CHAPTER V: COMPARATIVE EVALUATION OF PROPERTIES OF VARIOUS DEXTRINS PREPARED WITH AND WITHOUT CATALYST.
COMPARATIVE EVALUATION OF PROPERTIES OF VARIOUS DEXTRINS PREPARED WITH AND WITHOUT CATALYST.

WITOUT CATALYST

It will be worthwhile at this stage to review the various properties of the dextrins prepared from starch, gum guar, and starch-guar mixtures by heating with or without acid catalyst and to see whether the results give any indication about the reactions taking place during dextrinization. The differences in the properties of the two types of dextrins are quite marked. Thus, while pyrodextrin (British gum) shows maximum solubility of 82% in 3 hours' time after which the value drops progressively with time of dextrinization, the canary type of dextrins show 100% solubility in an hour's time after which there is no decrease in this value. Similarly, there are significant differences in the ferricyanide values of the two types of dextrins. The ferricyanide number of the pyrodextrins keeps on increasing with time of dextrinization attaining a value of 34 after 8 hours of heating while in the acid catalyzed dextrinization the maximum value of 17.5 is obtained in one hour after which there is a sharp fall to 14 units, and thereafter there is no change. $\beta$-Amylalysis of pyrodextrins keeps on going down with increasing time of roasting, the 8-hour sample having a $\beta$-amylolysis of only 1. Dextrinization of starch in the presence of acid, on the other hand, results in increasing resistance to $\beta$-amylase action upto 2 hours after which the value remains unchanged. The minimum $\beta$-amylolysis value obtained for canary type of dextrin was 17. From these results it can be said that in acid dextrinization there is
rapid hydrolysis of the glycosidic bonds followed by recombination of the fragments and in this process some new glycosidic linkages are produced. Most of the reaction appears to be over in 2 hours.

Dextrinization at higher temperatures in the absence of catalyst, on the other hand, requires more time. Such dextrins have a more branched structure than canary dextrins and there is no evidence of recombination of fragments with reducing end groups. Thus, while in acid dextrinization the main reaction is hydrolysis-recombination in pyro dextrinization at higher temperatures in the absence of catalyst, transglycosidation through perhaps levoglucosan is the predominant reaction.

The increase in reducing power with concomitant decrease in solubility in the case of pyro dextrin may be due to formation of insoluble substances arising from polymerization of degraded fragments such as glucose, hydroxymethyl furfural etc.

Pyro dextrinization of gum guar results in a tremendous increase in reducing power with concomitant lowering in intrinsic viscosity. These changes are accompanied by considerable darkening of the products. The other noticeable thing is the progressive insolubility in water of guar dextrin as the time of roasting is increased. This could be due to either debranching of the polymer or reaction between gum fragments and protein component.

Dextrinization of starch-guar mixtures follows a pattern of changes in reducing power, intrinsic viscosity and solubility similar to that of starch. The starch-guar dextrins differ slightly in terms of $\beta$-amylolysis value. Higher $\beta$-amylolysis value may be due to the quenching effect of gum on starch dextrinization.
As to the mechanism of pyrodextrinization of starch in the absence of any catalyst, it appears that the first reaction is that of hydrolysis, which is caused by the presence of substantial amounts of water in polysaccharide, some acidic or potentially acidic material and high temperature. The hydrolysis results in the cleavage of the glycosidic bonds producing carbonium ion (I) (see Fig. 22). The latter can then react in one of the three ways (1) combine with water to give a reducing end group (II), (2) react with -CH₂OH group of the same glucose unit to give 1,6-anhydroβ-D-glucopyranose (III) and (3) react with hydroxyl group (preferentially the primary hydroxyl) of neighboring glucose residue resulting in the formation of a branched polymer (IV). III can further react by attack of an anion of anhydro glucose residue from the backside of the anhydro ring to give 1,6-α-linkage.

If the 1→6 glycosidic linkage is formed by the participation of I, the anomeric configuration of the glucosidic bond would be both α and β.

Inasmuch as Wolfson and Thompson have isolated α-1→6 linked disaccharide (isomaltose) in much greater proportion than β-1→6 linked disaccharide (gentiobiose) it appears that the major path of dextrinization is via levoglucosan. Support for this mechanism is also forthcoming from the isolation of levoglucosan from pyrodextrin. However, it is to be noted that isomaltose is more stable to acid hydrolysis than gentiobiose, and therefore, greater yield of isomaltose cannot alone favour the mechanism of dextrinization via levoglucosan.
Fig. 2.2: Mechanism of Dextrinization
The mechanism of acid dextrinization will essentially be the same as pyrodextrinization except that the hydrolytic reaction is faster. This is then followed by considerable recombination and some transglycosidation.
A. COPOLYMERIZATION OF CORN STARCH WITH GUM KARAYA

As mentioned earlier, although extensive studies have been carried out on dextrinization of starch and mechanism of dextrinization, very little work has been done on modification of other carbohydrate polymers by dextrinization. The studies on dextrinization of starch have shown that transglycosidation is a major pathway by which dextrins are formed. It is also known that hydrolytic cleavage of the glycoside bonds takes place to varying degrees preceding the transglycosidation reaction. Considering the theory of dextrinization, it would be reasonable to assume that if during the course of dextrinization of starch, other substances having sites for acetal formation were present polymers would be formed incorporating the fragments of both compounds. Thus, if a polyfunctional substance such as a monosaccharide is added during dextrinization, it can react (a) through its potential aldehydic group at C1 with a hydroxyl group of an anhydroglucose unit, (b) as an alcohol with the reducing end of a starch fragment or (c) simultaneously through its carbonyl and hydroxylic functions.

Christensen\(^{44}\) has prepared a number of dextrins containing sugar units not present in the original polysaccharide by heating an acidified mixture of a polysaccharide such as amylopectin with xylose, galactose, and lactose. He also prepared codextrins by dextrinization of galactomannan (gum guar) with glucose and maltose and also from mannan and lactose. The new dextrins were fractionated and selected fractions were hydrolysed. Identification of the monosaccharides in the hydrolyzate showed that the added monosaccharide had become an integral part of the new dextrin.
Doubts have been raised about this claim on the basis that the presence of foreign sugars in the hydrolyzates results from acid reversion with the formation of new homopolysaccharides containing only the added monosaccharides. Inadequate purification of the dextrin may also lead to a mixture of dextrins derived from the polysaccharide and a polymer generated from the added sugar by acid reversion. These doubts on codextrin formation from the mixtures of polysaccharides and monosaccharides by dextrinization under acidic condition were removed by the work of Fischer.(45)

Fischer carried out two "tracer" types of experiments. In the first experiment, amylopectin was dextrinized in the presence of uniformly-labelled radioactive glucose in a mole ratio of 6,000 to 1. The acidified mixture was heated at 140° for 3 hours. The radioactivity counts for the reaction mixture, dissolved in known volume of water, before and after purification varied from 1609 to 1165, counts per minute. These results indicated that 72.4% of labelled glucose was incorporated in the dextrin.

The second experiment was concerned with the dextrinization of amylopectin in the presence of 2,3,6-tri-O-methyl-D-glucose. The reaction was carried out as usual. The resultant dextrin was purified by extracting it with solvents known to dissolve any 2,3,6-tri-O-methyl-D-glucose, or polysaccharide resulting from acid reversion of this trimethyl sugar. Methoxyl determination on the purified product showed that 5.6% of the methylated sugar had been incorporated into the dextrin when the mole ratio of amylopectin to 2,3,6-tri-O-methyl-D-glucose was 2:1.
To gain additional information, Fischer co-dextrinized amylopectin with D-galactose under acidic conditions in the usual manner in the mole ratio of 2.5:1. The product was fractionated from aqueous solution by ethanol, and further purification was carried out by acetylation. The purified codextrin contained 13.5% D-galactose and had a molecular weight of approximately 6,000 (D.P. 50) as determined by method of Unrau and Smith. The purified product when subjected to periodate oxidation, reduction and hydrolysis showed that for every mole of galactose there were 3 molar proportions of glucose, 15 of glycerol and 47.8 of erythritol. Further proof came from methylation experiments of codextrin. Fully methylated codextrin on hydrolysis gave, 2,3,4,6-tetra-(16.5%), 2,3,4-tri-(2.5%), 2,3,6-tri-(56.1%), 2,4,6-tri-(0.9%), 2,3-di-(8.8%), 3,6-di-(1%), 2,6-di-(2.8%), and trace amounts of 2-O, 3-O and 6-O-methyl-D-glucose. Also present in the hydrolysate were 2,3,4,6-tetra-(5.7%), 2,4,6-tri-(1.2%), 2,4-di-(1.5%) and a trace of 6-O-methyl-D-galactose. From the variety of tri- and di-O-methyl sugars which have been characterized, it is apparent that the branching is highly diversified as evidenced by single linkages at C5, C4 and C6 and branch linkage combinations involving carbons 4 and 6, 2 and 4, 3 and 6, and 3 and 4 in the glucose and galactose residues.

Parmar has studied physicochemical properties of codextrins prepared from starch and gum karaya. Indirect evidence from these studies reveal that copolymerization between starch and gum karaya takes place when they are dextrinized together in presence of acid. Studies were therefore undertaken to provide more positive evidence for copolymerization when the abovementioned polysaccharides are dextrinized together.
A starch-karaya co dextrin, prepared in the presence of an acid catalyst, was fractionated by treating with such reagents as barium hydroxide, \( \alpha \)-amylase and so on. The fraction resistant to action of \( \alpha \)-amylase was studied in greater detail. It was subjected to acetylation and acetylsalt, after deacetylation, was resolved on a charlad column. The various oligosaccharides were identified. The identification and characterization of oligosaccharides containing glucose and galactose clearly shows that copolymerization takes place when starch and gum karaya are codextrinized.

**EXPERIMENTAL**

**Materials**

**Starch:** Corn starch used for codextrinization was kindly supplied by Anil Starch Products, Ahmedabad (India). It had the following percentage analysis:

- Moisture 11.6
- Solubility 1
- Ash 0.1
- Free Acidity 0.1 (ml. of 0.1N NaOH/100 g of starch)
- Protein 0.4 (N x 6.24)

**Gum Karaya:** Gum karaya, in the form of nodules was obtained from Laboratory Chemical Company, Bombay (India). It was powdered and passed through 200 mesh sieve before use. The gum had the following analysis:

- Moisture 16%
- Acetyl content 15.6%
- Ash 4.6%
- Carboxyl content 42%
- Protein 0.25% (N x 6.24)

Acid number 22.5 and pH of an aqueous slurry, 5.5.
Upon hydrolysis it produced L-rhamnose, D-galactose and D-galacturonic acid and small amounts of D-glucuronic acid.

\(-\)Amylase: \(-\)Amylase used for degradation of the codextrin was obtained from General Biochemicals, Chagrin Falls, Ohio, U.S.A.

**General Methods**

**Evaporation:** All evaporations were carried out under reduced pressure and at low temperature (bath temp. 55-60°C).

**Paper Chromatography:** Paper chromatography was carried out by the descending technique on Whatman filter paper No.1 using the following solvent system (v/v).

\[\begin{align*}
A & \quad \text{Butan-1-ol-pyridine-water (6:4:3)} \\
B & \quad \text{Ethyl acetate-pyridine-water (5:2:5)}
\end{align*}\]

The following reagents were used to locate sugars on chromatograms:

(a) Acetonic silver nitrate-ethanolic sodium hydroxide

(b) p-Anisidine hydrochloride

(c) Diphenylamine urea-phosphoric acid

(d) Periodate-benzidine

**Paper Electrophoresis:** Electrophoresis was carried out on Whatman No.1 filter paper strips at 600 volts for 3 hours in 0.05M sodium tetraborate solution. The sugars on the paper were detected by reagent b.

**Hydrolysis:** Acid hydrolysis of the samples was carried out in 1N H_2SO_4 in sealed tubes at 95°C for 12-16 hours. The hydrolysates were
neutralized with barium carbonate and then filtered. The filtrates, after deionizing with Amberlite IR-120 (H⁺) and IR-45 (OH⁻) resins, were concentrated for examination.

**Acetyl Value:** The determination of acetyl groups was carried out by the ethyl acetate method of Freudenberg and Harder.

**Acid Number:** Acid number was determined according to the procedure of Thrune.

**Uronic Acid:** Uronic acid content of the gum was determined by the method of Barker et al. The gum was treated with 13% hydrochloric acid and the evolved carbon dioxide was absorbed in a standard sodium hydroxide solution. The excess of alkali was then titrated against standard hydrochloric acid solution.

**Gas Chromatography:** Gas-liquid partition chromatography was carried out on a Pye Argon gas chromatograph using four feet glass column of acid washed Gas-chrom (80-100 mesh) containing 1.5 percent each of ethylene glycol succinate polyester and GE-XF at 175°C; argon flow 60 ml/minute.

**Preparation of Starch-Karaya Codextrin:** To a mixture of corn starch (300 g) and gum karaya (200 g), hydrochloric acid (22.6 ml; 8.8N) was added. The mixture was well blended to ensure uniform distribution of the acid throughout the mass. The mixture was screened through a 60 mesh sieve to remove any lumps, and then used for dextrinization. The pH of the mixture, as a 12% slurry in ethanol-water (50:50), was 3.5. The mixture was heated in the dextrinization unit as described earlier at 153 ± 3°C, for 6 hours. The lid of the unit was kept partly open for
the first hour to facilitate the removal of moisture; thereafter it was kept closed. The codevitrin, obtained as above, was dark brown in colour and had the following analysis: Solubility (67), 70.8, reducing power (Ferri cyanide value) (132), 15.95.

RESULTS AND DISCUSSION

Fractionation of Starch-Karava Codevitrin

Codextrin (70 g.) was extracted with water (300 ml. x 3) on a boiling water bath. The water-insoluble portion was separated by centrifugation. It was then washed successively with rectified spirit, absolute ethanol, ethyl ether and petroleum ether (b.p. 40-60°), each three times, to yield a brown product (24 gm., Fr. I). To the aqueous supernatant increasing amounts of saturated barium hydroxide solution were added. The barium complex which separated, was centrifuged. The complex was suspended in water (400 ml.), and after cooling it in ice-salt bath, was decomposed by adding cold 0.2N hydrochloric acid. The resulting solution was poured into excess of ethanol to precipitate the codevitrin. It was then dried by solvent exchange in the usual manner. In this way, three fractions (II-IV) were obtained. The supernatant from fraction IV, which did not give any further insoluble complex with barium hydroxide was acidified with hydrochloric acid (2N). The acidified solution was then dialysed against distilled water till free from acid. The dialysate was concentrated, and then poured into excess of ethanol to give fraction V. Each fraction (50 mg.) was hydrolysed and the hydrolysate, after neutralization, was examined chromatographically in solvents A and B using spray reagents a and b. The details of fractionation as well as the sugars produced on hydrolysis of the
various fractions are given in Table I.

**Table I: Fractionation of Starch-Karaya Codexin with Barium Hydroxide**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Ml. of Ba(OH)$_2$ solution added</th>
<th>Yield in grams</th>
<th>Sugars produced on hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>-</td>
<td>24.0</td>
<td>Galactose, rhamnose, galacturonic acid and traces of glucose.</td>
</tr>
<tr>
<td>II</td>
<td>360</td>
<td>4.0</td>
<td>Glucose and small amounts of galactose and rhamnose.</td>
</tr>
<tr>
<td>III</td>
<td>130</td>
<td>8.6</td>
<td>Glucose and small amounts of galactose and rhamnose.</td>
</tr>
<tr>
<td>IV</td>
<td>270</td>
<td>7.8</td>
<td>Glucose and traces of galactose.</td>
</tr>
<tr>
<td>V</td>
<td>-</td>
<td>18.0</td>
<td>Glucose and traces of galactose.</td>
</tr>
</tbody>
</table>

**α-Amylolytic of Starch-Karaya Codexin**

Codexin (80 gm.) was extracted with hot water (750 ml.) and the insoluble portion was centrifuged off. The clear supernatant was treated with saturated solution of barium hydroxide (1000 ml.) and the resulting insoluble barium complex was centrifuged off. The complex was decomposed by acid in the usual manner. The aqueous acidic solution was poured into excess of ethanol to obtain a dextrin fraction (19 gm.). It was dissolved in water (500 ml.) and the solution thus obtained was dialyzed against distilled water. The nondialyzable portion was concentrated and then poured into ethanol to give a dextrin fraction (15.2 gm.). The latter was dissolved in citrate-phosphate buffer (1500 ml., pH 5.8), and after adding α-amylase (1.5 g.), the mixture was incubated at 38°C for 3 days. After the reaction was over, the enzyme was destroyed by heating the mixture in a
boiling water bath. The insoluble material was centrifuged off, the supernatant was concentrated to a small volume (50 ml.) and then poured into four volumes of ethanol. The precipitate thus obtained was dissolved in water (50 ml.) and the resulting solution poured into excess of ethanol, to yield a codextrin fraction (9.3 gm.). This was once more subjected to the action of \( \alpha \)-amylase, as described earlier, to obtain an \( \alpha \)-amylase-resistant codextrin (7.1 gm.).

The purified codextrin (50 mg.) was hydrolyzed with \( \text{N} \) sulphuric acid and the hydrolysate examined by paper chromatography employing developing solvents A and B, and spray reagents a and b. The hydrolysate showed the presence of glucose, galactose, rhamnose and galacturonic acid.

**Acetolysis of \( \alpha \)-Amylase-Resistant Codextrin**

The dextrin (7.0 gm.) was shaken with a mixture of acetic acid (50 ml.), acetic anhydride (40 ml.) and concentrated sulfuric acid (1.5 ml.) for four days at room temperature. The brown reaction mixture was poured with stirring on crushed ice, and the mixture allowed to stand for 5 hours with occasional stirring after which period it was extracted with chloroform and the chloroform extract washed successively with a solution of sodium bicarbonate and with water. The extract, after drying (\( \text{Na}_2\text{SO}_4 \)), was evaporated to a thick syrup (4.5 gm.). The syrup was dissolved in methanol (200 ml.) and a catalytic amount of sodium was added. The alkaline solution was neutralized with glacial acetic acid and then deionised with Amberlite IR-120 (\( \text{H}^+ \)) resin. The resulting solution was concentrated to a syrup which on paper chromatographic examination using solvents A and B, and
spray reagents a, b and d showed the presence of oligosaccharides in addition to glucose, galactose and rhamnose.

**Separation of Sugars Produced on Acetolysis of Codextrim on Charcoal**

(i) **Separation on a charcoal bed**

The mixture of sugars in the acetolyzate was fractionated on a charcoal bed composed of paper pulp and charcoal. The monosaccharides were removed by elution with water while the oligosaccharides were desorbed by 50% aqueous ethanol. The ethanolic eluate was concentrated to afford a mixture of oligosaccharides (1.7 gm.).

(ii) **Separation of oligosaccharides on a charcoal column**

The mixture of oligosaccharides (1.7 gm.) was put on a charcoal-celite (50:50) column. The column was eluted successively with 5, 10, 15, 20 and 50% aqueous ethanol. The various fractions obtained were analysed by paper chromatography. Similar fractions were combined, and wherever necessary, further purification was carried out by chromatography on paper or by paper electrophoresis. The results of fractionation of sugars on charcoal column are shown in Table II.
<table>
<thead>
<tr>
<th>Oligosaccharide</th>
<th>Rg.(^a)</th>
<th>Mg.(^b)</th>
<th>Eluant</th>
<th>Yield (g.)</th>
<th>Sugars produced on hydrolysis</th>
<th>Probable oligosaccharide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.78</td>
<td>0.32</td>
<td>5% aq. ethanol</td>
<td>0.311</td>
<td>Glucose</td>
<td>Maltose</td>
</tr>
<tr>
<td>2</td>
<td>0.64</td>
<td>0.69</td>
<td>&quot;</td>
<td>0.015</td>
<td>&quot;</td>
<td>Rg value corresponds to isomaltose and maltotriose, but Mg value clearly indicates isomaltose. Reaction with reagent (g) shows (1 \rightarrow 6) linkage.</td>
</tr>
<tr>
<td>3</td>
<td>0.54</td>
<td>0.77</td>
<td>&quot;</td>
<td>0.010</td>
<td>Glucose &amp; galactose</td>
<td>Melibiose; a (1 \rightarrow 6) linkage is indicated by reagent (g) and by Mg value.</td>
</tr>
<tr>
<td>4</td>
<td>0.48</td>
<td>-</td>
<td>5% &amp; 10% aq. ethanol</td>
<td>0.044</td>
<td>Glucose</td>
<td>Maltotetraose</td>
</tr>
<tr>
<td>5</td>
<td>0.38</td>
<td>0.30</td>
<td>10% aq. ethanol</td>
<td>0.033</td>
<td>Glucose</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>0.34</td>
<td>0.25</td>
<td>15% aq. ethanol</td>
<td>0.023</td>
<td>Glucose &amp; galactose</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>0.30</td>
<td>0.23</td>
<td>15% &amp; 20% aq. ethanol</td>
<td>0.025</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Higher oligosaccharides</td>
<td>-</td>
<td>50% aq. ethanol</td>
<td>0.45</td>
<td>Glucose</td>
<td>Higher malto oligosaccharides.</td>
</tr>
</tbody>
</table>

\(^{a}\) Rg = Rate of movement of oligosaccharides on paper with respect to glucose in solvent (\(A\)).

\(^{b}\) Mg = Rate of movement of oligosaccharide on pherogram with respect to glucose in 0.05M sodium tetraborate.
Identification of Melibiose

The disaccharide had the same mobility on paper and on pherogram as that of melibiose. Upon hydrolysis it produced equal amounts of glucose and galactose. The amounts of galactose and glucose were estimated by NaBH₄ reduction followed by acetylation and examination of the mixture of alditol acetates by gas chromatography, using a column of 1.5% GE-XF and 1.5% ethylene glycol sebacate polyester on Chromosorb-W at 175° (152).

To a solution of the disaccharide (2 mg.) in water (2 ml.) sodium borohydride (2 mg.) was added and the mixture allowed to stand at room temperature for an hour. After the reduction was complete, the mixture was acidified with glacial acetic acid and the resulting solution, after removal of Na ions by Amberlite IR 120 (H⁺), was distilled repeatedly with methanol to remove boric acid. The reduced disaccharide was hydrolysed with N H₂SO₄ in the usual manner.

The neutral hydrolyzate on paper chromatographic analysis employing solvent A, and spray reagent a showed the presence of galactose only, indicating that the reducing end in the disaccharide was glucose.

The disaccharide (5 mg.) was dissolved in N, N-dimethylformamide (DMF, 2 ml.) and to it were added methyl iodide (5 ml.) and silver oxide (1 g.). The mixture was shaken in the dark for 24 hours. The reaction mixture was poured into chloroform and the inorganic impurities were centrifuged off. The clear chloroform solution was evaporated to a syrup which was methylated once more by the procedure described above using methyl iodide (5 ml.) and silver oxide (500 mg.).
The methylated disaccharide was refluxed with 3% methanolic hydrogen chloride for 8 hours. The methanolyzate was neutralized with silver carbonate, and the inorganic salts were removed by centrifugation. The clear supernatant was then evaporated to a syrup.

**Examination of Methylated Sugars by Gas-Liquid Chromatography**

The mixture of methyl ether methyl glycosides, obtained as above, was resolved by gas-liquid chromatography using a column of acid-washed Gaschroom containing 1.5% each of ethylene glycol succinate polyester and GE-XF. The analysis revealed the presence of (1) methyl 2,3,4,6-tetra-O-methyl-β-D-galactoside, (2) methyl 2,3,4-tri-O-methyl-β-D-glucoside and (3) methyl 2,3,4-tri-O-methyl-α-D-glucoside. (Fig. 1). The ratio calculated on the basis of peak areas of (1):(2) + (3) was found to be 1:1. This indicated that, in the disaccharide, galactose unit was linked to glucose by a 1→6 glycosidic bond.

From the foregoing evidence, the disaccharide is designated 6-O-α-D-galactopyranosyl-D-glucopyranose (melibiose).

**Identification of O-α-D-Galactopyranosyl-(1→6)-O-D-Glucopyranosyl-(1→4)-D-Glucose**

Elution of the carbon column with 15% aqueous ethanol gave a number of oligosaccharides (Table II). These fractions were examined in greater detail. Fraction 6 (28 mg., Table II) showed more than one spot when examined chromatographically on paper using solvent A. The fraction when examined by paper electrophoresis showed the presence of a major component having a 
Mg of 0.25. This component was, therefore,
Gas-liquid chromatogram of methylated and methanolized disaccharides. (1) Methyl 2,3,4,6-tetra-O-methyl-D-galactoside, (2) Methyl 2,3,4-tri-O-methyl-β-D-glucoside, and (3) Methyl 2,3,4-tri-O-methyl-α-D-glucoside.

Fig. 1
separated by paper electrophoresis in the usual way. The resulting syrup (11 mg.) gave a single spot with reagent a when examined chromatographically on paper in solvent A. However, it gave two spots on the paper chromatogram developed by solvent B (R_maltose, 0.4 and 0.24). The mixture was separated on paper using solvent B to yield the faster moving oligosaccharide (7 mg.). Acid hydrolysis of the oligosaccharide gave galactose and glucose in the proportion of 1:2. The ratio of the constituent sugars was determined as described earlier.

The trisaccharide was reduced with sodium borohydride in the same way as described earlier for melibiose. The reduced trisaccharide upon hydrolysis followed by paper chromatographic examination (solvent A spray reagent a) showed the presence of glucose and galactose. This showed that the reducing end of the trisaccharide was a glucose residue.

Trisaccharide (3 mg.) was permethylated and methanolyzed as described earlier. The resulting mixture of methyl glycosides was examined by gas-liquid chromatography on a column consisting of acid-washed Gas-chrom containing 1.5% each of ethylene glycol succinate polyester and GE-XF. The analysis revealed the presence of methyl 2,3,4,6-tetra-O-methyl-α-D-galactoside (1), methyl 2,3,4-tri-O-methyl-β-D-glucoside (2), methyl 2,3,6-tri-O-methyl-β-D-glucoside (3), methyl 2,3,4-tri-O-methyl-α-D-glucoside (4), and methyl 2,3,6-tri-O-methyl-α-D-glucoside (5) (Fig. 2), the compounds 1, 2 + 4, and 3 + 5 being in approximately equal proportions. On the basis of the above results two structures (I) and (II) may be written for the trisaccharide.

\[
\text{Gap-(1 \rightarrow 6)-Galp-(1 \rightarrow 4)-Gal} \quad \text{and} \quad \text{Gap-(1 \rightarrow 4)-Galp-(1 \rightarrow 6)-Gal}
\]

(I) \hspace{2cm} (II)
To decide which of the two structures, I and II, represents the trisaccharide the following experiments were carried out.

The trisaccharide was reduced with sodium borohydride in the usual manner and the resulting trisaccharide alcohol was methylated and methanolysed according to procedures described earlier. The methylated fragments were examined by gas-liquid chromatography to show the presence of methyl 2,3,4,6-tetra-O-methyl-α-D-galactoside (1), methyl 2,3,4-tri-O-methyl-β-D-glucoside (2), 1,2,3,5,6-penta-O-methyl-D-glucitol (3) and methyl 2,3,4-tri-O-methyl-α-D-glucoside (4). (Fig. 3). Calculation of the peak areas showed that compounds 1, 2 + 4, and 3 were present approximately in equal proportions. An authentic sample of 1,2,3,5,6-penta-O-methyl-glucitol was prepared by methylation and hydrolysis of permethylated maltitol. These results clearly show that the reducing end of the trisaccharide is a glucose residue, which is linked to another glucose unit by a 1→4 glycosidic bond. The trisaccharide is therefore assigned the structure (I). Inasmuch as the disaccharide melibiose, identified earlier, and starch contain α-glucosidic bonds, the trisaccharide, isolated from the codein, is designated as 0-α-D-galactopyranosyl-(1 →6)-0-α-D-glucopyranosyl-(1 →4)-D-glucose.

Oligosaccharides can be synthesized from their monomer sugar units by means of enzymes. They can also be formed by the action of acid and heat on the monosaccharides (acid reversion). In order to test whether the disaccharide and trisaccharide, isolated and identified as described earlier, had arisen from copolymers of starch and gum
Gas-liquid chromatogram of reduced, methylated and methanolyzed hirundocharide. (1) Methyl 2,3,4,6-tetra-\(\alpha\)-methyl-\(\beta\)-d-galactoside, (2) Methyl 2,3,4,6-tetra-\(\alpha\)-methyl-\(\beta\)-d-glucoside, (3) Methyl 2,3,4,6-tetra-\(\alpha\)-methyl-\(\beta\)-d-glucoside, and (4) Methyl 2,3,4,6-tetra-\(\alpha\)-methyl-\(\beta\)-d-glucoside.

Fig. 3
karaya or they were products resulting from enzymic transglycosidation, the following experiment was carried out.

**Action of α-Amylase on a Mixture of Glucose and Galactose**

A mixture of glucose (75 mg.) and galactose (75 mg.) was dissolved in citrate-phosphate buffer (pH, 5.8; 15 ml.) and to the solution α-amylase (15 mg.) was added. The mixture was then placed in a water bath at 37° for 5 days. During storage, the mixture was shaken occasionally. After digestion, the solution was heated in a boiling water bath for 1 hour to destroy the enzyme. The mixture was filtered, and deionized by successive treatments with Amberlite IR 120 (H+) and IR 45 (OH-) ion exchange resins. The solution was then concentrated and examined by paper chromatography using solvent A and spray reagent d. The paper chromatogram showed the presence of glucose, galactose, and an oligosaccharide (Rf, 0.24). The oligosaccharide was isolated by preparative paper chromatography. Hydrolysis of the oligosaccharide with acid followed by paper chromatography of the hydrolyzate, using solvent A and spray reagent a showed the presence of glucose only. These results thus showed that although the enzyme had the capacity to bring about synthesis of glucose disaccharide, it was not capable of synthesizing \( \text{ga} \rightarrow \text{gl} \) disaccharides or trisaccharides.

**CONCLUSION**

The fractionation and fragmentation analyses of starch-karaya codextrin resulting in the isolation and identification of 6-0-\( \alpha \)-D-galactopyranosyl-D-glucose (melibiose) and O-\( \alpha \)-D-galactopyranosyl-
(1 → 4)-D-glucose clearly show that copolymerisation of the molecules of starch and gum karaya or fragments thereof takes place during dextrinization of the starch-karaya mixture. This study thus supports and extends the findings of Smith et al. (110), that copolymerization takes place when polysaccharides are heated with other carbohydrate substances in the presence of acid. It is, however, for the first time that copolymerisation between two polysaccharides has been demonstrated.
B. STUDIES ON GUAR GUM DEXTRIN
It has been shown earlier that gum guar inhibits the dextrinization of starch in the starch-guar mixture. In order to understand the role of gum guar in the starch-guar mixture during dextrinization, it was considered of interest to examine the chemical changes that take place when guar gum is dextrinized alone but in the presence of an acid catalyst. Inasmuch as extensive studies on dextrinization have been carried out only on starches, which are glucans, having a majority of $\alpha 1 \rightarrow 4$ linkages, it was thought of interest to see how a predominantly $\beta 1 \rightarrow 4$ linked polysaccharide would behave during dextrinization.

Review of the past literature reveals that very little work has been done on the dextrinization of polysaccharides other than starch. Cellulose is the most widely known and extensively used polysaccharide of commerce. Structural studies have revealed that it is a polymer in which D-glucose units are linked to each other by $\beta 1 \rightarrow 4$ glucosidic linkages. Christensen\(^{(44)}\) made an attempt to dextrinize cellulose and its derivatives such as hydrocellulose and methylcellulose. In each case 4 gm. of material was sprayed with hydrochloric acid (0.13 ml. 2.2N) and aged for 12 hours in a closed glass container. The material, after ageing, was roasted at 140° (bath temp.) with stirring for 15 hours. The resultant dextrins obtained from cellulose, hydrocellulose and methylcellulose differed in solubility from each other. Cellulose gave water solubles to the extent of 1.49 and 8.4% respectively, while methylcellulose did not
give any soluble product even in hot water. The soluble products were examined chromatographically. In all the cases, water-soluble products gave 5 spots when detected with Tollen's reagent. (154) No attempt was made to characterize these spots in terms of sugars.

To secure evidence for the process of transglycosidation during dextrinization reaction, purified guar gum was dextrinized by Fischer. (45) The purified gum (3.2 g) was ground in a mortar and sprayed with hydrochloric acid (0.16 ml., 2.2N). Acidified material was thoroughly mixed and aged overnight in a closed glass container. After ageing, the gum was heated for 3 hours in a nitrogen atmosphere with stirring. The dextrin was purified and then subjected to periodate oxidation, reduction and hydrolysis. Chromatographic analysis of the hydrolysate revealed the presence of mannose, glycerol and erythritol.

It is well-known that guar gum is a galactomannan. The constituent sugars, namely, mannose and galactose are oxidized when treated with periodate. Detection of mannose, after periodate oxidation of guar dextrin, clearly indicates that the original mannose units of gum guar are rearranged during dextrinization in such a way that they become immune to the action of sodium meta periodate. This may be due to the formation of new glycosidic linkages involving the hydroxyl groups at C3 or C2 and C4 of the mannose unit.

Dextrinization of galactomannan (gum guar), for industrial applications, has been patented by Swanson. (40) Claim is made for
the application of modified guar gum, as sizing or coating material for cellulosic sheet products. The modification consists in roasting guar powder (50-200 mesh) in the presence or absence of an added catalyst. Roasting is carried out until such time that the guar dextrin as a one percent aqueous dispersion prepared by heating at 85°C and then cooling to 30°C, gives a viscosity of about 15 centipoises. The viscosity of unmodified product by this method varies between 600 and 700 centipoises.

The present investigation deals with structural studies on guar dextrin with a view to understanding the changes that take place when the guar gum is dextrinized. In Part I of this thesis it was observed that the physicochemical changes that occur when gum guar is subjected to treatment of heat in absence and presence of acid, differ markedly from those which take place in the case of starch. The most striking and interesting behaviour noticed is the decrease in solubility with progressive dextrinization. Similar observation has been made in the dextrinization of other seed polysaccharides such as tamarind kernel polysaccharide, locust bean gum, gum karaya and gum ghatti.

**MATERIAL AND METHODS**

Gum guar (sample A), obtained from the local market, was screened through 100 mesh sieve and was used without further purification.

(a) **Preparation of the Dextrin**

To guar gum powder (300 g.) was added 2N hydrochloric acid (15 ml.), and after thorough mixing, the gum was roasted in a dextrinizer unit (described earlier) at 155°C for 8 hours.
(b) **General Methods**

All evaporations were carried out at 55-60°C under reduced pressure.

**Paper Chromatography:** Paper chromatography was carried out by the descending technique on Whatman No.1 filter paper using the following solvent systems (V/V):

(A) Butan-1-ol:pyridine:water (6:4:3)

(B) Butanone-water azeotrope

The following reagents were used to locate sugars on chromatograms:

(1) Acetonic silver nitrate-ethanolic sodium hydroxide

(2) p-Anisidine hydrochloride

(3) Periodate-benzidine

**Hydrolysis:** Acid hydrolysis of the samples was carried out with 1N H₂SO₄ in sealed tubes at 95-100°C for 12-16 hours. The hydrolysates were neutralized with barium carbonate and then filtered. The filtrates, after deionizing with Amberlite IR-120 (H⁺) and IR-45 (OH⁻) resins, were concentrated for examination.

**Determination of Sugar Ratio:** Determination of sugar ratio in the hydrolysates of various fractions was carried out by the phenol-sulphuric acid method of Smith et al.

**Preparation of Standard Curves for Galactose and Mannose:** Dried sugar (40 mg.) was dissolved in water (1 l.) and aliquots of the solutions containing 5 to 50 micrograms of the sugars were taken in triplicate.
and the volume was made up to 2 ml. with water. Required amounts of 80% aqueous phenol was added to each solution followed by sulfuric acid (5 ml.) taking care that the acid fell straight onto the solution. After thorough mixing, the tubes were allowed to stand for 15 minutes and then cooled in running water. A water blank for each sugar was also prepared in the same way and was used to adjust the colorimeter (Spectronic 20) to zero. The absorbance was read at 490 nm. The amount of phenol used for galactose and mannose was 0.04 ml. and 0.05 ml. of 80% aqueous solution respectively. The standard curves for galactose and mannose are given in Fig. 4.

Determination of Sugar Ratios in Guar Gum and Its Dextrin Fractions:
The mixtures of sugars obtained on hydrolysis of the gum and its dextrin fractions were resolved by paper chromatography, using solvent A. The different sugar zones, as revealed by guide strips, were cut out and extracted with equal amounts of water for an hour. The extract was filtered through a glass wool plug. Aliquots of the filtrate were taken and the sugar estimated according to the procedure outlined earlier for pure sugar solutions. A portion of the paper chromatogram, having no sugar on it but equal to the area of sugar-containing zone, was extracted with water. This extract served as blank for colorimetric readings.

Fractionation of the Dextrin

The dextrin (100 gm.) was extracted with methanol in a soxhlet extractor, till the extract gave negative test with Molisch reagent. The extract was then evaporated to constant weight, to obtain a thick syrup.
Absorbance

Fig. 4: Standard Curves of Sugars

I Mannose
II Galactose

Micrograms of Sugar
From the residue left after methanol extraction, 80 g. was taken for further fractionation. The residual dextrin was extracted with water (1800 ml.) at room temperature for 5 hours with mechanical stirring. The soluble and insoluble portions were separated by centrifugation. The clear supernatant was concentrated and poured over excess of ethanol to give fraction I.

The insoluble portion of the dextrin was then extracted three times with hot water (95°, 5 x 1500 ml.). Each time the soluble and insoluble portions were separated by centrifugation. The extracts were concentrated and poured over excess of ethanol to afford fractions II-IV. The residual dextrin was extracted successively with 15% and 17% aqueous sodium hydroxide (750 and 300 ml., respectively) in an atmosphere of nitrogen. Dextrins were recovered from these extracts by precipitation with acidified ethanol. These fractions are designated V and VI respectively. The residue left after extraction with 17% sodium hydroxide was washed with acidified ethanol, to get fraction VII. All these fractions were dried by solvent exchange technique i.e. the precipitates obtained were trituted with 95% ethanol, absolute ethanol, ethyl ether and pet. ether (b.p. 40-60°) in this order, and then dried over calcium chloride in vacuum.

Analysis of the Fractions

The various fractions were hydrolyzed and the neutral, ion-free hydrolyzates were chromatographed on paper using solvent A and the developed chromatograms, after drying, sprayed with reagents 1, 2 and 3. All the fractions showed the presence of galactose, mannose, glucose and xylose. The latter two sugars were present only in trace amounts.
The methanol extract showed presence of galactose and mannose.

Many investigators (22-33) have studied gum guar from different points of view but no one has reported the presence of glucose and xylose. In order to establish their presence as constituents of the gum, many gum samples, obtained from various suppliers, were hydrolysed. All these samples showed the presence of glucose and xylose in addition to galactose and mannose. The results of fractionation are given in Table III.

**TABLE III. FRACTIONATION OF GUAR DEXTRIN**

<table>
<thead>
<tr>
<th>Fraction Number</th>
<th>Weight in gm.</th>
<th>$\Delta^D$</th>
<th>Ratio of Galactose: Mannose</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>15.2</td>
<td>+47°</td>
<td>1:2</td>
</tr>
<tr>
<td>II</td>
<td>8.0</td>
<td>+51°</td>
<td>1:1.75</td>
</tr>
<tr>
<td>III</td>
<td>7.5</td>
<td>+41°</td>
<td>1:1.55</td>
</tr>
<tr>
<td>IV</td>
<td>4.6</td>
<td>+45°</td>
<td>1:1.6</td>
</tr>
<tr>
<td>V</td>
<td>38.0</td>
<td>+36°</td>
<td>1:1.6</td>
</tr>
<tr>
<td>VI</td>
<td>1.2</td>
<td>not observable</td>
<td>1:1.4</td>
</tr>
<tr>
<td>VII</td>
<td>1.2</td>
<td>n.</td>
<td>-</td>
</tr>
</tbody>
</table>

As all these fractions showed the presence of glucose and xylose, Fractions I to IV were further fractionated.

Sub-fractionation of Fractions I to IV: All the fractions were dissolved separately in water (500 ml.) and to the clear solution an increasing volume of ethanol was added to obtain further fractions or sub-fractions. The precipitated fractions were removed by centrifugation and the precipitates were dried in the usual way.
Hydrolysis of the sub-fractions and chromatographic examination of the hydrolysates showed the presence of galactose, mannose, glucose and xylose, the latter two sugars being in trace amounts only.

The foregoing analysis established the presence of polysaccharides other than galactomannan in the crude gum guar, hence further study was carried out on purified gum guar.

**STUDIES ON DEXTRANIZATION OF PURIFIED GUAR GUM**

**Purification of Guar Gum**

Guar gum powder (100 gm.) was dispersed in water (15 litres) with mechanical stirring. The stirring was continued for 2 hours as during this period guar gum solution obtains maximum viscosity which is not much increased on prolonged aging. The viscous solution was then centrifuged to remove lumps as well as other impurities. To the clear viscous solution Fehling solution was added dropwise with constant stirring. The gel-like copper complex was filtered on muslin cloth, washed with dilute Fehling solution and squeezed to remove excess of the reagent. The complex was then suspended in water (4 litres), cooled in ice-salt bath, and with stirring, acidified with cold 1N HCl. The resulting viscous solution was then filtered through a coarse sintered glass funnel and to the clear solution ethanol (10 litres) was added to precipitate out the gum as a white fibrous mass. The latter, without drying, was fractionated three times more with Fehling solution. The final product was washed with 95% aqueous ethanol, absolute ethanol, ethyl ether and petroleum ether (40-60°) each twice in this order to yield a white fibrous polysaccharide (40 gm.).
Analysis of Purified Guar Gum

The purified product was hydrolysed in the usual way. The neutral hydrolysate on chromatographic analysis using solvent A and reagent 1 showed the presence of galactose and mannose, but trace amounts of glucose and xylose were also detectable. The ratio of galactose to mannose in the purified gum was found to be 1:1.26. The presence of polysaccharide(s) containing glucose and xylose and a higher proportion of galactose in gum guar are findings which are different from those of earlier workers. Though the ratio of galactose:mannose (1:1.26) is different from earlier workers it is very close to the value (1:1.3) reported by Hui & Neukom for the cold-water soluble portion of gum guar. The gum was also analysed for protein content, which was found to be negligible. The ash content of the gum was found to be 0.17%.

Dextrinization of Purified Gum Guar

Purified gum guar, obtained as a white fibrous mass, was powdered manually and the powder was sieved through 50 mesh sieve. Guar powder (30 gm.) was suspended in 60 ml. of acetone containing 0.9 ml. of 1.99N hydrochloric acid. After thoroughly stirring the slurry, the solvent was removed by filtration and dried at room temperature. The gum was put in the flask (250 ml.) of a rotary vacuum film evaporator and dextrinized at 153°C, the flask rotating at a speed of 95 r.p.m. In order to remove the moisture and the residual acetone, vacuum from a water aspirator was applied to the dextrinizer flask for the first 20 minutes. Thereafter, the dextrinization was carried out for four hours. The yield of dextrin was 28 gms.
Fractionation of the Dextrin

Guar dextrin (26 gm.) was extracted with methanol repeatedly in a Soxhlet extractor until the extract gave negative test with Molisch reagent. Methanol extracts were combined and evaporated to constant weight (Fr. I, 6.92 gm.). The dextrin left after methanol extraction was suspended in water (1 litre). The resulting slurry was stirred for 2 hours. The soluble and insoluble portions were separated by centrifugation. The clear supernatant was concentrated to one-fourth of the original volume and the polysaccharide was precipitated by pouring it over excess of ethanol (Fr. II, 2.67 gm.). Cold-water insoluble dextrin was extracted with hot water (1 litre, 95°) for one hour with constant stirring. The resulting paste was centrifuged to obtain hot water-soluble and insoluble portions. The clear supernatant was concentrated as usual, and the polysaccharide precipitated by pouring over excess of ethanol (Fr. III, 2.81 gm.). The hot water-insoluble dextrin was extracted successively with 10% sodium hydroxide (350 ml.) and 17% sodium hydroxide (200 ml.) in an atmosphere of nitrogen. From the extracts fractions IV (4.81 g.) and V (2.71 g.) were isolated by pouring them into acidified ethanol. The residue was washed with acidified ethanol and then with ethanol till free from acid.

All fractions were dried by washing successively with aqueous ethanol, absolute ethanol, ether and petroleum ether 40-60° followed by drying under vacuum.

Determination of Galactose:Manrose Ratio in Various Fractions

Each fraction (100 mg.) was hydrolysed with 1N sulfuric acid in
the usual way. The hydrolysate was neutralised with BaCO₃ and
deionised by giving successive treatments with resins IR-120 (H⁺) and
IR-45 (OH⁻). Neutral, ion-free hydrolysate was analysed for sugars
by paper chromatography using solvent A and detecting reagents 1 and 2.
Almost all fractions showed the presence of galactose and mannose with
very faint spots for glucose and xylose when treated with spray
reagent 1. The ratio of galactose to mannose in the hydrolysates was
determined by the method of Smith et al. (156).

The results of fractionation in respect of yield and galactose:
mannose ratio are given in Table IV.

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Solvent Extracted with</th>
<th>Quantity of Solvent</th>
<th>Yield (g.)</th>
<th>Galactose:Mannose</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Methanol Soxhlet Extraction</td>
<td>0.82</td>
<td>1:0.07</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Cold water 1 litre</td>
<td>2.67</td>
<td>1:1.36</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Hot water 1 litre</td>
<td>2.61</td>
<td>1:1.56</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>10% NaOH 350 ml.</td>
<td>4.81</td>
<td>1:2</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>17% NaOH 200 ml.</td>
<td>2.71</td>
<td>1:1.42</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>Residue</td>
<td>8.05</td>
<td>1:1.44</td>
<td></td>
</tr>
</tbody>
</table>

Periodate Oxidation of Guar Gum and Its Dextrins

Purified guar gum (0.107 gm.) and guar dextrin fractions IV
(0.129 gm.) and VI (0.232 gm.) were oxidised with 0.01M sodium meta-
periodate in the dark at 10°. The periodate consumption and formic
acid production were followed in the usual manner. After 315 hours
the consumption of periodate and liberation of formic acid was constant.
The results are given in Table V.

**TABLE V: PERIODATE OXIDATION OF GUAR GUM AND GUAR DEXTRINS**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Consumption of periodate (mole/mole)</th>
<th>Liberation of formic acid (mole/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guar Gum</td>
<td>1.29</td>
<td>0.25</td>
</tr>
<tr>
<td>Guar dextrin (Fr.IV)</td>
<td>1.22</td>
<td>0.23</td>
</tr>
<tr>
<td>Guar dextrin (Fr.VI)</td>
<td>1.27</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Reduction and Hydrolysis of the Periodate-Oxidized Guar Gum and Dextrins

The remaining solution from the periodate oxidation was evaporated to dryness following the removal of iodate and periodate ions, reduction with sodium borohydride (200 mg.) for more than 12 hours, and acidification with acetic acid. After the borates were removed, the polyalcohol was hydrolysed with N sulfuric acid (10 ml.) in a sealed tube as usual. Subsequent neutralization, deionization and concentration yielded a colourless syrup. Examination of guar dextrin hydrolysates by paper chromatography using solvent A and spray reagents 1, 2 and 3 furnished mannose and galactose in addition to glycerol and ethylene glycol. No galactose or mannose was found in case of gum guar.

**Methylation of Guar Dextrin (Fraction IV)**

Guar dextrin, fraction IV (3.5 gm.) was suspended in water (50 ml.) and to this suspension 40% sodium hydroxide (250 ml.) and dimethyl sulfate (60 ml.) were added dropwise with stirring in an atmosphere of nitrogen. For the first three hours the reaction was carried out at
low temperature (15-20°) and in a nitrogen atmosphere. Thereafter, the reaction was carried out at room temperature. The reaction mixture was stirred overnight and then heated at 95° for an hour to decompose excess of dimethyl sulfate. After cooling in an ice-salt bath, the excess of alkali was partially neutralised with cold 10N sulfuric acid, and finally with acetic acid. Sodium sulfate was removed by filtration. The filtrate was extracted with chloroform (200 x 2) for 12 hours, and the chloroform extract evaporated to a syrup (80 mg.). Aqueous solution left after chloroform extraction was dialysed first against tap water and then against distilled water. Most of the polysaccharide which remained with sodium sulfate was recovered by dialysis. Both the dialysates were combined and concentrated to a small volume (50 ml.). Chloroform extract and the dialysate were combined, and once again methylated by adding dropwise 40% sodium hydroxide (250 ml.) and dimethyl sulfate (60 ml.). After overnight stirring the reaction mixture was worked up as described above, to give a partially methylated guar dextrin (0.1 g.) along with unreacted dextrin.

It was observed that the dextrin obtained from sodium sulfate after dialysis, swelled in dimethyl sulfoxide, hence it was further methylated by the method of Srivastava et al. (159).

**Methylation by the DMSO Method (159)**

The combined partially methylated and unreacted guar dextrin, obtained as described earlier, was suspended in DMSO (100 ml.) and shaken for three hours. To the resulting swollen mass, sodium hydroxide pellets (30 gm.) and dimethyl sulfate (60 ml.) were added.
during a period of six hours with constant vigorous stirring. The reaction mixture was stirred overnight and then heated to 95° for an hour to decompose excess of dimethyl sulfate. Water was added to dissolve the residual sodium hydroxide. It was then cooled in ice-salt bath and partially neutralised with cold 10 N sulfuric acid. Final neutralization was done by dilute acetic acid. The reaction mixture was filtered and the filtrate extracted with chloroform. The chloroform extract was washed with water, dried over Na₂SO₄ and evaporated to yield the methylated product (0.340 gm.). The infrared spectrum of the methylated product showed presence of hydroxyl absorption. The byproduct, sodium sulfate, was dissolved in a small volume of water and dialysed first against tap water and then against distilled water, until the dialysate gave a negative test for sulfate ions. The dialysate was concentrated and poured over excess of ethanol to give guar dextrin (0.95 gm.).

**Purdie Methylation** (180)

The product obtained as a chloroform extract after DMSO method was dissolved in methyl iodide (10 ml.) and refluxed with silver oxide (6.0 gm.), the latter being added in small amounts over a period of six hours. The reaction mixture was filtered and worked up in the usual manner. One more such methylation yielded a product (0.520 gm.) which showed very little hydroxyl absorption in the infrared.

**Fractionation of Methylated Guar Dextrin**

The product (0.520 gm.), obtained as above, was dissolved in acetone (10 ml.) and to it was added dry ether (15 ml.) to precipitate
out inorganic impurities. The impurities were centrifuged off. To the supernatant increasing amounts of petroleum ether was added and the material which separated was centrifuged off. In this way, fractions I, II and III were obtained. After the third fraction was removed, further addition of petroleum ether to the supernatant did not yield any precipitate. The supernatant was therefore evaporated to give fraction IV. The fractions I, II, III and IV amounted to 0.019, 0.058, 0.22 and 0.005 gms. respectively.

Hydrolysis of Methylated Guar Dextrin

Methylated guar dextrin (Fraction III, 20 mg.) was suspended in 72% sulfuric acid for an hour at room temperature. It was diluted with water (10 ml.) and heated on a boiling water bath for 4 hours. The hydrolysate, after neutralization with BaCO$_3$ was deionized by passing through Amberlite IR-120 (H$^+$) and IR-45 (OH$^-$) resins and the resulting solution evaporated. The hydrolysate on chromatography in solvent B and spraying with reagent 2 showed the presence of a number of methylated sugars as shown in Table VI.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Spot No.</th>
<th>R*MG Found</th>
<th>R*MG Reported Value</th>
<th>Probable Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guar dextrin</td>
<td>1</td>
<td>0.99</td>
<td>1.0</td>
<td>2,3,4,6 Tetra-O-methyl-D-mannose</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.85</td>
<td>0.87</td>
<td>2,3,4,6 Tetra-O-methyl-D-galactose</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.78</td>
<td>0.77</td>
<td>2,3,4 Tri-O-methyl-D-mannose</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.71</td>
<td>0.71</td>
<td>2,4,6 Tri-O-methyl-D-mannose</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.61</td>
<td>0.64</td>
<td>2,3,6 Tri-O-methyl-D-mannose and/or 2,3,6 Tri-O-methyl-D-galactose</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.52</td>
<td>-</td>
<td>Unidentified</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.46</td>
<td>0.46</td>
<td>2,3,4 Tri-O and/or 2,4,6 Tri-O-methyl-D-galactose</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.39</td>
<td>-</td>
<td>Unidentified</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>0.29</td>
<td>0.28</td>
<td>2,5 Di-O-methyl-L-mannose</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.18</td>
<td>-</td>
<td>Unidentified</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>0.08</td>
<td>-</td>
<td>Unidentified</td>
</tr>
</tbody>
</table>

*R*MG = Tetra-O-methyl-D-glucopyranose.
Identification of Methylated Sugars by Gas-Liquid Chromatography

Hydrolyzate of methylated guar dextrin was analysed by gas-liquid chromatography, on a four-foot column of 10% butanediol succinate* at 150° on a Pye Argon Chromatograph. The following methylated sugars were identified by their retention time and their ratios determined by areas under the peaks. (Fig. 5).

<table>
<thead>
<tr>
<th>Methylated Sugar</th>
<th>Retention Time RT</th>
<th>Molar Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 2,3,4,6 Tetra-O-methyl-D-mannose</td>
<td>0.99</td>
<td>1.0</td>
</tr>
<tr>
<td>2. 2,3,4,6 Tetra-O-methyl-D-galactose</td>
<td>1.26</td>
<td>7.0</td>
</tr>
<tr>
<td>3. 2,3,4 Tri-O-methyl-D-mannose</td>
<td>2.18</td>
<td>2.5</td>
</tr>
<tr>
<td>4. 2,3,6 Tri-O-methyl-D-galactose</td>
<td>2.34</td>
<td>2.0</td>
</tr>
<tr>
<td>5. 2,4,6 Tri-O-methyl-D-mannose</td>
<td>2.69</td>
<td>0.25</td>
</tr>
<tr>
<td>6. 2,4,6 Tri-O-methyl-D-galactose</td>
<td>2.89</td>
<td>4.0</td>
</tr>
<tr>
<td>7. 2,3,6 Tri-O-methyl-D-galactose</td>
<td>3.31</td>
<td>10.0</td>
</tr>
<tr>
<td>9. Unidentified</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>10. 2,3,4 Tri-O-methyl-D-galactose</td>
<td>5.5</td>
<td>2.5</td>
</tr>
<tr>
<td>11. 2,3 Di-O-methyl-D-mannose</td>
<td>11.12</td>
<td>8.0</td>
</tr>
</tbody>
</table>

*This column was used previously for lipid analysis. However, when a fresh column of butanediol succinate was used, the resolution was not good.

2,3,6 Tri-O-methyl-D-galactose gave two peaks corresponding to α- and β-anomers, the latter being more prominent. The α-anomer had the same retention time as methyl 2,4,6 tri-O-methyl-α-D-galactose. Attempt was made to separate them on a programmed column of 10% neopentyl glycol succinate but without success.
<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Probable Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,3,4,6 Tetra-O-methyl-D-mannose</td>
</tr>
<tr>
<td>2</td>
<td>2,3,4,8 Tetra-O-methyl-D-galactose</td>
</tr>
<tr>
<td>3</td>
<td>2,3,4 Tri-O-methyl-D-mannose</td>
</tr>
<tr>
<td>4</td>
<td>2,4,6 Tri-O-methyl-D-mannose</td>
</tr>
<tr>
<td>5</td>
<td>2,3,6 Tri-O-methyl-D-mannose and/or 2,5,6 Tri-O-methyl-D-galactose</td>
</tr>
<tr>
<td>6</td>
<td>Unidentified</td>
</tr>
<tr>
<td>7</td>
<td>2,3,4 Tri-O- and/or 2,4,6 Tri-O-methyl-D-galactose</td>
</tr>
<tr>
<td>8</td>
<td>Unidentified</td>
</tr>
<tr>
<td>9</td>
<td>2,3 Di-O-methyl-D-mannose</td>
</tr>
<tr>
<td>10</td>
<td>Unidentified</td>
</tr>
<tr>
<td>11</td>
<td>Unidentified</td>
</tr>
</tbody>
</table>

Fig. 5