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# Chemical Structures of Green Coffee Bean Polysaccharides

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The chemical structures of green coffee bean polysaccharides have been determined by methylation analysis of whole beans and of extracted polymer-rich fractions. Ground beans were completely solubilized in 4-methylmorpholine *N*-oxide, and the Hakomori methylation procedure was applied directly. The polysaccharide fraction in Robusta beans constitutes 48% of the dry weight and is principally composed of three polymers: arabinogalactan, mannan, cellulose. The arabinogalactan has a β1→3-linked galactan main chain with frequent arabinose and galactose residue containing side chains, whereas the mannan resembles cellulose in that it has a linear β1→4-linked structure. Only occasional single-residue galactose side chains are present in the mannan fraction. Polysaccharide compositions of Robusta and Arabica beans are similar; the only significant difference is the higher content (approximately 3%) of arabinogalactan in the former type.

On a dry-weight basis, almost half of the green coffee bean is made of polysaccharides. In spite of the obvious importance of this fraction with respect to coffee processing, a complete structural characterization of the polysaccharides in green coffee beans has not yet been published. The most detailed structural studies are those of Wolfrom et al. (1960, 1961) and Wolfrom and Patin (1965) who identified an arabinogalactan, a mannan, and cellulose in green coffee. The arabinogalactan was characterized as having a β1→3-linked galactan main chain with two unit (Araβ1→3-Gal1-) side chains. The mannan was shown to be a linear, β1→4-linked polymer with a comparatively low molecular weight (7000) and a low degree of side chain substitution. Hashimoto (1971) used galactanase enzymes to probe the structure of coffee arabinogalactan. He verified the β1→3-galactan backbone and concluded that about two-thirds of the galactose residues were substituted at C6 either with (Araβ1→3-Gal1-) side chains or single arabinose (furanoside or pyranoside forms) or galactose residues.

These studies utilized pure polymers isolated from coffee beans in small yields, and the structures determined were not necessarily representative of the whole polysaccharide fraction that produced arabinose, galactose, and mannose on hydrolysis. A number of publications on coffee polysaccharides have been produced by Thaler's group [see, for example, Thaler (1979) and references therein] and characterization is as the hydrolysis products: arabinose, galactose, mannose, and glucose. Both mannan and galactomannan have been referred to as components of coffee beans (Clifford, 1985); however, structural evidence for the latter polymer type has not been produced. One of the objectives of this study was to characterize the polymeric fraction that yields mannose on acid hydrolysis.

By applying high-resolution GC-MS, the classical methylation procedure can be used for the structural analysis of complex polysaccharide mixtures. Also, it has been shown that, by nondegradative presolubilization in the *N*-oxide solvent, 4-methylmorpholine *N*-oxide, it is possible to utilize the Hakomori methylation procedure to obtain a linkage analysis of cell wall material (Joseleau et al., 1981). In this publication, the application of these methods to define the structure of coffee bean polysaccharides is described.

## EXPERIMENTAL SECTION

**Polysaccharide Content.** Ground, green Ivory Coast Robusta coffee beans were Soxhlet-extracted overnight with chloroform/

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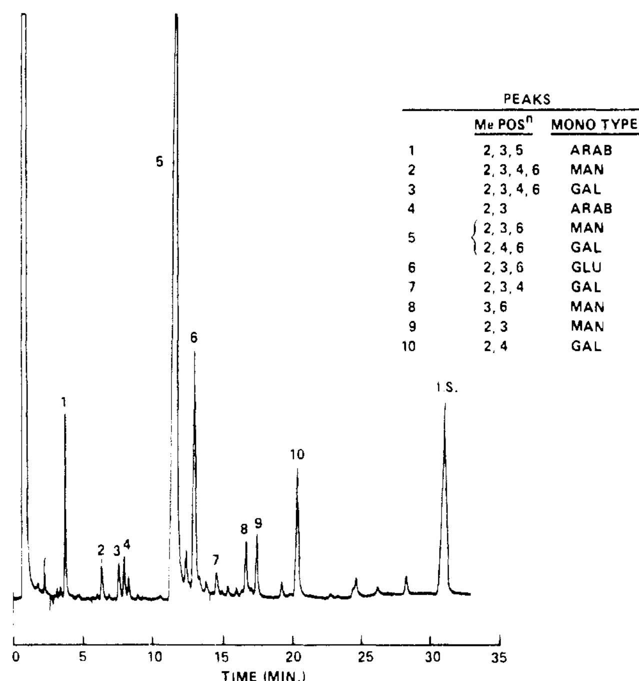



**Figure 1.** GC separation of partially methylated alditol acetates from methylation analysis of green Robusta coffee beans.

methanol (2:1) and petroleum ether (5 h) to remove lipids and with aqueous ethanol (95%, overnight) to remove low molecular weight carbohydrate. Samples (20 mg) were hydrolyzed by solubilization in 72% (v/v) sulfuric acid (sonication, 10 min, room temperature), addition of internal standard (erythritol), dilution to 8% acid, and heating (105 °C, 3 h, sealed tube). Excess barium carbonate was added to neutralize, the filtrate was concentrated, and the resultant monosaccharides were converted to the corresponding alditol acetate derivatives. Analysis was by GC using 25-m OV225 or SP2330 glass capillary columns.

**Methylation Analysis.** Samples of ground coffee were solubilized in 4-methylmorpholine *N*-oxide and then methylated by the procedure of Hakomori using the potassium methylsulfinyl methide ion as suggested by Phillips and Fraser (1981). Sample (10 mg) was Vortex-mixed with 4-methylmorpholine *N*-oxide (0.5 g) and solubilized by heating under nitrogen (120 °C, 5 min).

Dimethyl sulfoxide (0.5 mL) was added to the partially cooled, still liquid, sample. Potassium methylsulfinyl methide (0.5 mL) was slowly added while stirring under an atmosphere of nitrogen with a surrounding ice bath to keep the sample temperature below 25 °C. The mixture was allowed to stir at room temperature overnight. The sample was then placed in a small ice–water bath under a stream of nitrogen and methyl iodide (0.3 mL) added very slowly. After the mixture was stirred for 30 min, water (1 mL) was added and the sample dialyzed (2000 MW cutoff) overnight and freeze-dried. The procedure was repeated until methylation was complete (monitored by IR); a total of three methylations was often necessary. The methylated polysaccharides were acid-hydrolyzed (as above) to partially methylated monosaccharides. These were converted to alditol acetates and analyzed by GC (Figure 1). Identification was by means of capillary GC–MS using published mass spectra (Bjorndal et al., 1967) and by comparison to standards prepared by methylation and hydrolysis of polysaccharides of known structure. For quantitation, response factors were taken from Sweet et al. (1975). Trimethylsilyl alditols (Freeman et al., 1972) were also used to quantitate the partially methylated monosaccharide hydrolysates. These derivatives were made by treating the alditols with Tri-Sil Z (Pierce) at 70 °C for 10 min. GC separation was on a 30-m J&W DB-1 bonded-phase fused silica capillary column. With this procedure, alditol–TMS derivatives of 2,3,6-trimethylmannose and 2,4,6-trimethylgalactose, which coeluted as their alditol acetates, were separated.

**Table I. Linkage Analysis of Robusta Green Bean Polysaccharides**

| isomer[a] | linkage posn | mol/100 mol |
|---|---|---|
| 235 A | 1 | 7.2 |
| 2346 M | 1 | 1.3 |
| 23 A | 1,5 | 2.8 |
| 2346 GAL | 1 | 1.8 |
| 236 M | 1,4 | 40.1 |
| 246 GAL | 1,3 | 20.3 |
| 236 GLC | 1,4 | 14.6 |
| 234 GAL | 1,6 | 0.6 |
| 36 M | 1,2,4 | 2.6 |
| 23 M | 1,4,6 | 2.9 |
| 24 GAL | 1,3,6 | 5.9 |

[a] Key: A = arabinose; M = mannose; GAL = galactose; GLC = glucose. 235 A = 2,3,5-tri-*O*-methylarabinose, etc.

**Table II. Polysaccharide Analysis of Green Bean Extracts**

| fraction[a] | yield,[b] wt % | polysaccharide profile,[c] wt % | | | |
|---|---|---|---|---|---|
| | | arabinose | galactose | glucose | mannose |
| 1 | 7.6 | 0.5 | 66.2 | 4.4 | 7.9 |
| 2 | 2.4 | 1.9 | 33.4 | 3.0 | 37.2 |
| 3 A | 9.4 | 0 | 3.3 | 1.7 | 94.0 |
| 3 B | 0.5 | 0.4 | 2.7 | 2.1 | 86.0 |
| 3 R | 8.5 | 0 | 0 | 55.6 | 13.2 |

[a] Refer to Scheme I. [b] With respect to dry green bean weight. [c] Calculated as anhydromonosaccharides.

## RESULTS AND DISCUSSION

The polysaccharide content of the green Robusta beans was 48.1%. Expressed as the monosaccharides produced on hydrolysis, this fraction was made up of mannose (22.4%), galactose (12.4%), glucose (8.7%), and arabinose (4.0%), with minor quantities of rhamnose (0.3%) and xylose (0.2%). Methylation analysis of the beans gave the linkage distribution given in Table I. It can be seen that the glucose and mannose residues are 1,4-linked with the mannan, showing a low degree of branching at the 2- and 6-positions. The presence of tetramethylmannose indicates terminal mannose residues on side chains and/or chain ends. The arabinogalactan is 1,3-linked with frequent side chains at the C6-position containing arabinose and probably galactose residues. All of the arabinose residues are in the furanoside form, and most of them are terminal. Good agreement between the relative monosaccharide yields from the methylation analysis and from direct hydrolysis of the beans confirms the accuracy of the methylation procedure. The data suggest that the polysaccharide fraction of coffee beans consists of arabinogalactan, mannan, and glucan with only minor amounts of other polysaccharides.

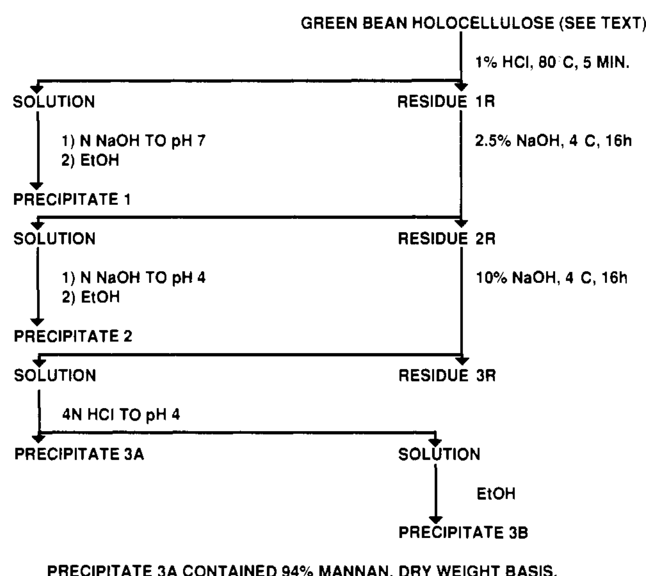
**Isolation of Mannan.** A sequential fractionation procedure, based on a delignification treatment, an acid wash, and subsequent alkali extraction, was used to isolate pure coffee mannan. Ground, defatted, green Robusta coffee beans were hot water extracted and then delignified with weakly acidic sodium chlorite solution, according to the method of Wolfrom and Patin (1965), to give a white holocellulose. Most of the arabinogalactan polymer was solubilized, in a partially hydrolyzed form, by washing with dilute hydrochloric acid (1%, 80 °C). The mannan was then isolated in discrete fractions by extraction (overnight, 4 °C) with 2.5 and 10% sodium hydroxide solutions. Addition of ethanol to the 2.5% NaOH extracts led to a precipitate containing arabinogalactan and mannan. Neutralization of the 10% NaOH extracts led to rapid formation of a white precipitate, which was removed by filtration after the mixture was allowed to stand over-

**Table III. Methylation Analysis of Partially Hydrolyzed Coffee Arabinogalactan**

| hydrolysis time,[a] h | residual AG[b] | relative mol, % | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 235 A[c] | 23 A | 35 A | 2346 GAL | 246 GAL | 234 GAL | 24 GAL |
| 0 | 1 | 15.4 | 9.8 | 2.1 | 5.0 | 44.5 | 1.9 | 21.5 |
| 5 | 0.743 | 10.8 | 7.1 | 2.4 | 10.0 | 44.5 | 4.2 | 20.0 |
| 24 | 0.560 | 3.0 | 1.8 | 1.4 | 20.9 | 47.1 | 6.8 | 18.8 |

[a] Arabinogalactan fraction (0.5%) in 0.005 N oxalic acid at 100 °C. [b] Residual arabinogalactan, after dialysis (8000 MW cutoff) and freeze-drying. [c] See Table I.

**Scheme I. Isolation of Mannan from Green Coffee Beans**




night at 4 °C. A further fraction was obtained by addition of ethanol to the filtrate. The precipitates were all washed with ethanol and diethyl ether before drying. The reaction sequence is outlined in Scheme I. The mannan studied in this work was the fraction precipitated by neutralization of the 10% NaOH extracts (precipitate 3A, contained 94% mannan by weight).

**Mannan Structure.** Carbohydrate analysis of the polymeric fractions isolated in Scheme I is shown in Table II. The highest yield was obtained on neutralization of the 10% NaOH extracts. This fraction also had a high mannan content; 94% was determined as mannose. Methylation analysis yielded terminal mannose (1 mol), terminal galactose (0.1), 1,4-linked mannose (12.3), and 1,4,6-linked mannose (0.1). The low yield of terminal galactose residues and branched mannose residues showed that this polymer contained only about 1 one-residue galactose stub at C6 per 100 mannose residues. There is no sign of branching at the C2-position. Methylation analysis of the mannans extracted at lower alkali concentrations also failed to indicate a higher density of branches at C6 or any branches at C2, suggesting that the slightly higher branching density apparent in the whole-bean analysis is due to incomplete methylation or to linkages, possibly non-carbohydrate in nature, labile to the acid or alkali treatments used in the polymer preparation. However, removal of the one-unit galactose side chains by hydrolysis in the acid-washing step was considered unlikely because the mannose to galactose ratio in a locust bean galactomannan was unaltered by the same treatment.

The average dp for the mannan preparation was only 13 on an end group basis. This indicated some depolymerization during isolation as the average mannan dp from the whole-bean methylation analysis was 35. The dp of the native mannan may even be higher as solubilization in the *N*-oxide has been known to reduce polysaccharide molecular weight (Joseleau et al., 1981). However, the absence of terminal glucose, which would be an indicator for depolymerized cellulose, suggests that depolymerization in the *N*-oxide solvent was not extensive. The low value of optical rotation of the mannan, $[\alpha]^{15}_{589}$ −53° (*c* 0.1, 1 N NaOH), is similar to the value for ivory nut mannan, $[\alpha]^{15}_{589}$ −47° (Aspinall et al., 1953), and is characteristic of a β-linked chain. The low level of side chains emphasizes that mannan is the correct terminology for the polymeric fraction producing mannose on acid hydrolysis, and there is no indication of a substituted mannan such as galactomannan in coffee beans.

**Isolation of Arabinogalactan.** Extraction of holocellulose with dilute acid (e.g., hydrochloric or trichloroacetic acids) led to significant solubilization of the arabinogalactan polymer. However, treatment of the obtained extracts with ethanol gave only a low yield of a galactose-rich precipitate, indicating that the labile arabinose residues had been removed by hydrolytic scission as more soluble, low molecular weight fragments. A lower yield, but less degraded preparation, was obtained by prolonged aqueous extraction of hot water extracted beans in the presence of protease enzymes. Ground beans (10 g) in water (300 mL) were heated to 90 °C for 30 min. The residue from filtration was stirred in pH 7 Tris buffer (300 mL) to which was added a protease enzyme (Pronase E, Sigma, 0.03%) and a drop of toluene. After 3 days, the mixture was filtered and the solution freeze-dried. A yield of 0.48 g was obtained, which on hydrolysis gave yields of arabinose (19.8%, weight basis, as anhydromonosaccharide), galactose (48.2%), mannose (0.8%), and rhamnose (1.1%). This preparation was used directly for the methylation studies.

**Arabinogalactan Characterization.** Quantitative saccharification and methylation analysis showed that the chemical structure of the isolated arabinogalactan correlated with that indicated by direct bean methylation. 1→3-Linked galactose residues form the structural basis of the polymer and about one-third of those units are branched at the C6-position. The possibility of covalent linkages to protein is suggested by the improved soluble yield obtained on treatment with protease. The linkage analysis is characteristic of a type II arabinogalactan; these polysaccharides have a β1→3-linked galactan main chain and are often covalently linked to proteins in plant cell walls (Clarke et al., 1979), usually by glycosidic linkages from galactose to the free hydroxyl of serine, threonine, or hydroxyproline residues or from arabinose to hydroxyproline residues. Extraction of green beans with 10% NaOH (16 h, 3 °C) led to solubilization of only 44% of the arabinogalactan, and even after heating in 20% NaOH overnight at 100 °C, 45% of the polymer remained in the insoluble fraction. This indicates that if the polymer is bound via glycosidic linkages, then those to the hydroxyproline residues would be most likely, as the other linkages would be scissioned at high pH by β-elimination. However, although amino acid analysis indicated a protein content of 9.5%, hydroxyproline content was

negligible, suggesting the presence of other intramatrix linkages to the polymer. Stability to alkali could also be due to other linkages within the main chain, as 1→3-linked polysaccharide chains are rapidly depolymerized zipwise from the free reducing group at high pH (Young and Sarkanen, 1977). For example, it has been suggested that arabinose residues may be situated between blocks of galactose linkages in type II arabinogalactans (Young and Sarkanan, 1977). The nonterminal arabinose residues present in the coffee polymer could possibly be situated in the main chain.

Table III shows the results of methylation analysis of residual polymeric arabinogalactan following mild hydrolysis with dilute oxalic acid. As hydrolysis proceeds, the proportion of terminal arabinose residues decreases in the residual fraction while there is a corresponding increase in terminal galactose residues; i.e., the majority of side chains terminate in galactose residues after partial hydrolysis. This supports earlier reports (Wolfrom and Patin, 1965; Hashimoto, 1971) that most side chains contain galactose (bound to main chain) and arabinose residues. Thus, the polymer is principally a β1→3-linked galactan chain with frequent short side chains linked at C6 to galactose residues 1→3-linked to terminal arabinose residues. The polymer also contains some nonterminal arabinose residues (linked at C2 and C5) as well as a small proportion of 1→6-linked galactose residues. These linkages have all been identified in type II arabinogalactans (Stephen, 1983).

**Other Polysaccharides.** Digestion of green beans with α-amylase subsequent to boiling in water led to very low yields of glucose and maltose (total <0.5%), indicating a negligible starch content. This observation, together with the inertness to alkali and dilute acid extraction (Table II), confirms that the coffee glucan is cellulose.

Application of the carbazole colorimetric procedure (Bitter and Muir, 1962) indicated that the pectin content of green beans was low (<1%). As is typical with all plant-derived substrates, minor quantities of other polysaccharides, as evidenced by the low yields of rhamnose and xylose, are also present.

**Polysaccharides in Arabica Beans.** Polysaccharide profiles, expressed as the monosaccharides produced on hydrolysis, were similar for Robusta and Arabica coffee beans. The principal difference was in the arabinogalactan content, which tended to be higher (average 17% vs 14%) for Robusta beans. Mannan and cellulose contents were typically about 22% and 8%, respectively, for both bean types. Methylation analysis also showed that the chemical structures of the Arabica bean polysaccharides were essentially the same as those in the Robusta beans. It is interesting to note that although the polymeric carbohydrate content of the Arabica beans is lower than that of Robusta beans, Arabica beans generally have a higher content of low molecular weight carbohydrate, which is principally sucrose (Clifford, 1975).

**Registry No.** Cellulose, 9004-34-6; β-(1→4)-mannan, 9052-06-6; arabinogalactan, 9036-66-2.

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