XIV), 1,6-anhydro-2,3,4-tri-O-trimethylsilyl-β-D-galactofuranose (T-XVII) and 1,6-anhydro-2,3,4-tri-O-trimethylsilyl-β-D-mannopyranose (T-XVIII). The only dimer observed is produced from the pyrolysis of the mannose backbone.

This dimer, 4-O-2,3,4,6-tetra-O-trimethylsilyl-β-D-mannopyranosyl-1,6-anhydro-2,3-di-O-trimethylsilyl-β-D- mannopyranose (T-XIX) gives the quasimolecular ion at m/e 774.

5. Structural Analysis

For rapid structural analysis of polysaccharides the trimethylsilyl derivatives of the off-line pyrolysis products were analysed on a high resolution thirty meter DB-5 GC column with high temperature programming. This approach provided unique retention times for each anhydro-monosaccharide and disaccharide isomer encountered in this study. Identification of many of the chromatographic peaks was provided by comparison of retention times of trimethysilyl derivatives of anhydro sugar standards and from mass spectral data (Section 4.2).

Amylose

Amylose is a plant polysaccharide composed of a linear chain of α-D-(1→4) linked glucan units. The average degree of polymerization of an amylose molecule is 200-350 units (42). This polysaccharide is the principal food reserve for the plant kingdom (1). The gas chromatogram of the trimethylsilyl derivative of amylose pyrolysate (Figure 5.1) results in two major peaks with retention times corresponding to 1,6-anhydro-2,3,4-tri-O-trimethylsilyl-β-D-glucopyranose (T-I) and 4-O-(2,3,4,6-tetra-O-trimethylsilyl-α-D-glucopyranosyl)-1,6-anhydro-2,3-di-O-trimethylsilyl-β-D-glucopyranose (T-VIII). The large peaks at the front are due to remaining silylating reagent, extraction with hexane results in a cleaner chromatogram with no loss of product. The small peak before the anhydro monomer is due to an anhydro glucopyranose dehydration product, namely 5-hydroxymethyl-2-furaldehyde (T-IV) (Fig. 5.2). Identification of peaks was performed through comparison with trimethylsilyl derivatives of standards as well as mass spectral analysis of both underivatized and methylated pyrolysate oils (see section 4.2.2).

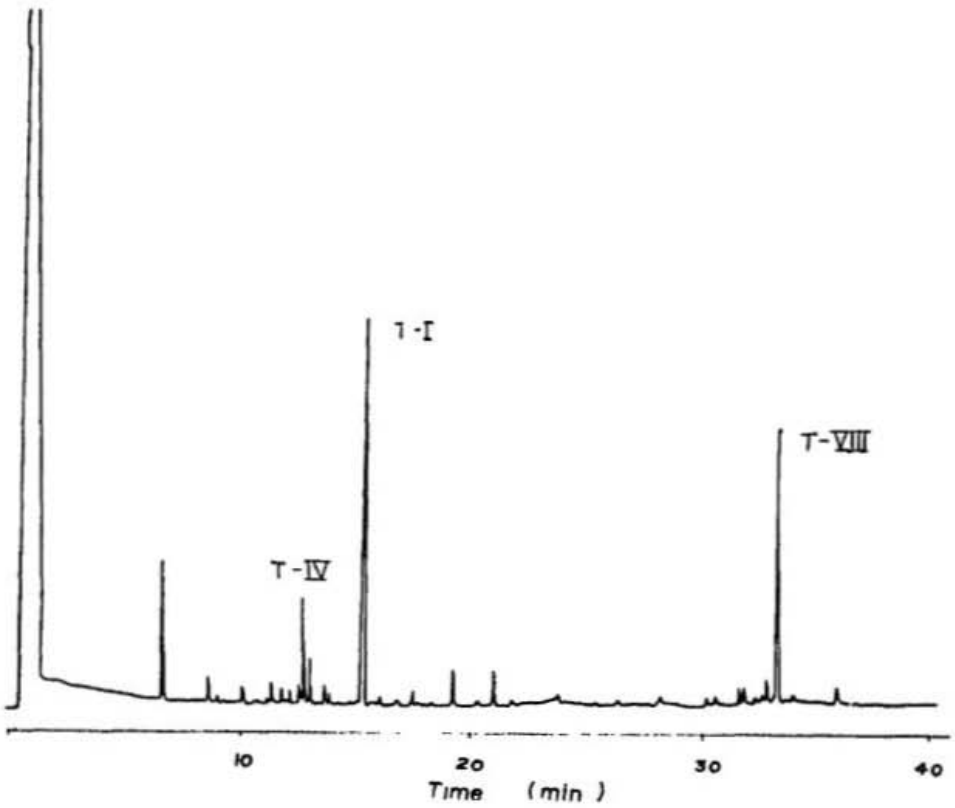


Figure 5.1. Gas Chromatogram of the Trimethylsilyl Derivative of Amylose Pyrolysate.

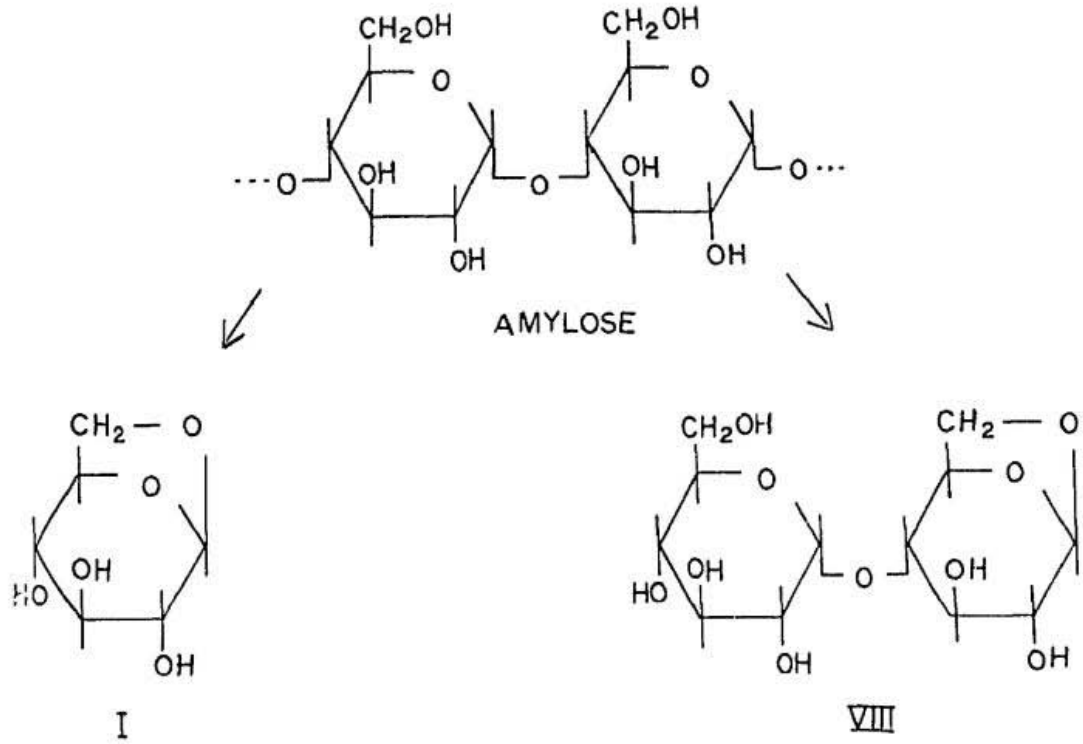


Figure 5.2. Structures of Amylose and its Major Pyrolysis Products.

β-cyclodextrin

β-Cyclodextrin or cycloheptoamylose (Figure 5.3) results in a pyrogram almost identical to that of amylose. The only differences are slight variations in the relative amounts of 1,6-anhydro-2,3,4-tri-O-trimethylsilyl-β-D-glucopyranose (T-I) and 4-O-(2,3,4,6-tetra-O-trimethylsilyl-β-D-glucopyranosyl)-1,6-anhydro-2,3-di-O-trimethylsilyl-β-D-glucopyranose (T-VIII) (Figure 5.4). The monomer to dimer ratio is one for amylose and 1.5 for β-cyclodextrin.

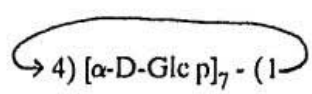


Figure 5.3. Structure of β-cyclodextrin.

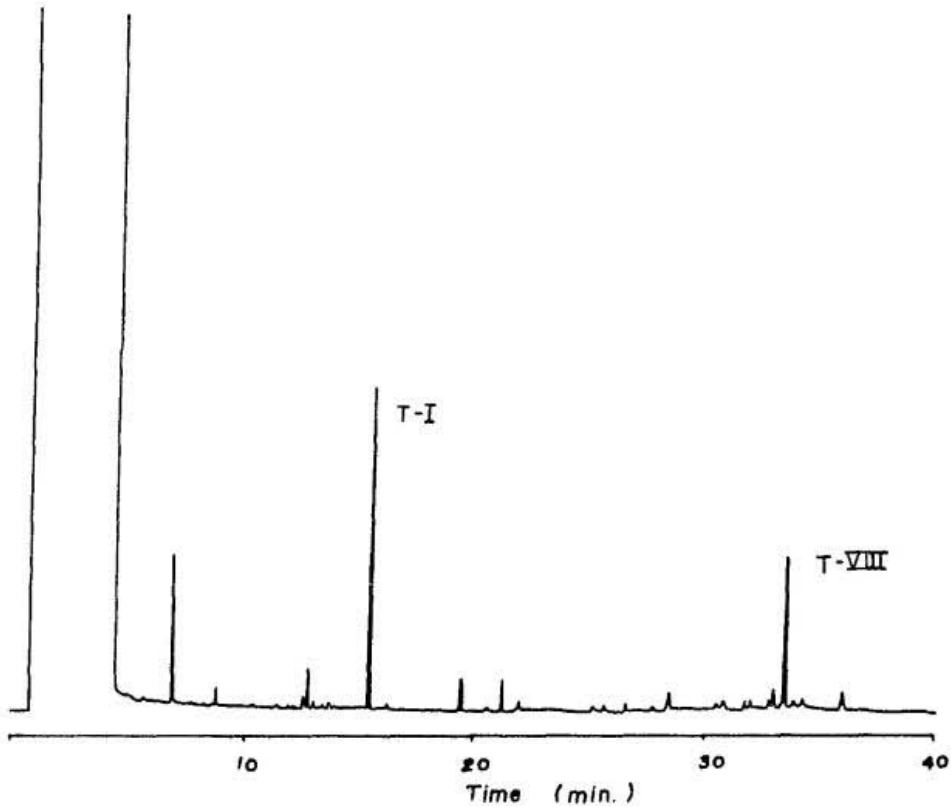


Figure 5.4. Gas Chromatogram of Trimethylsilyl Derivative of β-cyclodextrin Pyrolysate.

Amylopectin

Amylopectin is a branched polymer with an amylose backbone with about five percent (1→6)-α-linked glucopyranosyl units. The average branch length consists of twenty to thirty residues (42) (Fig. 5.5).

The chromatogram of the trimethylsilyl derivative of amylopectin pyrolysate resembles that of amylose with slightly lower yields of anhydro dimer. No unique anhydro dimer peaks are detected that reflect the presence of the (1→6) linkage (Figure 5.6).

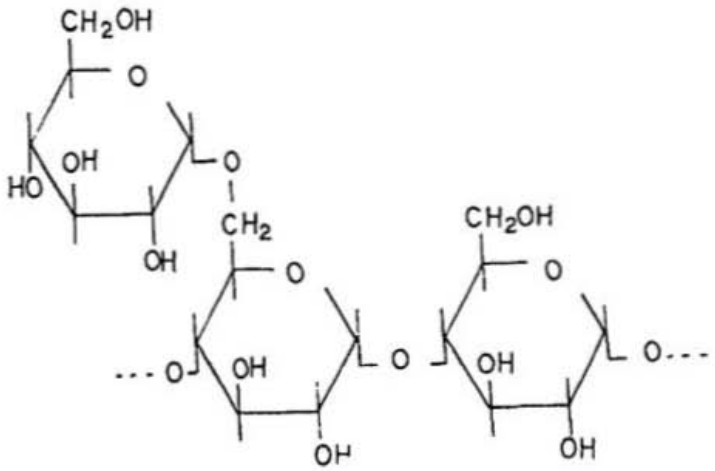


Figure 5.5. Structure of Amylopectin.

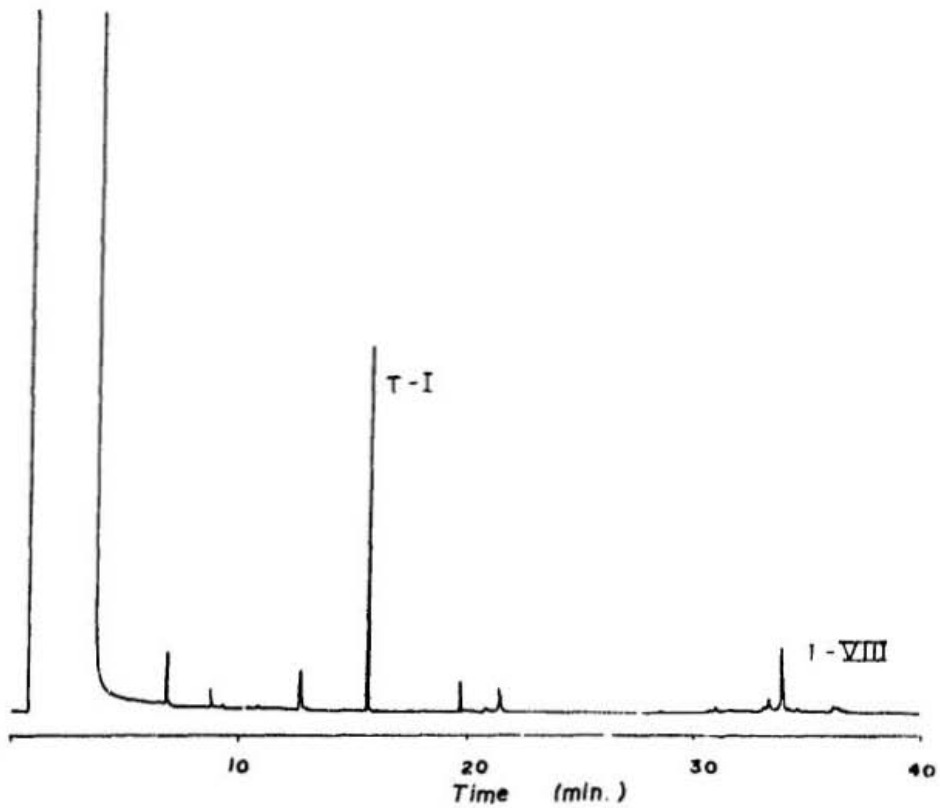


Figure 5.6. Gas Chromatogram of the Trimethylsilyl Derivative of Amylopectin Pyrolysate.

Glycogen

Glycogen is the storage polysaccharide of animals, it is structurally similar to amylopectin comprised of a (1→4)-α-D-glucopyranose backbone and (1→6)-α-D-branch points (42). The average chain length of glycogen is shorter than amylose and fewer branch points are present (Figure 5.7).

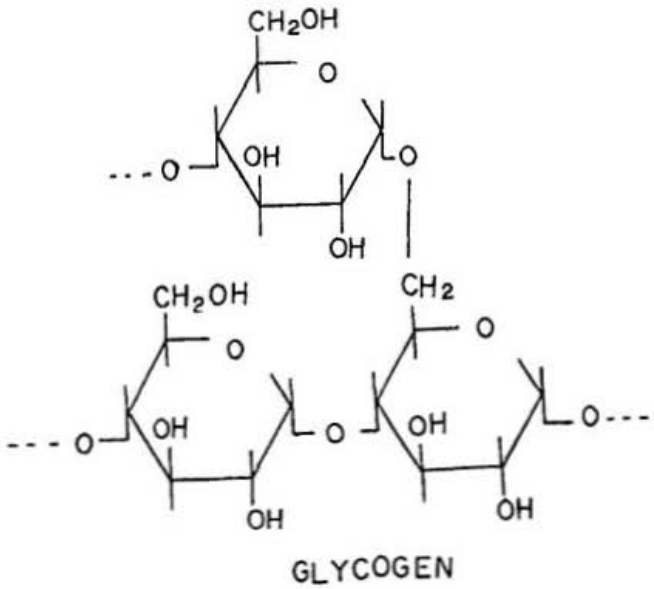


Figure 5.7. Structure of Glycogen.

When glycogen is subjected to thermal depolymerization analysis the resultant chromatogram of the trimethylsilyl derivative is similar to that of trimethylsilylated amylose pyrolysate (Figure 5.8). The major peaks are 1,6-anhydro-2,3,4-tri-O-trimethylsilyl-β-D-glucopyranose (T-I) and 4-O-(2,3,4,6-tetra-O-trimethylsilyl-α-D-glucopyranosyl)-1,6-anhydro-2,3-di-O-trimethylsilyl-β-D-glucopyranose (T-VIII). Several smaller unidentified peaks occur in this chromatogram that are not detected in that of amylose pyrolysate.

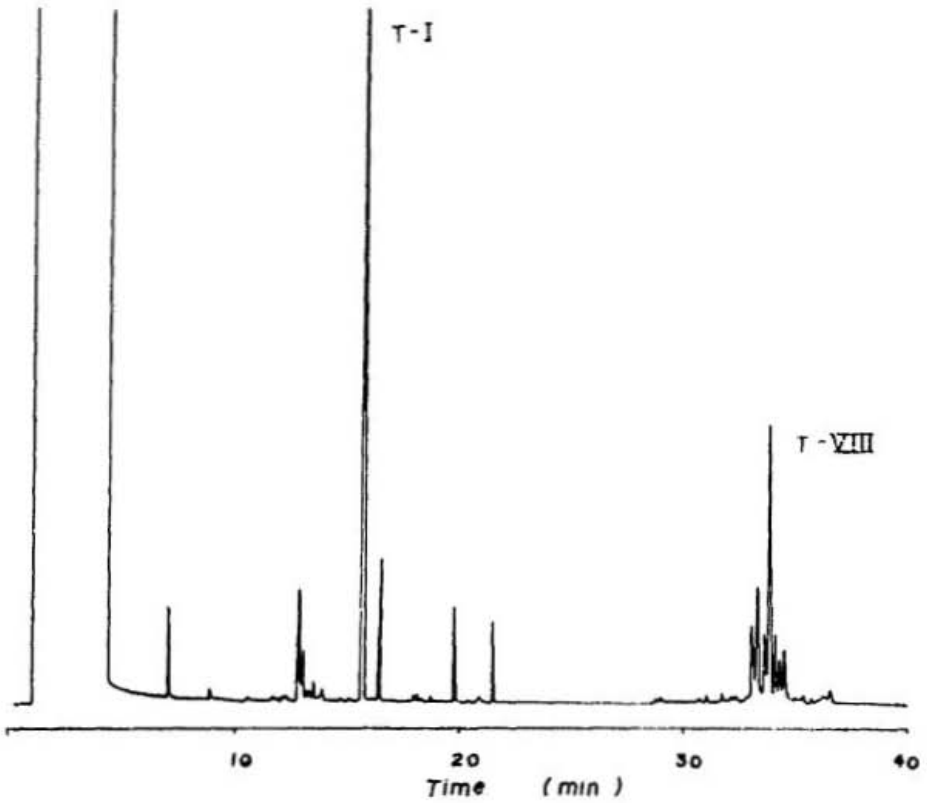


Figure 5.8. Gas Chromatogram of the Trimethylsilyl Derivative of Glycogen Pyrolysate.

Cellulose

Cellulose is the major component of wood and the most abundant natural organic substance. It is a structural polysaccharide providing rigidity to species of the plant kingdom. Cellulose is comprised of a linear chain of (1→4)-β-D-glucose units with a degree of polymerization up to 10,000 units. (Fig. 5.9) (1,42).

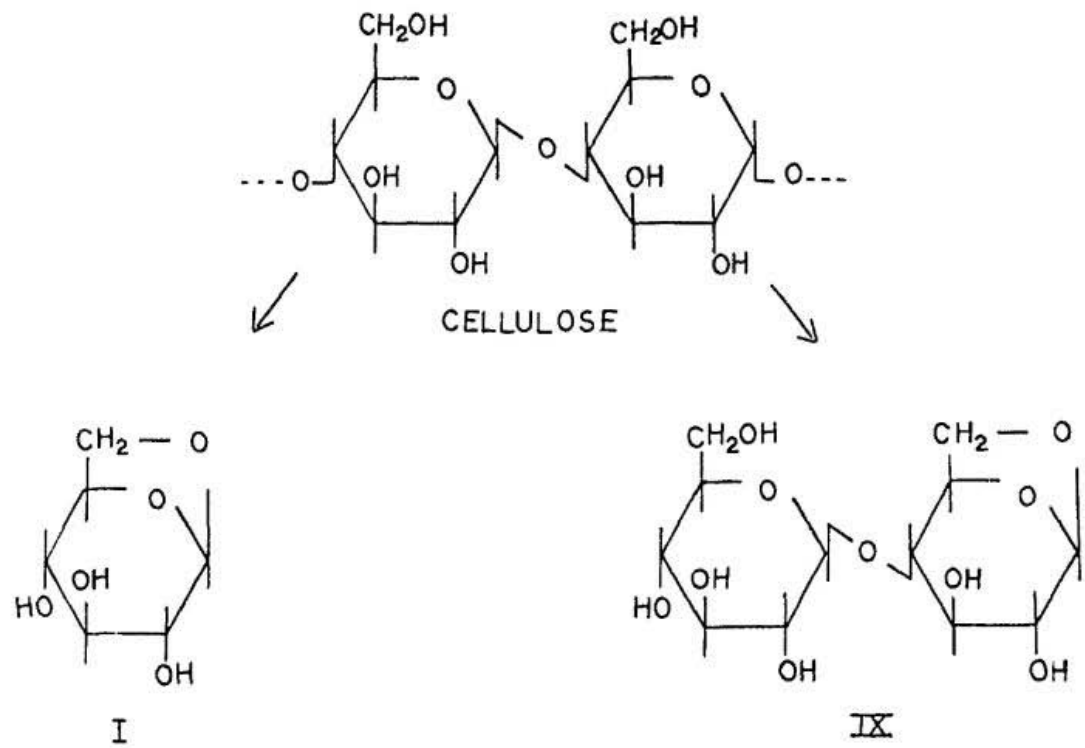


Figure 5.9. Structure of Cellulose and its Major Pyrolysate Products.

Analysis of the derivatized pyrolysate resulted in two major peaks (Fig. 5.10); due to 1,6-anhydro-2,3,4-tri-O-trimethylsilyl-β-D-glucopyranose (T-I) and the β-D-(1→4)-dimer, 4-O-(2,3,4,6-tetra-O-trimethylsilyl-β-D-glucopyranosyl)-1,6-anhydro-2,3-di-O-trimethylsilyl-β-D-glucopyranose (T-IX). The retention time of the β-D-(1→4) linked dimer peak was 34.5 minutes, almost one-half minute later than that of the α-D-(1→4) dimer. Identification of peaks was through comparison of GC retention times with authentic standards and mass spectral analysis (see section 4.2.2).

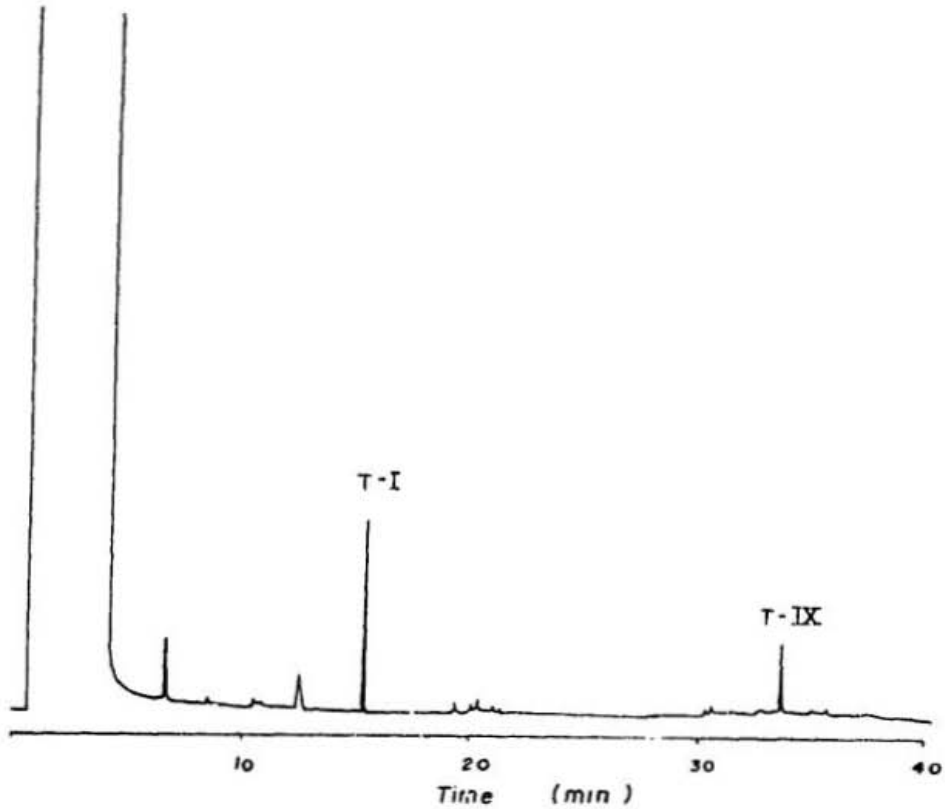
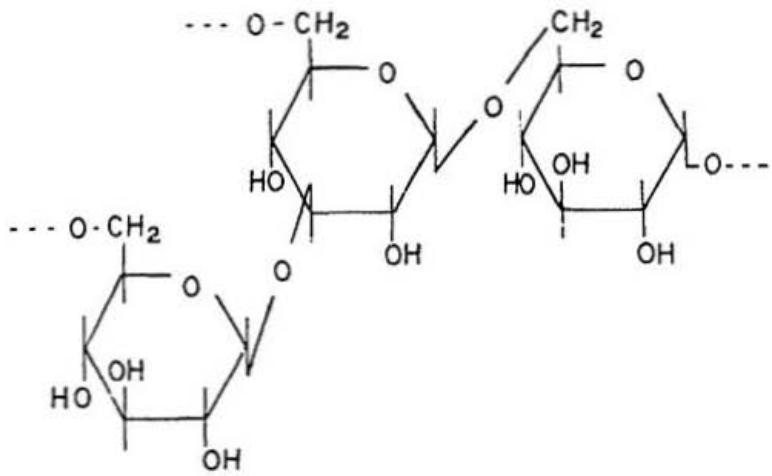


Figure 5.10. Gas Chromatogram of the Trimethylsilyl Derivative of Cellulose Pyrolysate.

Dextran

Dextrans are bacterial polysaccharides which consist of (1→6)-α-D-linked glucose units in the backbone (43). Some naturally occurring dextrans are linear in nature while others contain significant quantities of (1→4) or (1→3) linked D-glucose branch points (Fig. 5.11). The pyrogram of dextran (Fig. 5.12) resulted in one major peak, 1,6-anhydro-2,3,4-tri-O-trimethylsilyl-β-D-glucopyranose (T-I). No dimer was isolated for this (1→6) linked polymer due to substitution at position six in the glucopyranose molecule.



DEXTRAN

Figure 5.11. Structure of Dextran.

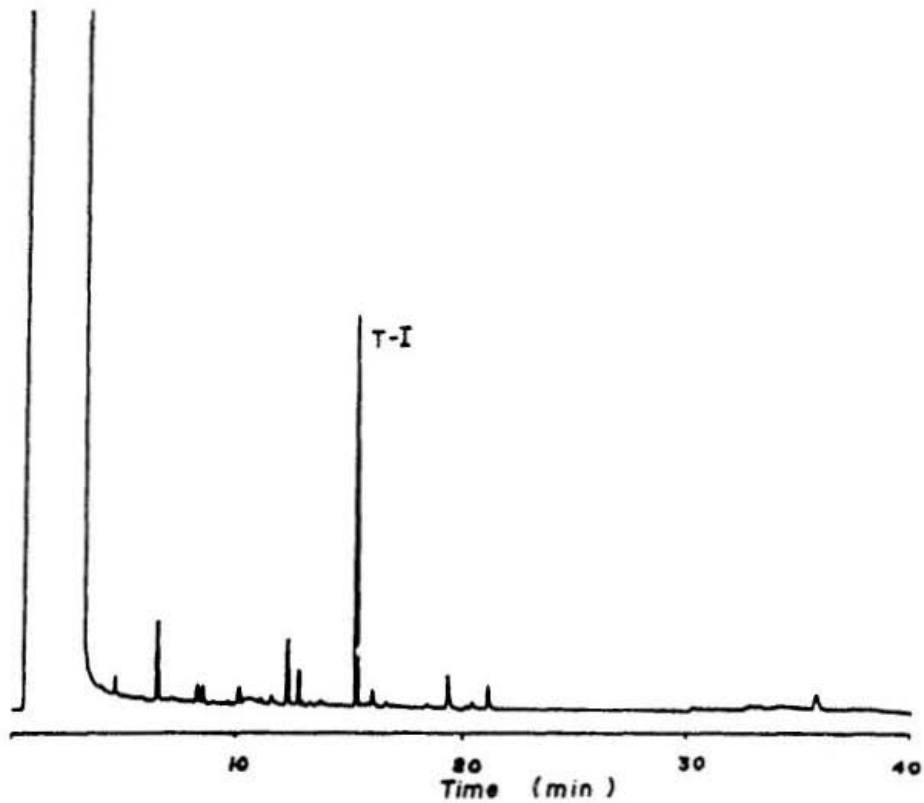


Figure 5.12. Gas Chromatogram of the Trimethylsilyl Derivative of Dextran Pyrolysate.

Laminarin

Laminarin is a water soluble reserve polysaccharide isolated from algae. It is a linear polymer with a degree of polymerization of about thirty-one. This polymer consists of (1→3) linked β-D-glucose units (Fig. 5.13) (42,44).

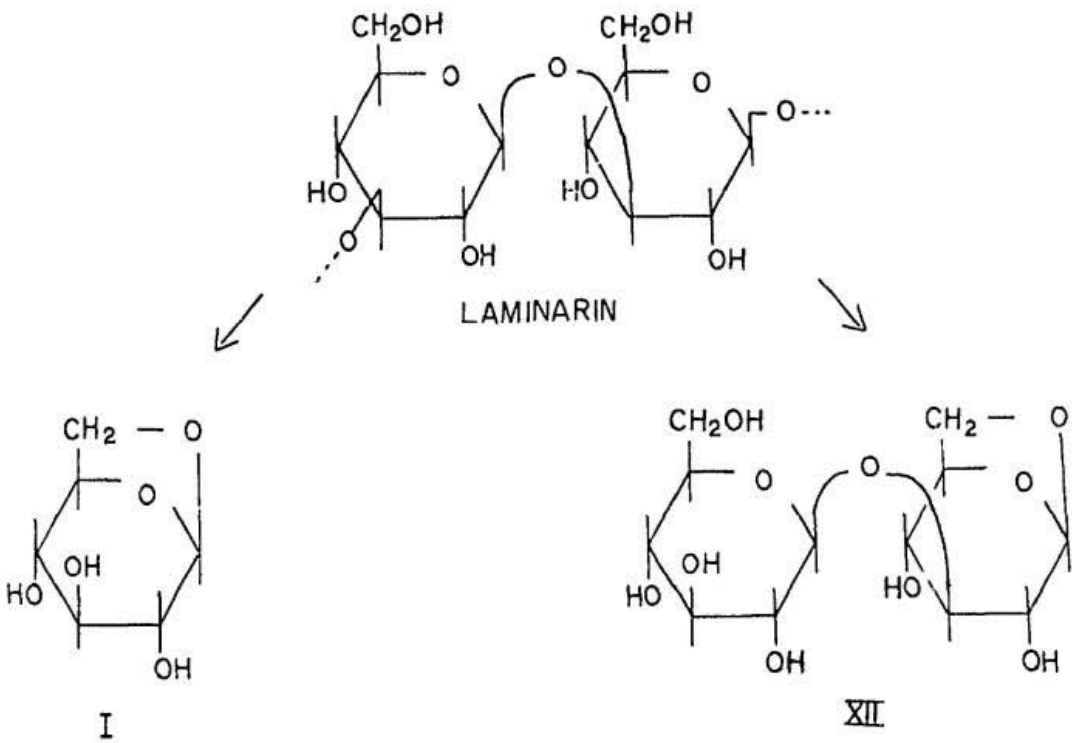


Figure 5.13. Structure of Laminarin and Its Major Pyrolysis Products.

The pyrogram (Fig. 5.14) consists of peaks corresponding to 1,6-anhydro-2,3,4-tri-O-trimethylsilyl-β-D-glucopyranose (T-I) and the (1→3)-β-D-glucose dimer, 3-O-2,3,4,6-tetra-O-trimethylsilyl-β-D-glucopyranosyl-1,6-anhydro-2,4-di-O-trimethylsilyl-β-D-glucopyranose (T-XX). This dimer is only about fifteen percent as abundant as the anhydro monomer. The lower yield of the (1→3) dimer compared to the (1→4) dimers may be attributed to the stability variations in the two linkages. The retention time for this dimer is 33.4 minutes, over one-half minute before the (1→4)-α- and one minute before the (1→4)-β-anhydro dimers.

Identification of the dimer was inferred through the structure of the original polymer.

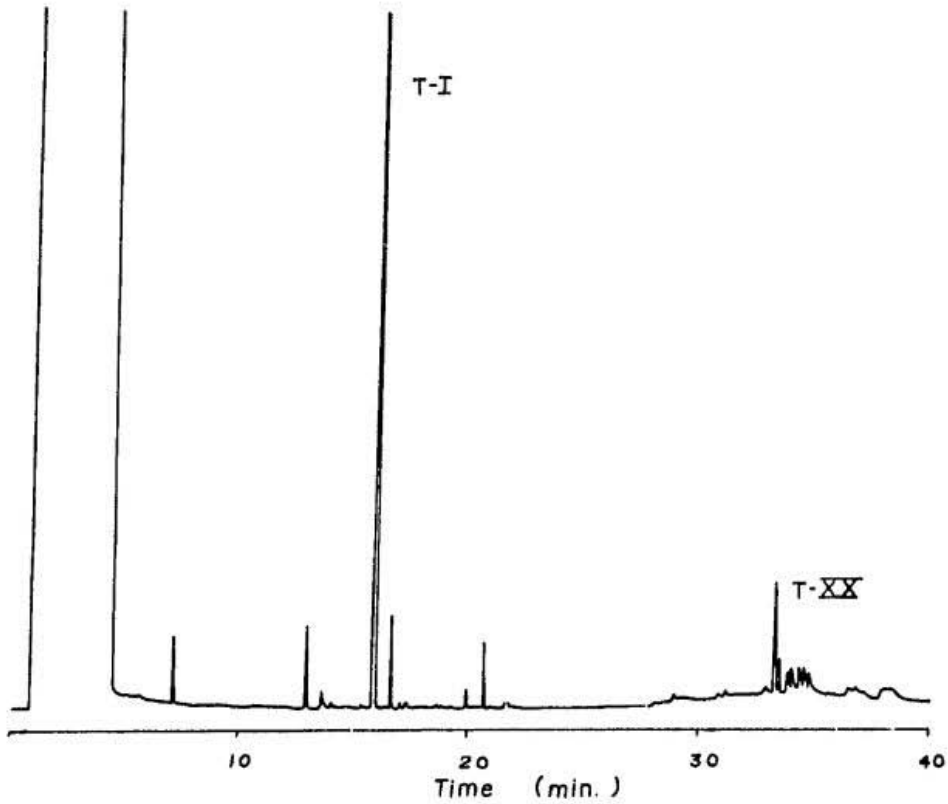


Figure 5.14. Gas Chromatograph of the Trimethysilyl Derivative of Laminarin Pyrolysate.

Lichenan

Lichenan is a glucan found in moss. The linkages are all β-D but the repeating unit is a (1→3) linked cellotriose (Fig. 5.15) (43). The chromatogram of the trimethylsilyl derivative of lichenan pyrolysate reflects this structure giving major peaks corresponding to 1,6-anhydro-2,3,4-tri-O-trimethylsilyl-β-D-glucopyranose (T-I), 3-O-(2,3,4,6-tetra-O-trimethylsilyl-β-D-glucopyranosyl)- 1,6-anhydro-2,4-di-O-trimethylsilyl- β-D-glucopyranose (T-XX) and 4-O-(2,3,4,6-tetra-O- trimethylsilyl- β-D-glucopyranosyl- 1,6-anhydro-2,3-di- O-trimethylsilyl- β-D-glucopyranose (T-IX) (Fig. 5.16). The ratio of products are 3.6 to 1 for the (1→4) dimer

compared to the (1→3) dimer, similar to the linkage ratios in the original polysaccharide. Identification of the dimer (T-XX) was inferred from the structure of the polymer.

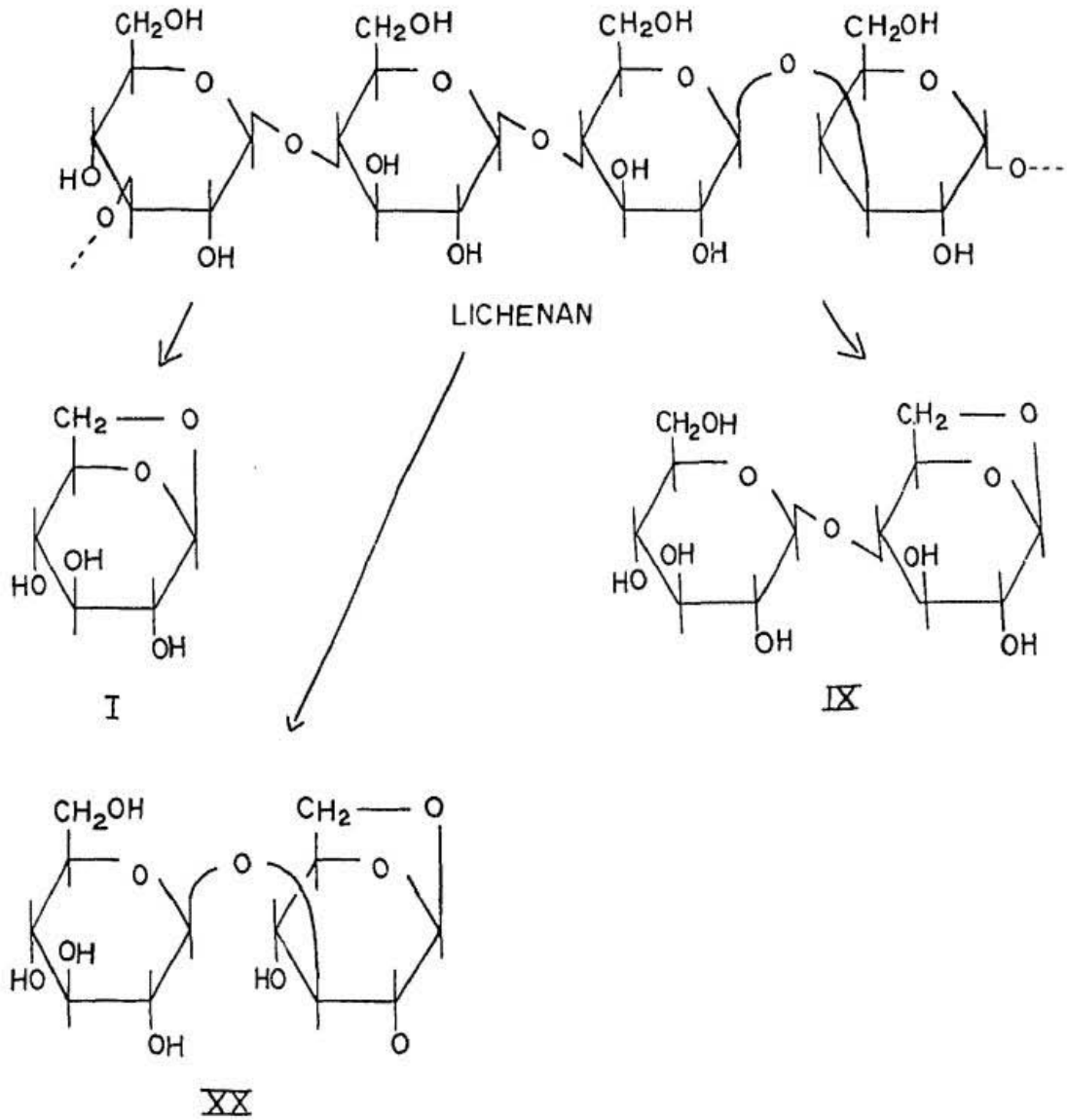


Figure 5.15. Structures of Lichenan and Its Major Pyrolysis Products.

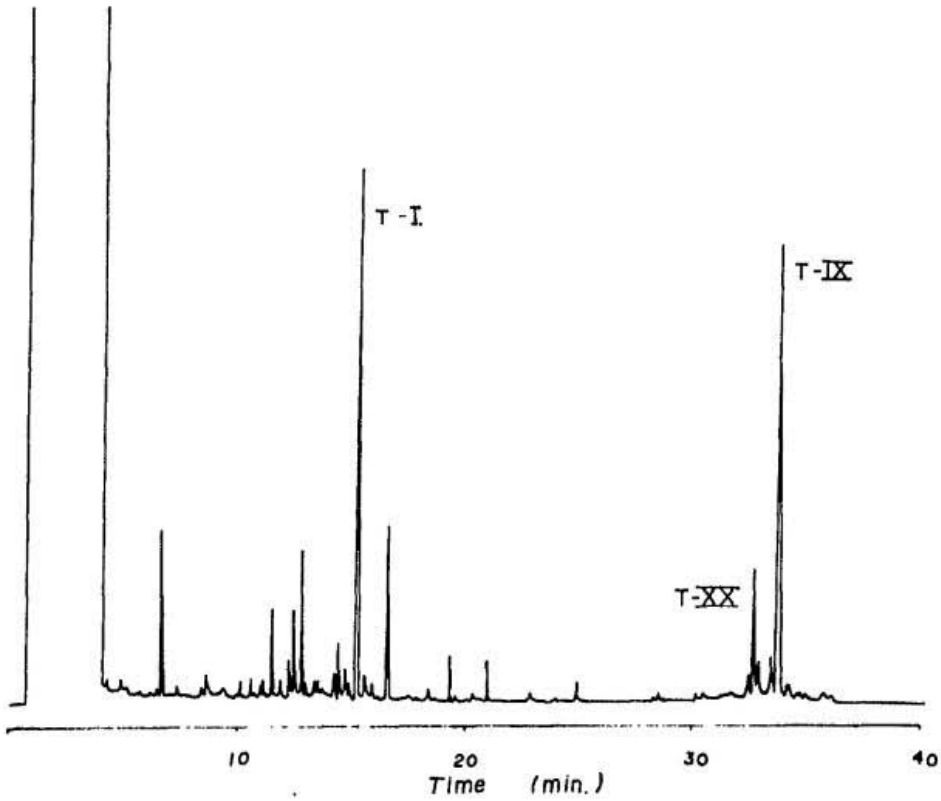


Figure 5.16. Gas Chromatogram of the Trimethylsilyl Derivative of Lichenan Pyrolysate Products.

Nigeran

Nigeran is a regular linear copolysaccharide, it is an α-D-glucan with alternating (1→3) and (1→4) linkages (Fig. 5.17) (42). Again, this pyrogram (Fig. 5.18) indicates a large proportion of 1,6-anhydro- 2,3,4-tri-O- trimethylsilyl- β-D-glucopyranose (T-I) in the pyrolysate oil. Two dimers of equal intensity occur, they are 3-O-(2,3,4,6- tetra-O- trimethylsilyl- α-D-glucopyranosyl)- 1,6-anhydro- 2,4-di-O- trimethylsilyl- β-D-glucopyranose (T-XXI) and 4-O-(2,3,4,6- tetra-O- trimethylsilyl- α-D- glucopyranosyl) -1,6-anhydro-2,3-di-O-trimethylsilyl-β-D-glucopyranose (T-VIII). The linkages and ratios of these products quantitatively reflect the proposed structure for the nigeran polymer. Peaks were identified by mass spectral analysis of the trimethylsilyl derivative of the pyrolysate (see section 4.2.3).

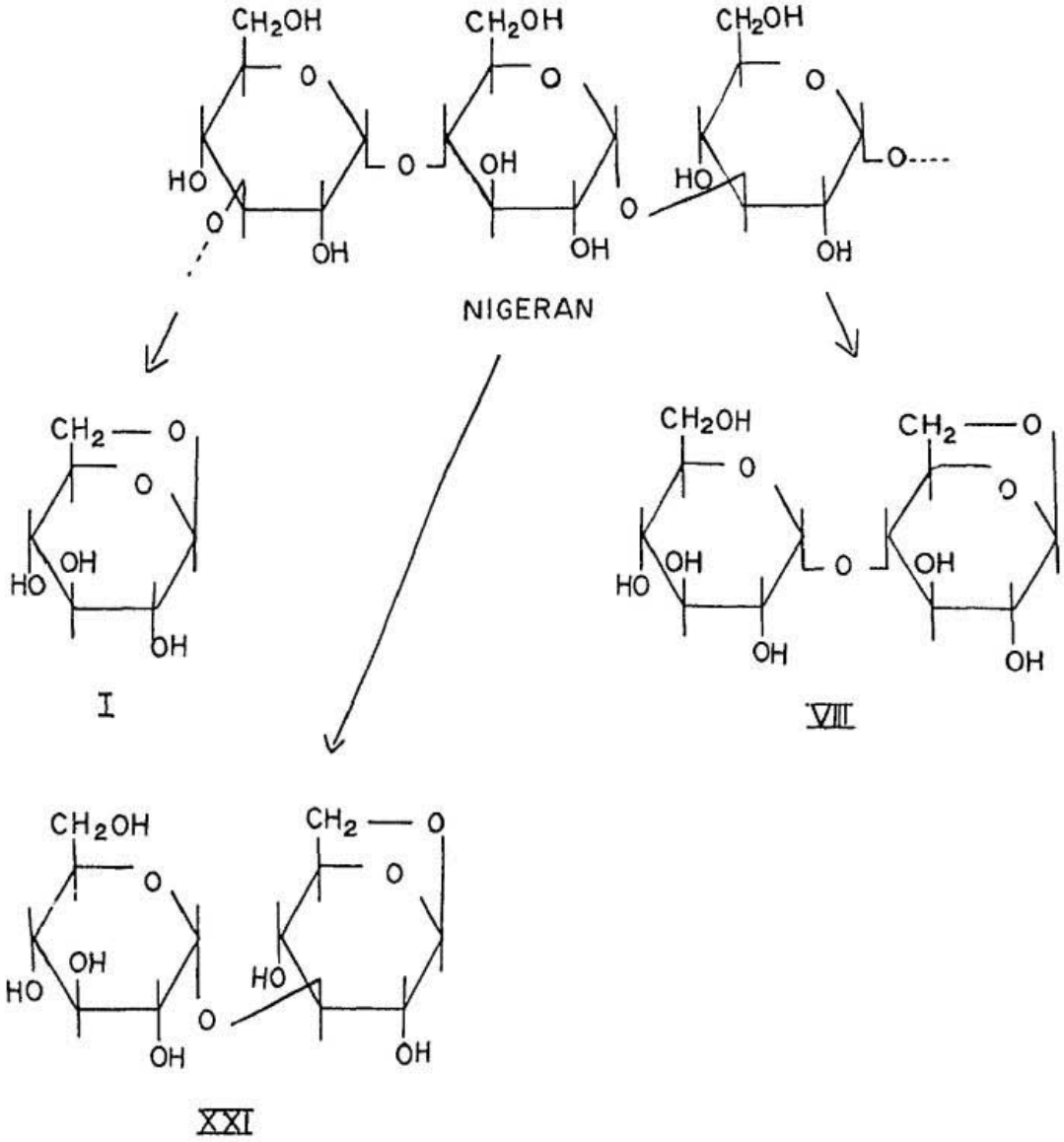


Figure 5.17. Structures of Nigeran and Its Major Pyrolysis Products.

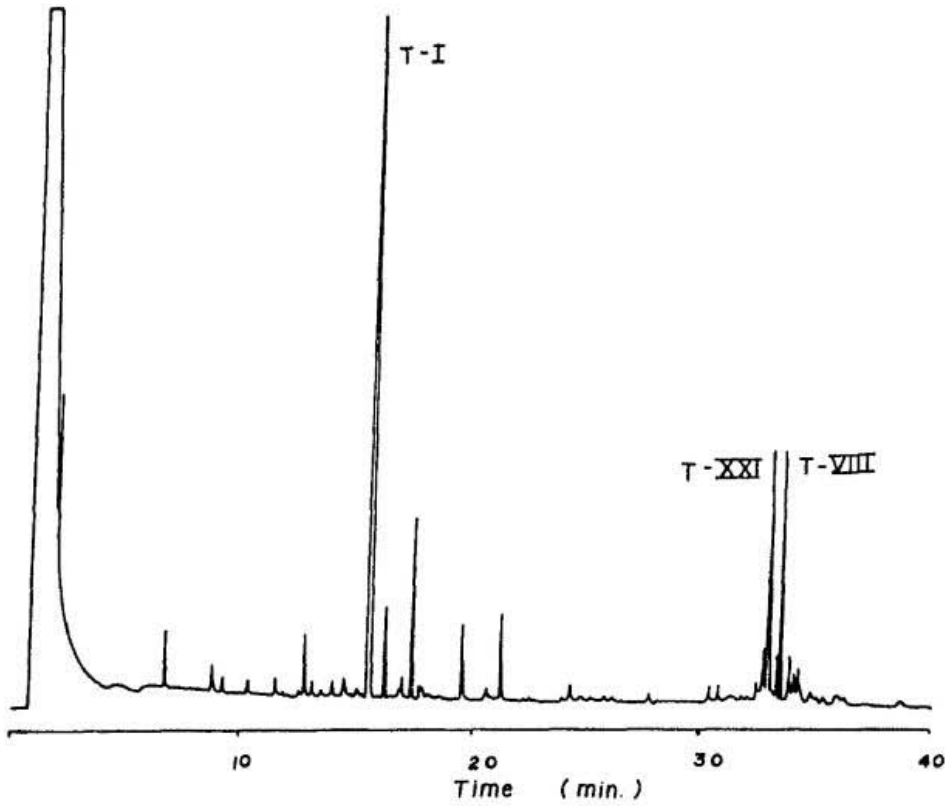


Figure 5.18. Gas Chromatogram of the Trimethylsilyl Derivative of Nigeran Pyrolysate Products.

Pullulan

The fungal polysaccharide, pullulan, is a linear polysaccharide composed of mostly (1→6) linked maltotriose units with some (1→6) linked maltotetraose (Fig. 5.19). The (1→6) linkages impose a greater flexibility than the straight chain (1→4)-α-glucan, amylose. This added linkage also serves to increase the solubility of this polysaccharide (44,45).

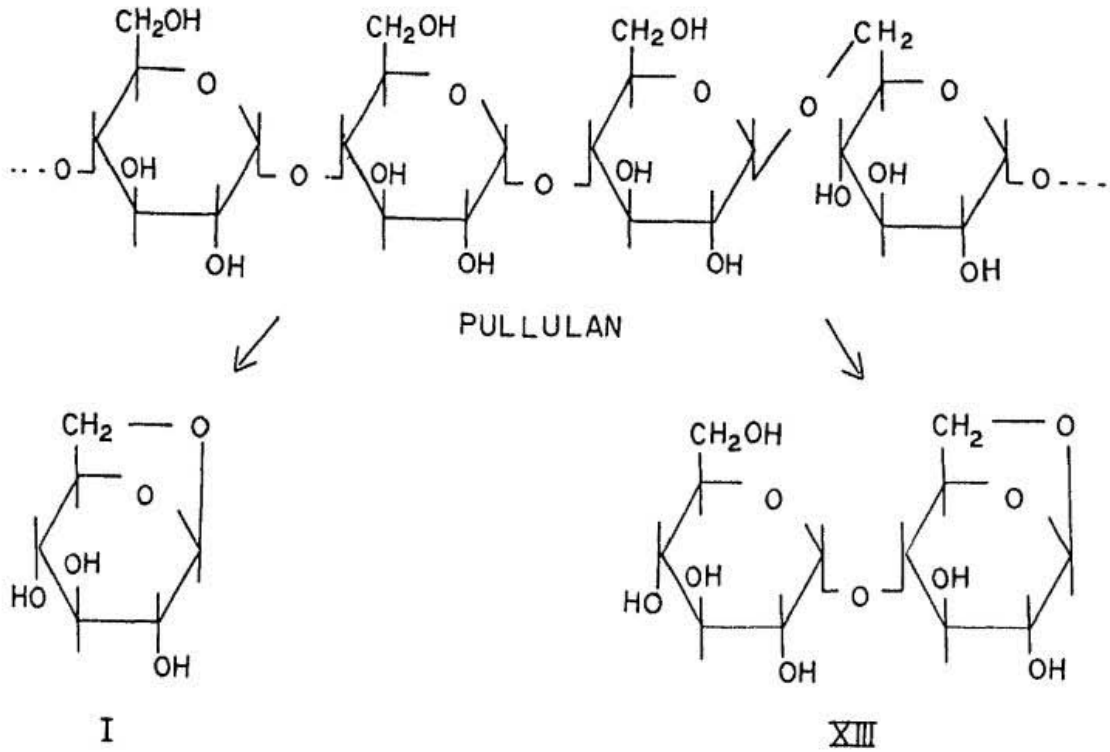


Figure 5.19. Structures of Pullulan and Its Major Pyrolysis Products.

The trimethylsilyl derivative of the pyrolysate contains two major components: 1,6-anhydro- 2,3,4-tri-O- trimethylsilyl- β-D-glucopyranose (T-I) and 4-O-(2,3,4,6- tetra-O-trimethylsilyl- α-D-glucopyranosyl)- 1,6-anhydro- 2,3-di- O-trimethylsilyl- β-D-glucopyranose (T-VIII) (Fig. 5.20). Again, no representation of a (1→6) linkage is observed.

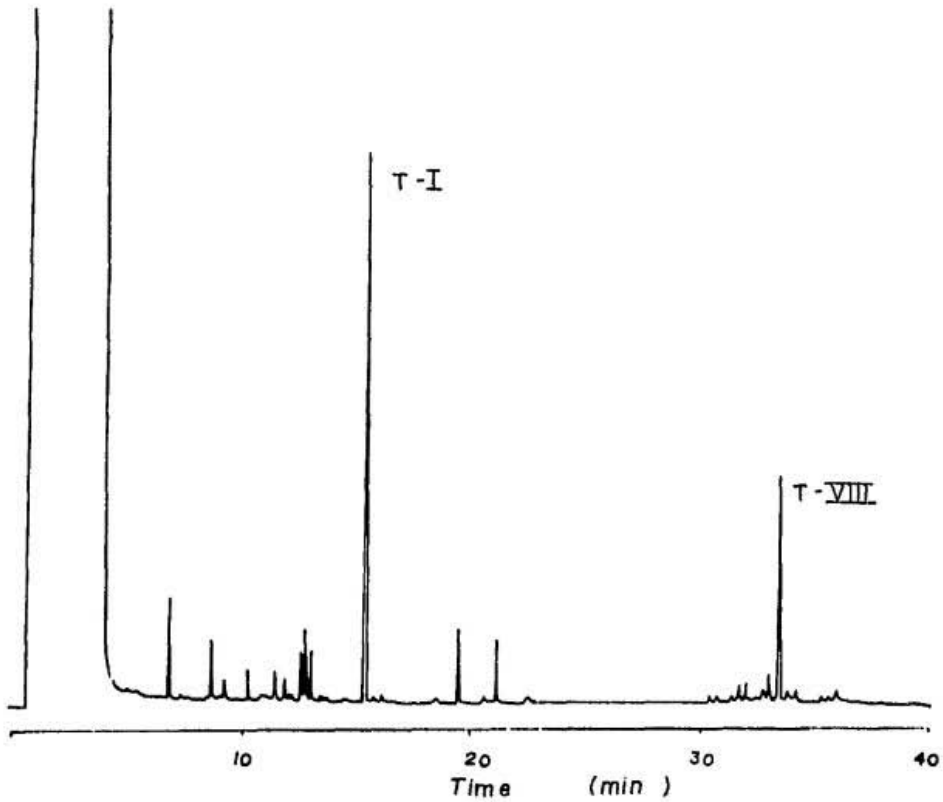


Figure 5.20. Gas Chromatogram of Trimethylsilyl Derivative of Pullulan Pyrolysate.

Ivory nut mannan

Ivory nut powder is a seed polysaccharide that consists of a mannan chain with galactose side chains. The backbone consists of (1→4) linked β-D-mannose units, the side chains are single units of α-D-galactose attached through a (1→6) linkage (Fig. 5.21) (42). The proportion of galactose in ivory nut mannan is very small with a ratio of 1:5 for galactose to mannose units.

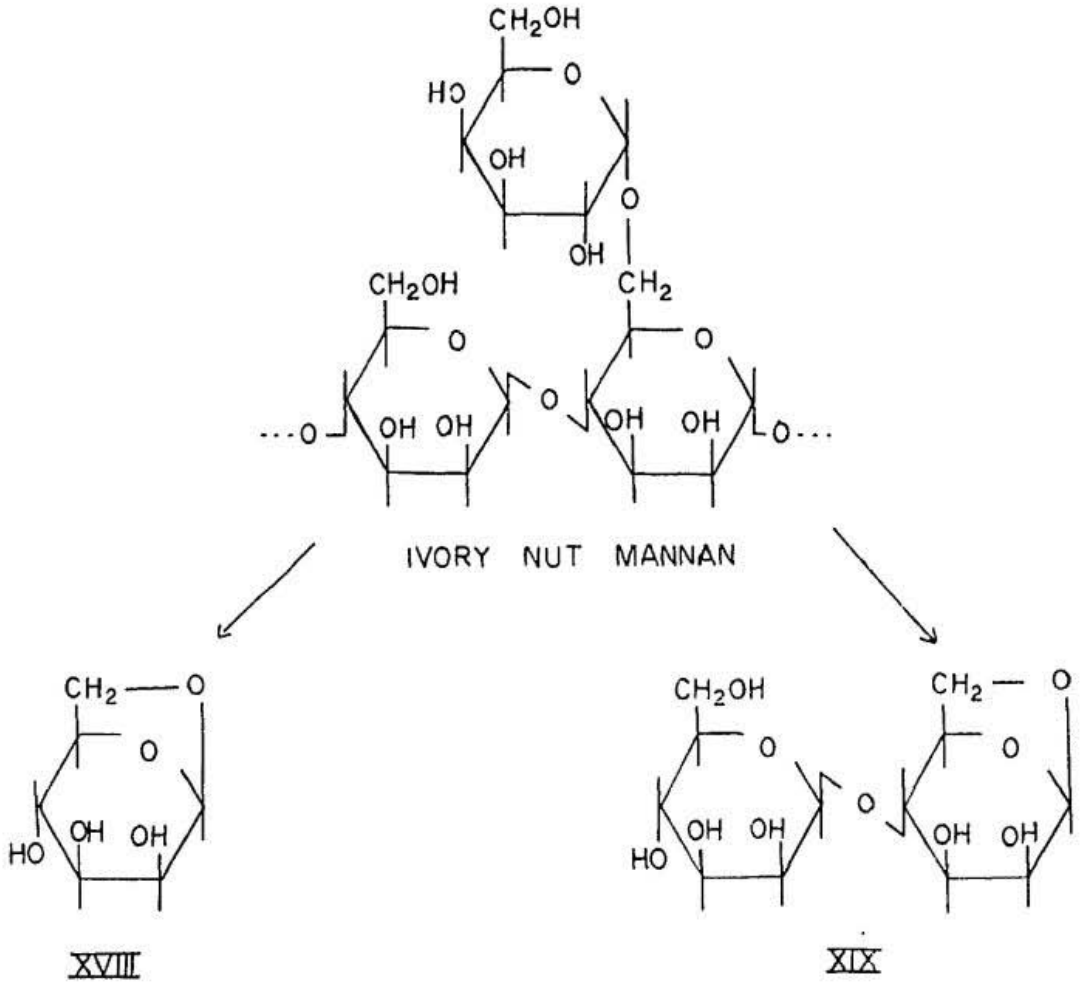


Figure 5.21. Structures of Ivory Nut Powder and Its Major Pyrolysate Products.

The pyrogram of ivory nut powder (Fig. 5.22) has a large peak contributed by 1,6-anhydro-2,3,4-tri- O-trimethylsilyl- β-D-mannopyranose (T-XVIII) and a peak about forty percent as intense as this due to the mannan dimer 4-O-2,3,4,6- tetra-O- trimethylsilyl- β-D-mannopyranosyl- 1,6-anhydro-2,3- di-O-trimethylsilyl- β-D-mannopyranose (T-XIX). There is no substantial amount of 1,6-anhydro galactose present in the pyrogram. The retention time of this dimer is thirty-six minutes, one and a half minutes past the latest eluting glucan. Identification of dimeric products was through inference of the structure of the original polysaccharide.

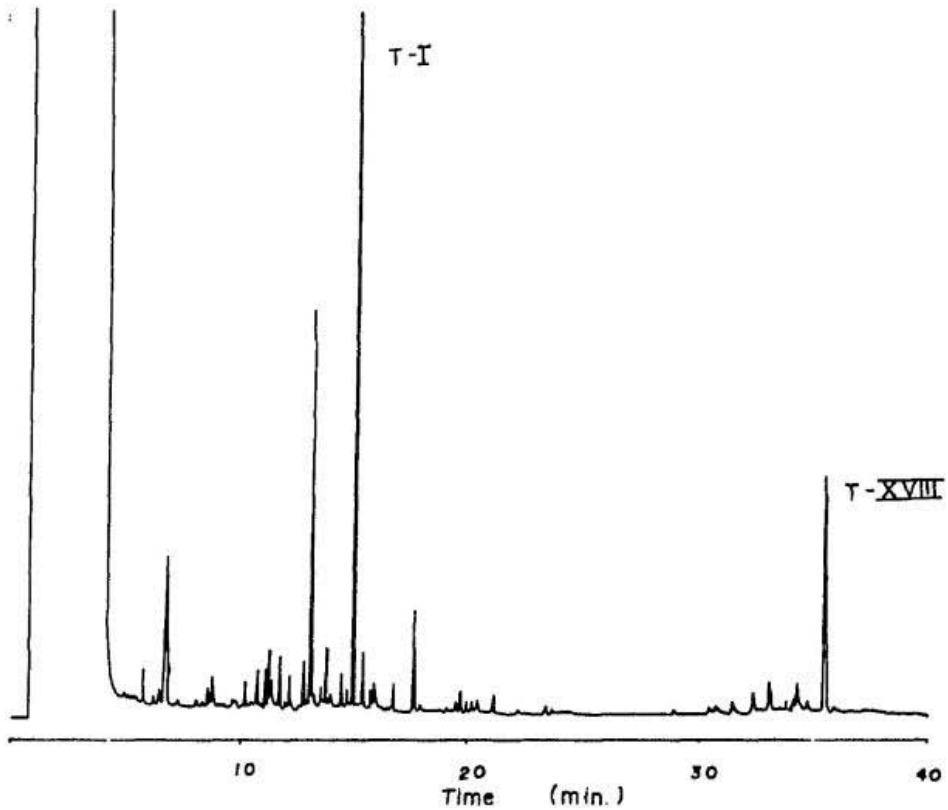


Figure 5.22. Gas Chromatogram of the Trimethylsilyl Derivative of Ivory Nut Pyrolysate.

Locust bean gum

Locust bean gum, a leguminous seed polysaccharide, is a galactomannan with a (1→4)-β-D-mannan backbone with α-D-galactose units linked to C-6 of approximately every fourth mannose residue (Fig. 5.23). The ratio of galactose to mannose is 1:3 (45).

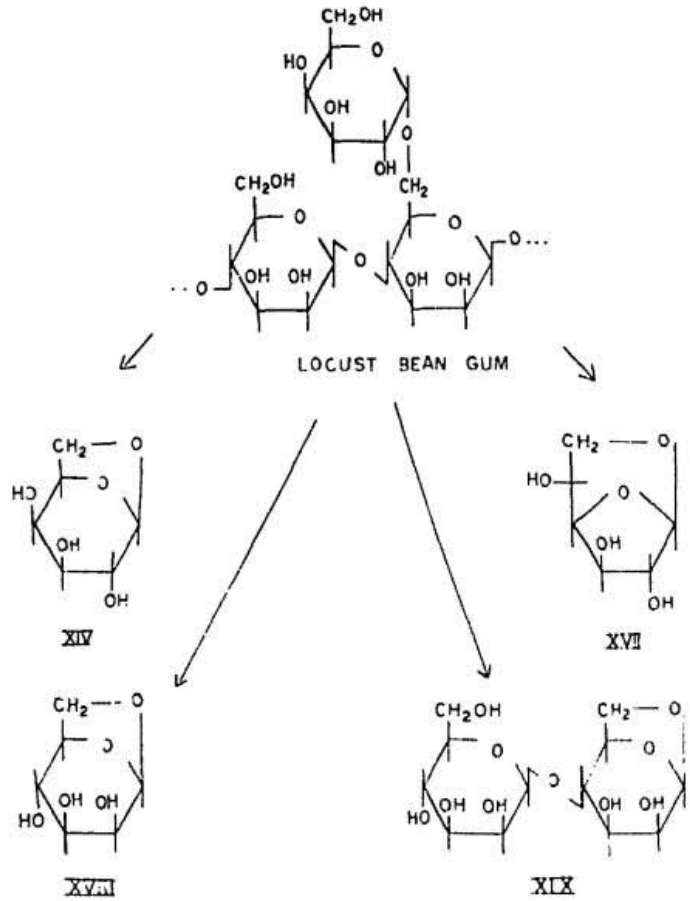


Figure 5.23. Structures of Locust Bean Gum and Its Major Pyrolysate Products.

The pyrolysis of locust bean gum results in three anhydro monosaccharides and one anhydro disaccharide (Fig. 5.24). There are two peaks due to galactose; these correspond to the pyranose (XIV) and furanose (XVII) ring structures with the first being 1,6-anhydro-2,3,4-tri-O- trimethylsilyl- β-D- galactopyranose (T-XIV) and the second 1,6-anhydro- 2.3,4-tri-O- trimethylsilyl- β-D- galactofuranose (T-XVII). The third anhydro monosaccharide, 1,6-anhydro- 2,3,4-tri- O-trimethylsilyl- β-D- mannopyranose (T-XVII), arises from depolymerization of the mannan backbone. Partial depolymerization of the backbone produces the only anhydro disaccharide, 4-O-2,3,4,6- tetra- O-trimethylsilyl- β-D- mannopyranosyl- 1,6-anhydro-2,3-di-O- trimethylsilyl- β-D-mannopyranose (T-XIX). The gas chromatographic peaks were

identified through mass spectral analysis of the TMS derivative of the pyrolysate oil (see section 4.2.3).

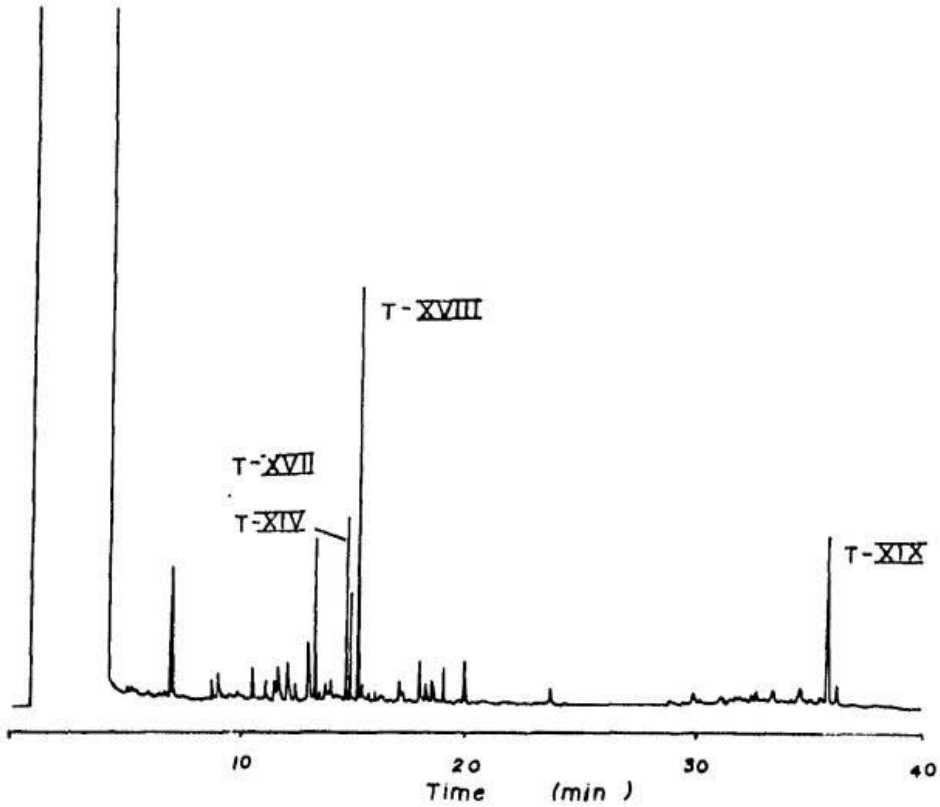


Figure 5.24. Gas Chromatogram of the Trimethylsilyl Derivative of Locust Bean Gum Pyrolysate Products.

Larch gum

Larch gum is an arabinogalactan obtained from the wood of the larch tree. It is a highly branched polymer containing arabinose and galactose in a 1:6 ratio (Fig. 5.25). The backbone is made up of units of β-D-galactose joined in a (1→3) linkage (45).

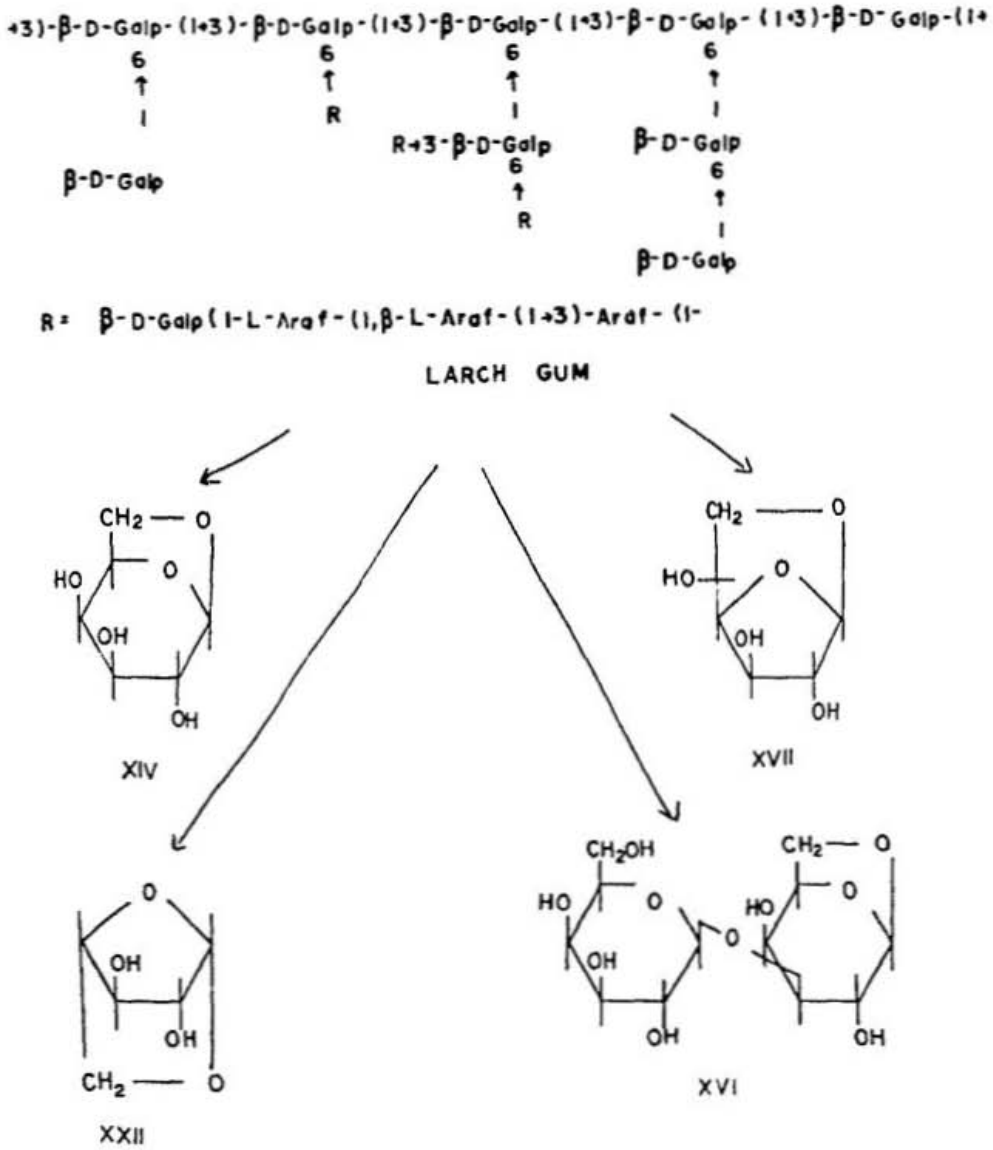


Figure 5.25. Structure of Larch Gum and Its Major Pyrolysis Products.

The chromatogram of the trimethylsilyl derivative of the pyrolysate indicates the presence of galactose through the anhydrogalactopyranose (T-XIV) and anhydrogalactofuranose (T-XVII) peaks (Fig. 5.26). A peak about ten percent of these combined intensities occurs at the beginning of the chromatogram; this peak is due to an anhydro pentose namely, 1,5-

anhydro- 2,3-di-O- trimethylsilyl- β-L- arabinofuranose (T-XXII). The dimer in this instance elutes about one minute after the mannose dimer seen in ivory nut and locust bean pyrolysates, this is most likely due to the galactose dimer 3-O-(2,3,4,6- tetra- O-trimethylsilyl- β-D-galactopyranosyl)- 1,6- anhydro- 2,4-di-O- trimethylsilyl- β-D- galactopyranose (T-XVI). Mass spectral analysis of the TMS derivative permitted interpretation of the GC peaks (see section 4.2.3).

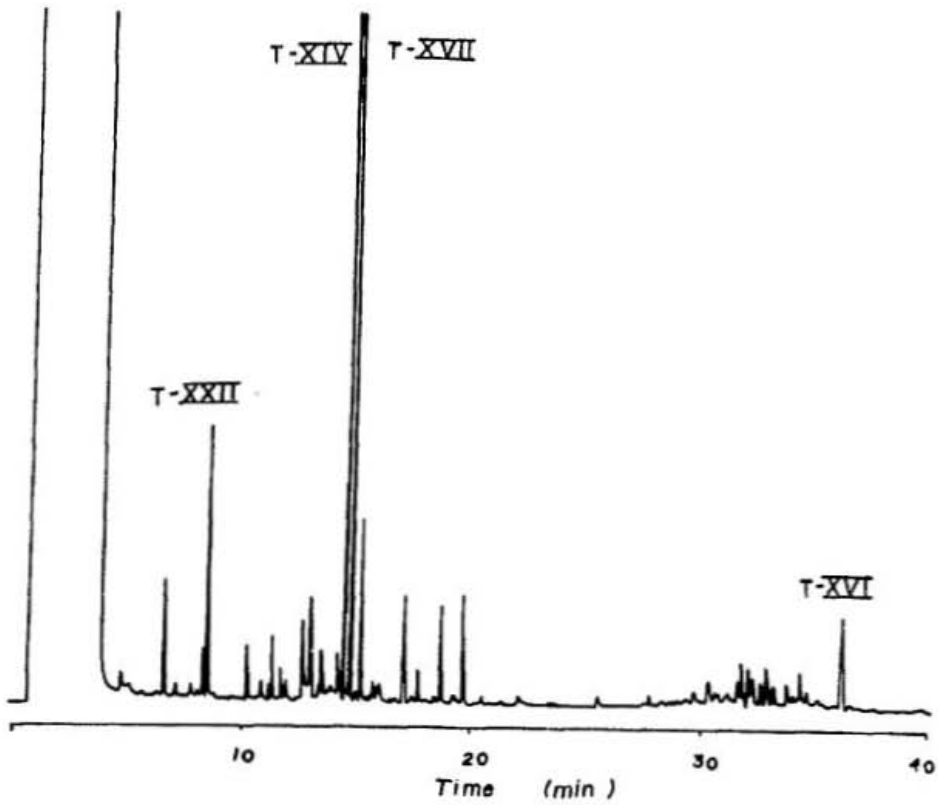


Figure 5.26. Gas Chromatogram of the Trimethylsilyl Derivative of Larch Gum Pyrolysate Oil.

6. Conclusion

These preliminary depolymerization studies of neutral polysaccharides showed promising results for the rapid screening of polymers for structural interpretation. This technique provides interesting applications for mechanistic studies of thermal depolymerization as well as finger printing of biopolymers and synthetic polymers. This approach to off-line thermal depolymerization analysis is a rapid micro scale technique requiring approximately one hundred micrograms of polymer and less than two hours for complete analysis. This experimental technique has many variables, such as choice of derivative, chromatographic technique and separation conditions, which may be selected to tailor analysis to the specific needs of the analyst.

Structural information was obtained from the presence of individual anhydro sugars in the pyrolysate oils. The monosaccharide building blocks of the polysaccharides could be interpreted from the anhydro monosaccharides present in the pyrolysates. The unique GC retention times of the TMS derivative of the anhydro dimers encountered in this study provided detail about both linkage position and anomeric configuration (Table 6.1). Identification of these dimeric species was provided by comparison with standards or through mass spectral confirmation. Semi-quantitative results may be extracted from chromatographic data. The ratios of anhydro monomers and dimers closely relate to the corresponding compositions in the structural features of the original polymer.

Table 6.1. Comparison of Retention Times of Trimethylsilyl Derivatives of Anhydro Dimers.

	Dimer	Retention Time (mins)
XXI	α-D-Glu-(1→3)-1,6-anh-β-D-Glu	33.0
XX	β-D-Glu-(1→3)-1,6-anh-β-D-Glu	33.4
VIII	α-D-Glu-(1→4)-1,6-anh-β-D-Glu	34.1
IX	β-D-Glu-(1→4)-1,6-anh-β-D-Glu	34.5
XIX	β-D-Man-(1→4)-1,6-anh-β-D-Man	36.0
XVI	β-D-Gal-(1→3)-1,6-anh-β-D-Gal	36.8

The identification of ring fragment products, that is a hexose unit with a portion of the neighbouring ring attached, provides additional possibilities for structural information and mechanistic distinction of polysaccharides. The ring fragment products found varied with structural differences presenting an interest for future studies of thermal depolymerization analysis of polysaccharides.

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