Polysaccharides from Instant Coffee Powder

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Two polysaccharide residues have been isolated from a commercial soluble coffee powder. An arabinogalactan was precipitated from aqueous solution, after removal of the mannan, by barium hydroxide precipitation. The mannan was isolated in a separate experiment by precipitation with Fehling solution. The constituent sugars and physical properties of the materials were determined. The L-arabinose content of the arabinogalactan was much lower than that previously found for the arabinogalactan of the green coffee bean.

Previous publications from this laboratory (Wolfrom *et al.*, 1960, 1961; Wolfrom and Patin, 1964, 1965) have dealt with the constituents of the roasted and green coffee bean. In the work herein recorded, the authors report a study of the polysaccharide residues from a commercial soluble coffee powder. This material was fractionated, and two polysaccharides were isolated. These components have been quantitatively analyzed for their sugar content, and their main physical properties have been determined.

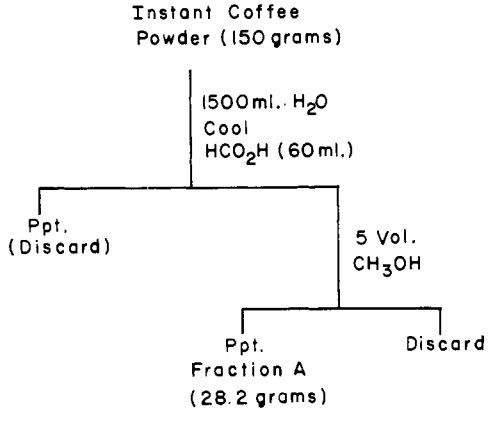
Materials and Methods

A quantity of an instant coffee powder, of American manufacture, sufficient for this study, was obtained in standard, commercial, sealed glass jars each with a net weight of 6 ounces (170 grams). The material was all from the same production lot.

Preliminary Fractionation (Scheme 1). One hundred and fifty grams of coffee powder was dissolved in 1500 ml. of water and cooled to 4° C., and 60 ml. of formic acid (96%) was added. This mixture was centrifuged at 4000 r.p.m. for 45 minutes at 4° C. The supernatant was decanted and the dark brown precipitate was discarded. Supernatant was treated with five volumes of methanol (100%). The light brown precipitate (fraction A) was removed by centrifugation, and the supernatant was discarded. The precipitate was washed with methanol and ethyl ether; yield, 28.2 grams (18.8% of starting material). This procedure was repeated.

SCHEME 1.

Preliminary Fractionation



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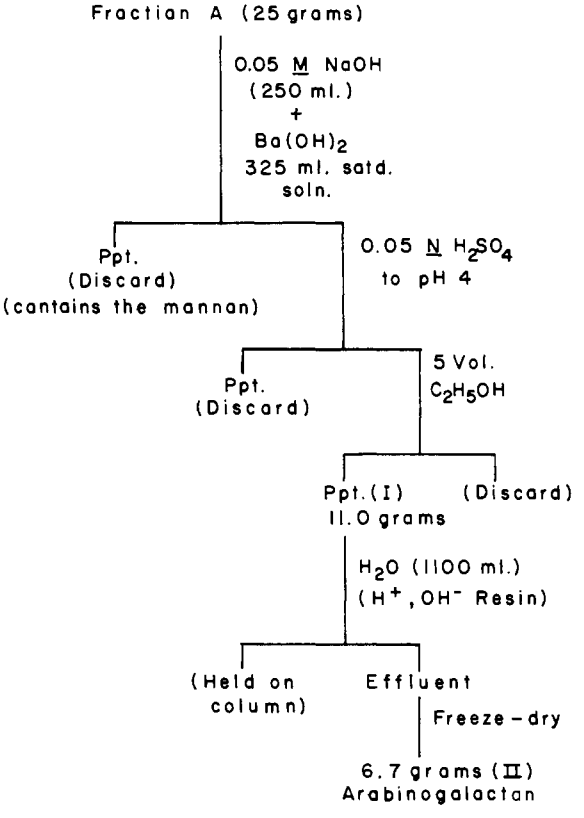
Isolation of an Arabinogalactan (Scheme 2). An amount of 25 grams of fraction A was dissolved in 250 ml. of 0.05*M* sodium hydroxide. To this was added 325 ml. of saturated aqueous barium hydroxide solution. After it had stood overnight, the brown precipitate formed was removed by centrifugation and discarded.

SCHEME 2

Isolation of Arabinogalactan



The supernatant was treated dropwise with 0.5*N* sulfuric acid until a pH of 4 was obtained. The precipitate was removed by centrifugation and discarded. The supernatant was poured into five volumes of absolute ethanol to give a precipitate (I); yield, 10.95 grams (8.24% of coffee powder).

Analysis: N, 0.54% (Dumas); ash, 5.1% (as sulfate).

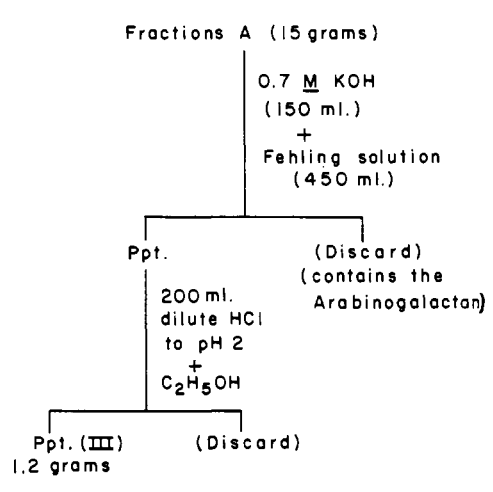
One gram of I was dissolved in 100 ml. of water. This solution was passed through a column (280 × 13 mm.) of Rexyn I-300 (H^+, OH^-) (Fisher Scientific Co., Fairlawn, N.J.). The column was eluted with water until the effluent gave a negative phenol–sulfuric acid test for reducing sugar (Hodge and Hofreiter, 1962). The effluent was then freeze-dried to obtain the arabinogalactan (II) as a nearly colorless, amorphous solid; yield, 0.61 gram; $[\alpha]_D^{18} + 9.5°$ (*c* 2.0, water).

Analysis: Calcd. for $(C_6H_{10}O_5)_{25}(C_5H_8O_4)_2$: C, 44.50; H, 6.17. Found: C, 43.72; H, 6.32; N, 0.32%; ash 0.8% (sulfate); molecular weight, 1796 (Mechrolab vapor pressure osmometer).

Acid Hydrolysis of the Arabinogalactan. One hundred milligrams of II was hydrolyzed with formic acid according to the method previously described (Wolfrom *et al.*, 1961). The concentrated neutralized hydrolyzate was diluted to exactly 10 ml. with water. Thirty microliters of this solution was chromatographed, along with standards, on a thin layer (200 × 200 × 0.25 mm.) of microcrystalline cellulose (Avicel) (Wolfrom *et al.*, 1965) using the solvent system ethyl acetate–acetic acid–water, 3:1:3 (v./v., nonaqueous phase) (Jermyn and Isherwood, 1949). After eight developments, with intermittent air-drying, the spots were visualized with aniline phthalate, and the sugars were determined quantitatively (Wolfrom *et al.*, 1966). Only galactose and arabinose could be detected, and these were in the ratio 25 to 2. These two sugars accounted for 90% of the theoretical recovery of the hydrolyzate. The degree of polymerization of the arabinogalactan was 11 as calculated from the analytically determined sugar ratio and the osmometrically determined molecular weights.

Isolation of Mannan (Scheme 3). Fifteen grams of fraction *A* (obtained from duplicating Scheme 1) was dissolved in 150 ml. of 4% potassium hydroxide, and 450 ml. of Fehling solution was added. The mixture was allowed to stand overnight in the cold, and the precipitate was removed by centrifugation and washed with 4% potassium hydroxide. The bluish green precipitate was suspended in 200 ml. of water,

SCHEME 3



and concentrated hydrochloric acid was added to pH 2. The precipitate dissolved, giving a brown solution. Absolute ethanol was added to give a final volume of 500 ml. The precipitate was removed by centrifugation and dried by washing with ethanol and ethyl ether; yield, 1.22 grams (III) (1.53% of coffee powder).

The material was reprecipitated by dissolving 0.50 gram in 50 ml. of 4% potassium hydroxide and adding 150 ml. of Fehling solution. The mannan was isolated from this complex as described above; yield, 0.468 gram (IV) of a cream colored, amorphous solid; $[\alpha]_D^{25} - 31 \pm 3°$ (90% formic acid).

Analysis: Calcd. for $C_6H_{10}O_5$: C, 44.46; H, 6.21. Found: C, 41.02; H, 6.73; N, 0.28%; ash, 1.78% (sulfate).

Acid Hydrolysis of the Mannan. Substance IV (0.0533 gram) was dissolved in 2 ml. of 72% sulfuric acid. After standing for 2 hours, the solution was diluted to 48 ml. and heated for 1 hour under pressure (15 p.s.i.). After it had cooled, the acid was neutralized with saturated barium hydroxide solution. The precipitate was removed by centrifugation and filtration. The supernatant was then evaporated to a sirup, which was refluxed for 1.5 hours with 200 ml. of absolute methanol. After filtration, the methanol was evaporated, and the sirup obtained was diluted to exactly 10 ml. with water.

Fifteen microliters of this solution was chromatographed as described for the arabinogalactan. A blank hydrolysis was performed on 4.89 mg. of D-mannose, and the hydrolyzate was chromatographed simultaneously. Only a slight trace of galactose could be detected visually in the mannan hydrolyzate, in addition to the main component mannose spot. The mannose represented 94% of the weight of the material in the precipitate (IV).

Results and Discussion

Previous work (Wolfrom *et al.*, 1961; Wolfrom and Patin, 1965) has shown that both a mannan and an arabinogalactan are present in the green coffee bean, and that the constituent sugars are D-mannose, L-arabinose, and D-galactose.

The dissolved coffee powder was first treated with formic acid to precipitate much of the high nitrogen content browning polymer in the solution. The methanol-insoluble fraction (A) of the supernatant was treated with saturated barium hydroxide solution to remove mannose-containing polymeric material (Meier, 1958). In the isolation of the arabinogalactan, the mannan fraction was discarded and in the isolation of the mannan the arabinogalactan was discarded. Because the mannan was separated from the arabinogalactan by precipitation with barium hydroxide, it was very difficult to isolate the mannan from this precipitate. Conversely, when the mannan was separated by precipitation with Fehling solution, no method was found for isolating the mannan from the alkaline copper mother liquor (Schemes 2 and 3).

The isolated arabinogalactan could be easily purified by an ion exchange resin to a low ash value. This material had a much lower arabinose content than the ara-

binogalactan isolated from green coffee (Wolfrom and Patin, 1965). This could be due to loss of easily cleaved arabinose end groups during processing. Another major point of difference is in the specific rotations of the two materials, owing perhaps to linkage changes and the lower arabinose content of the arabinogalactan material from the processed coffee.

The mannan was isolated from fraction A by precipitation with Fehling solution. To ensure that maximum separation from the arabinogalactan would be made, a reprecipitation was performed. The mannan isolated showed a trace of galactose present after hydrolysis. The mannan from green coffee also contained a small amount of D-galactose (Wolfrom *et al.*, 1961). Thaler (1957) isolated a material containing mannose and galactose from coffee powder; however, the galactose content of this was high relative to the mannose and it was decreased by reprecipitation. He reported no arabinose.

The specific rotation, in formic acid solution, of the mannan from fraction A ($-31 \pm 3°$) had a higher levorotatory value than the rotation ($-22°$) of the mannan isolated from green coffee (degree of polymerization 45) (Wolfrom *et al.*, 1960) but was close to that ($-28°$) of an ivory nut mannan (degree of polymerization 10–13) (Aspinall *et al.*, 1953).

Both of these polysaccharides are the survivors of the industrial roasting and extraction processes and should thus be considered as degraded; likewise, the nature of the linkages may have been altered during the processing. The nature of these linkages will be the subject of a further investigation.

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