Anal. Calcd. for $C_{28}H_{40}N_2OS$: C, 54.90; H, 6.54; N, 4.58; S, 5.23. Found: C, 54.75; H, 6.71; N, 4.68; S, 5.56.

2. In Liquid Ammonia.—When the above-described reaction was carried out in liquid ammonia (30 ml.) instead of DMF, at 100° for 15 hr., IX (0.45 g.) was obtained.

The Reactions of I with 2-Thiopyridone. A. In DMF.—2-Thiopyridone (0.75 g.) was dissolved in water (50 ml.) containing potassium carbonate (1.0 g.) and the water was removed under reduced pressure. The dried residue was added to a solution of I (2.5 g.) in DMF (40 ml.) and the solution was refluxed for 8 hr. After cooling, the solution was filtered and the filtrate was evaporated under reduced pressure. The residue was chromatographed on an alumina with benzene. The first elute yielded XIX. Recrystallization of crude crystals from *n*-hexane gave pure cubic crystals (0.2 g.), m.p. 69°, $[\alpha]^{25}D$ −0.7° (*c* 1.1130), λ_{max}^{Nujol} 1576 cm.$^{-1}$ (pyridine).

Anal. Calcd. for $C_{17}H_{23}NO_5S$: C, 57.79; H, 6.52; N, 3.97; S, 9.07. Found: C, 57.74; H, 6.62; N, 3.80; S, 9.10.

B. In Liquid Ammonia.—When the above reaction was carried out in liquid ammonia (20 ml.) at 100° for 20 hr., the same result was obtained.

1-S-Phenyl-2,3:4,6-di-O-isopropylidene-1-thio-α-L-sorbofuranose (XX).—A mixture of sodium (1 g.) and thiophenol (5 g.) was dissolved by adding methanol (*ca.* 6 ml.). The solution was mixed with a liquid ammonia (30 ml.) solution of I (10 g.) and the mixture was heated on a boiling-water bath for 25 hr. Ammonia was allowed to evaporate, and the residue was diluted with water, extracted with chloroform, washed with water, dried, and evaporated. Recrystallization of the residue from *n*-hexane gave XX (4.2 g.), m.p. 64°, $[\alpha]^{24.5}D$ −17.5° (*c* 1.058).

Anal. Calcd. for $C_{18}H_{24}O_5S$: C, 61.36; H, 6.82; S, 9.09. Found: C, 61.44; H, 6.86; S, 9.19.

The same result was obtained when DMF was used as a solvent.

The Reaction of I with Potassium Thiolacetate.—A solution of I (2 g.) and potassium thiolacetate (0.56 g.) in DMF (50 ml.) was refluxed for 2.5 hr. The solution was filtered, and the filtrate was evaporated. The residue was extracted with chloroform, the extract was washed with water and dried, and the solvent was removed. Distillation of the residue gave XXI (0.6 g.), b.p. 162° (7.5 mm.), $[\alpha]^{23}D$ −6.6° (*c* 0.992), λ_{max}^{film} 1672 cm.$^{-1}$ ($SOCOCH_3$).

Anal. Calcd. for $C_{14}H_{22}O_6S$: C, 52.80; H, 6.94; S, 10.04. Found: C, 52.76; H, 7.00; S, 9.69.

The Reaction of I with Potassium Ethylxanthate.—A solution of I (2 g.) and potassium ethylxanthate (0.8 g.) in DMF (40 ml.) was refluxed for 6 hr. After removal of solvent under reduced pressure, the residue was chromatographed on an alumina column with benzene. After the starting material was recovered from the benzene fraction, IX (0.5 g.) was obtained which was eluted with ether.

The Reaction of I with Potassium Thiocyanate.—A solution of I (8 g.) and potassium thiocyanate (8 g.) in DMF (50 ml.) was refluxed for 12 hr. The solution was filtered, and the filtrate was evaporated under reduced pressure. The residue was extracted with hot *n*-hexane. From the *n*-hexane-soluble part, XXIII (1.1 g.) was obtained. It had m.p. 148° (recrystallized from *n*-hexane), $[\alpha]^{25}D$ −23.4° (*c* 1.1152).

Anal. Calcd. for $C_{24}H_{38}O_{10}S_2$: C, 52.36; H, 6.91; S, 11.62. Found: C, 52.47; H, 7.02; S, 11.53.

Distillation of the *n*-hexane-insoluble part gave XXIV (1.5 g.), b.p. 170° (7 mm.), $[\alpha]^{25}D$ −10.6° (*c* 1.1028), λ_{max}^{film} 2245 cm.$^{-1}$ (SC≡N).

Anal. Calcd. for $C_{13}H_{19}NO_5S$: C, 51.83; H, 6.31; N, 4.65; S, 10.63. Found: C, 52.10; H, 6.43; N, 4.63; S, 10.62.

The Reaction of XII with Potassium Thiocyanate.—A mixture of potassium thiocyanate (8 g.), XII (8 g.), and DMF (40 ml.) was refluxed for 8 hr. The solution was filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was extracted with chloroform, the extract was washed with water and dried, and the solvent was removed. Recrystallization of the residue from *n*-hexane and chloroform gave colorless needles (2.8 g.), m.p. 89°, $[\alpha]^{24.5}D$ −27.5° (*c* 0.9431). Thus-obtained XIII was identical with an authentic sample.[2]

Acknowledgment.—The authors wish to express their deep gratitude to Dr. K. Takeda, director of this laboratory, for his encouragement.

Carbohydrates of the Coffee Bean. IV. An Arabinogalactan[1]

M. L. WOLFROM AND D. L. PATIN

Department of Chemistry, The Ohio State University, Columbus, Ohio 43210

Received May 17, 1965

Essentially all of the L-arabinose and D-galactose present in the green coffee bean (*Coffea arabica*) are shown to occur as a water-soluble arabinogalactan (2:5). The L-arabinose can be removed from this polysaccharide by dilute acid leaving a galactan. Methylation analysis of the arabinogalactan and of its component galactan can be interpreted tentatively as indicating I which has a backbone chain of β-D-(1→3)-linked galactopyranose units to some of which are attached at the C-6 position terminal L-arabinofuranose side chains containing an occasionally interposed (1→3)-linked D-galactopyranose entity.

The green coffee bean (*Coffea arabica*) has a high content (50–60%) of polysaccharides, the constituent sugars of which have been determined[2,3] to be D-mannose (preponderant), L-arabinose, D-galactose, and D-glucose. This communication is concerned with the nature of the L-arabinose and D-galactose content which is now established to be in the form of an arabinogalactan. Although the arabinogalactan is slightly water soluble, the polysaccharide is difficult to extract completely from the very hard green coffee bean, probably because of the cellular structure of this botanical entity. The arabinogalactan appears to be associated, at least in part, with the protein fraction,[4] likewise mainly water soluble, and with the characteristic coffee acids, largely aromatic in nature. We report some preliminary evidence that some of these acids may be esterified with the arabinogalactan since an alkaline treatment was required to purify the polysaccharide and since some spectral evidence for the presence of aromatic esters could be adduced.

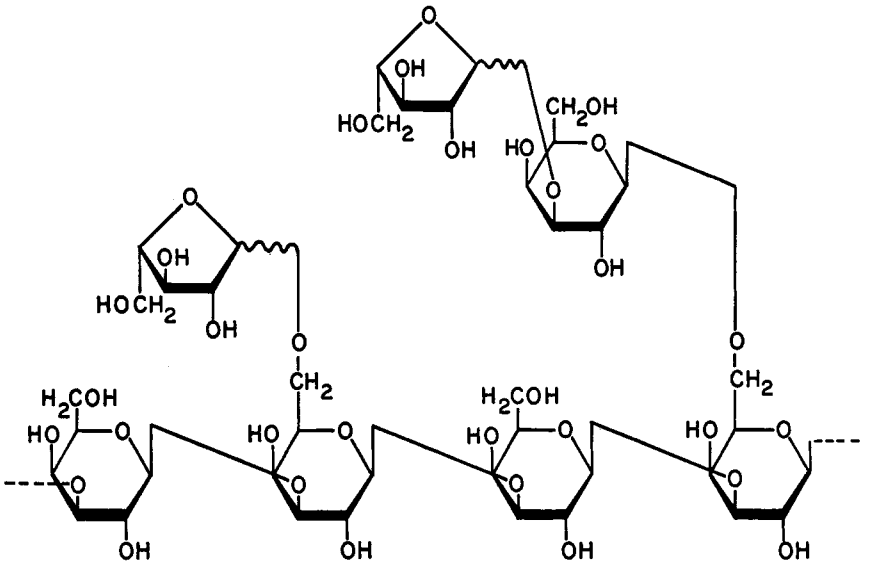
Two methods were employed in the isolation of the arabinogalactan from the green coffee bean. In both methods the ground beans were extracted successively[3] with ethanol–water, benzene–ethanol, water at 25°, and ammonium oxalate. In the one case (method A) the residue (fraction 1) from the above treatment was extracted with hot water, the extract was digested with proteolytic enzymes (chymotrypsin and trypsin to-

(1) Previous communication: M. L. Wolfrom and D. L. Patin, *J. Agr. Food Chem.*, **12**, 376 (1964). A preliminary report of this work has appeared in Abstracts of Papers, 145th National Meeting of the American Chemical Society, New York, N. Y., Sept. 1963, p. 24D.

(2) E. Schulze, E. Steiger, and W. Maxwell, *Z. Physiol. Chem.*, **14**, 227 (1890); E. Schulze, *ibid.*, **16**, 387 (1892); E. Schulze, *Chem. Ztg.*, **70**, 1263 (1893).

(3) M. L. Wolfrom, R. A. Plunkett, and M. L. Laver, *J. Agr. Food Chem.*, **8**, 58 (1960).

(4) R. L. Clements and F. E. Deatherage, *Food Res.*, **22**, 222 (1957).



I, coffee arabinogalacton (tentative formulation)

gether), and the product was isolated by dialysis. In method B the above fraction 1 was treated with sodium chlorite and acetic acid, as described previously,[3] and the resultant coffee holocellulose was extracted with hot water. The extract was concentrated, subjected to an alkaline treatment, and dialyzed. Both preparations involved alkaline treatment and dialysis which seemed to remove esters, apparently combined with the polysaccharide, the natures of which are under further investigation. The final preparation was a slightly water-soluble, levorotatory arabinogalactan, $[\alpha]^{25}D$ $-27°$ (c 0.5, water). By formic acid hydrolysis, the constituent sugars were obtained in 90% yield with a arabinose–galactose ratio very close to 2:5. It had been determined previously[3] that these sugars were present as L-arabinose and D-galactose. Three methylations, performed under nitrogen, with methyl sulfate and solid sodium hydroxide in dimethyl sulfoxide and *N*,*N*-dimethylformamide, with subsequent product isolation by dialysis, succeeded in introducing only 83.5% of the theoretical methoxyl content. Nevertheless, this etherified product sufficed to give a picture of the main structural features present in this plant polysaccharide.

The nearly permethylated ether was subjected to hydrolysis with formic acid, and the hydrolysate was investigated by paper chromatographic methods. Three main zones were located together with several very faint, slower moving ones. Isolative thin layer chromatography on microcrystalline cellulose[5] then served to isolate these three methyl ethers. The material in the fastest moving zone was identified as 2,3,5-tri-*O*-methyl-L-arabinose after oxidation to the aldonic acid which was characterized as its crystalline amide. The material in the slowest moving zone was identified as 2,4-di-*O*-methyl-D-galactose by its crystalline monohydrate and by its crystalline *N*-phenylglycosylamine. The intermediate zone material was found to be 2,4,6-tri-*O*-methyl-D-galactose by preparation, likewise, of its crystalline *N*-phenylglycosylamine. All identifications of crystalline products were made by comparative melting points and X-ray powder diffraction data with authentic specimens. The molar ratio of 2,4,6-tri-*O*-methyl-D-galactose to 2,4-di-*O*-methyl-D-galactose was found to be close to 3:2 by densitometric measurement on paper.

It was known[3] that mild acidity removed essentially all of the L-arabinose from the green coffee bean. This indicated that the L-arabinose was present in the furanoid form, a finding verified by the furanoid nature of the L-arabinose trimethyl ether obtained. Accordingly, the L-arabinose was so stripped from the arabinogalactan and the resultant galactan was subjected to methylation assay. The galactan was methylated twice by the method described above followed by one methylation by the procedure of Kuhn and Trischman.[6] This sufficed to yield a methyl ether containing approximately 80% of the calculated methoxyl content. It is then apparent that the hindrance to methylation lies in the galactan portion and not in the end-group L-arabinose units. The nearly permethylated galactan, isolated by dialysis, was then subjected to methanolysis, and the resultant methanolysate was investigated by thin layer chromatography on silica gel by comparison with a methanolysate prepared in a similar manner from the arabinogalactan. Two zones were present in the methanolysate from the methylated galactan that were not present in the other. On acid hydrolysis the material from each of these zones gave tetra-*O*-methylgalactopyranose, identified by thin layer chromatography. They were thus considered to be the anomeric methyl glycosides of tetra-*O*-methyl-D-galactose. The material in these zones was isolated by preparative thin layer chromatography on silica gel and was identified, after glycosidic hydrolysis of the combined zone material and reaction with aniline, as the crystalline *N*-phenylglycosylamine of tetra-*O*-methyl-D-galactopyranose, again by comparative melting point and X-ray powder diffraction data with an authentic specimen.

A formulation (I) which is in approximate agreement with the above data is shown. It can be seen from I that mild acid stripping of the terminal L-arabinofura-

(5) M. L. Wolfrom, D. L. Patin, and R. M. de Lederkremer, *Chem. Ind.* (London), 1065 (1964); *J. Chromatog.*, **17**, 488 (1965).

(6) R. Kuhn and H. Trischman, *Ber.*, **96**, 234 (1963).

nose units would uncover some side-chain D-galactose, although not in an amount equivalent to the L-arabinose present. Therefore D-galactose, pyranoid in form, is not present in all of the side chains. The D-galactose backbone is (1→3)-linked in which a pyranoid form is assumed. Present data are insufficient to allocate anomeric configurations to the units present, although the levorotations exhibited by the arabinogalactan and its methylated product is indicative of a β-D linkage between the D-galactose units. While the formulation presented is in general agreement with the facts obtained, further evidence is required to place it on a firm basis. Fragmentation to component oligosaccharide units would be desirable. The formulation is similar to those of other arabinogalactans found in plants.[7]

As noted above, essentially all the L-arabinose present in the green coffee bean can be removed by mild acidity[3] and can therefore be considered as being all present in the terminal furanoid units established by methylation techniques. The D-galactose-L-arabinose ratio, about 5:2, in the coffee holocellulose[8] is essentially the same as that found in the isolated arabinogalactan. It would thus appear that essentially all D-galactose units present in the coffee bean are combined in the arabinogalactan. A minimal content of the isolated arabinogalactan in the green bean can be calculated as 8.5% but is probably higher. A β-D-(1→4)-mannan has been isolated (5% of the green bean) and characterized.[8] This mannan fraction had a small content (about 2%) of galactose, a negligible amount. The mannan fraction isolated probably represents the more soluble and thus the less polymerized fraction of the mannan present; the more insoluble fraction awaits further investigation. The rather small D-glucose content of the green coffee bean can be closely accounted for as cellulose.[1] The seed belongs to that group containing relatively large amounts of mannan.[9]

Experimental Section

Isolation of an Arabinogalactan from the Green Coffee Bean. Method A.—Ground green coffee beans (*Coffea arabica*, Santos 4's) were partially deproteinized, as described previously,[3] by successive extractions with ethanol–water (80:20 v./v.), benzene–ethanol (2:1 v./v.), water (three times at 25°), and 0.5% aqueous ammonium oxalate (twice at 90°).

An amount of 800 g. of the residue obtained, after the treatment described above (fraction 1), was refluxed with water (20 l.) and 2-octanol (200 ml.) for 1 hr. The solids were removed by hot filtration through sintered glass and washed with 2 l. of water. The extract was divided equally and placed in two 12 l. flasks. To each flask was added tris(hydroxymethylamino)-methane and sufficient hydrochloric acid to obtain pH 8.5. Chymotrypsin (300 mg.) and trypsin (235 mg.) (Worthington Biochemical Corp., Freehold, N. J.) were added to each flask and the temperature was maintained for 12 days at 35–40° with stirring. The filtrate was concentrated under reduced pressure to several liters and dialyzed against distilled water for 9 days, concentrated under reduced pressure, and freeze dried: yield 19 g. This material showed free carbonyl absorption at 5.75 μ (KBr, Perkin-Elmer Infracord) and aromatic absorption at 260 and 275 mμ (maximum, dimethyl sulfoxide solution).

Anal. Found: N, 0.17 (Dumas); ash, <1.0.

An amount of 9.85 g. of the above product was stirred with 500 ml. of 0.2 *N* aqueous sodium hydroxide for 3 hr. at 25° and then for 3 hr. at 55–60°. The cooled solution was brought to pH 7.5 with sulfuric acid and extracted successively with the following solvents: chloroform (400 ml.), ethyl acetate (600 ml.), and ether (600 ml.). The aqueous phase was then dialyzed against distilled water for 15 days, and the dialysate was concentrated under reduced pressure and freeze dried: yield 5.6 g. (0.6% dry bean). This material exhibited no absorption at 260 and 275 mμ and no free carbonyl absorption by infrared (5.75 μ, KBr).

Method B.—The ground green coffee beans were extracted as described above and the residue from the ammonium oxalate treatment (fraction 1) was treated with sodium chlorite and acetic acid as described previously[3] to prepare the holocellulose of green coffee. An amount of 500 g. of this material was stirred with warm water (45 l.) and 2-octanol (100 ml.) overnight which caused thorough wetting of the material. It was then refluxed for 5 hr. and filtered through sintered glass and washed with water. The combined filtrate and washings were evaporated under reduced pressure to a dark brown solution (3 l.). The solution was made 0.2 *N* in sodium hydroxide and stirred at 60° for 4 hr. under a rapid stream of nitrogen. The solution was made neutral to litmus with sulfuric acid. The neutralized solution was dialyzed for 4 days against distilled water and the colored, clear dialysate was evaporated under reduced pressure to 1.5 l. and poured into cold 95% ethanol (final ethanol concentration, 70%). Upon addition of a few grains of sodium chloride, immediate flocculation occurred. The solids were isolated by repeated decantation and washing with 70% ethanol, slurried with water, and freeze dried to a light colored, fluffy solid: yield 11.4 g. (0.9% dry bean). This material exhibited no absorption at 260 or 275 mμ and no free carbonyl absorption by infrared (5.75 μ, KBr).

Characterization of the Arabinogalactan.—The same arabinogalactan was obtained by either isolation method A or B: $[\alpha]^{25}D$ −27° (*c* 0.5, water). The material was soluble in water at room temperature to the degree shown in the polarimetric determination.

The arabinogalactan (630 mg.) was heated with formic acid (100 ml.) for 4 hr. at steam-bath temperature. The formic acid was removed under reduced pressure and the resultant syrup was heated for 1 hr. at steam-bath temperature with 50 ml. of 1.5% sulfuric acid. The cooled solution was exactly neutralized with barium hydroxide and filtered, and the filtrate was concentrated under reduced pressure and made up to a volume of 25 ml. The quantitization of constituent sugars followed the procedure of Wilson.[10] Only arabinose and galactose were found: total yield 90%, molar ratio of arabinose–galactose 1:2.5. It had been determined previously[3] that the steric forms of these sugars were L-arabinose and D-galactose. For elementary analysis the material was dried over phosphorus pentaoxide at 78° and 0.5 mm. for 24 hr.

Anal. Calcd. for $2C_5H_8O_4 \cdot 5C_6H_{10}O_5$: C, 44.40; H, 6.70. Found: C, 44.79; H, 6.45; N, 0.2; ash, 0.35.

Methylation of the Arabinogalactan.—The arabinogalactan (4.5 g.), prepared by method B, was stirred for 24 hr. with dimethyl sulfoxide (300 ml.) which caused good solvation. *N*,*N*-Dimethylformamide (100 ml.) was added and the solution was cooled and swept with nitrogen. Dimethyl sulfate (36 ml.) and powdered sodium hydroxide (36 g.) were then added and the reaction was kept cold for 3 hr. Additional reagents (18 ml. of dimethyl sulfate and 15 g. of sodium hydroxide) were added after 22 and 30 hr., respectively. After a total reaction time of 55 hr., the reaction was dialyzed. The dialysate was concentrated and freeze dried: yield 5.3 g. The material was remethylated in the same manner: yield 5.0 g.

Anal. Calcd. for complete methylation: OCH_3, 43.7. Found: OCH_3, 30.5.

The twice methylated product dissolved very well in dimethyl sulfoxide (300 ml.). *N*,*N*-Dimethylformamide (100 ml.) was added and the solution was chilled and swept with nitrogen. Solid powdered sodium hydroxide (10 g.) and dimethyl sulfate (12 ml.) were added and the reaction was kept cold for 3 hr. Additional equal amounts of the above reagents were added after 7, 26, 32, 48, and 56 hr. The reaction was dialyzed after a total reaction time of 5 days. The nondialyzables were concentrated under reduced pressure to dryness, suspended in chloroform, and filtered through Celite 545. The clear amber filtrate and combined washing (1 l.) were concentrated to 10 ml. and poured into cold petroleum ether (b.p. 30–60°) (200 ml.). A

(7) T. E. Timell, *Advan. Carbohydrate Chem.*, **20**, 409 (1965).

(8) M. L. Wolfrom, M. L. Laver, and D. L. Patin, *J. Org. Chem.*, **26**, 4533 (1961).

(9) G. O. Aspinall, *Advan. Carbohydrate Chem.*, **14**, 448 (1959).

(10) C. M. Wilson, *Anal. Chem.*, **31**, 1199 (1959).

flocculent, white solid precipitated which was isolated by centrifugation: yield 2.1 g.

Anal. OCH_3, 36.5 (83.5% of theory).

Hydrolysis of the Methylated Arabinogalactan and Identification of the Products.—The methylated arabinogalactan (36.5% OCH_3, 0.835 g.) was refluxed for 3.5 hr. with 100 ml. of formic acid. The formic acid was removed by evaporation under reduced pressure and codistillation with water. The resultant syrup was refluxed for 3 hr. with 50 ml. of 1.5% sulfuric acid and neutralized with Duolite A-4 ion-exchange resin. The effluent was concentrated to a syrup and paper chromatographed using 1-butanol–ethanol–water (3:1:1 v./v.) with indication by aniline phthalate. Three main zones were observed (with several very faint, slower moving zones) with R_{TMG} (TMG = 2,3,4,6-tetra-*O*-methyl-D-glucose) 1.07, 0.76, and 0.575. The syrup was then loaded on paper by means of a microburet, and the relative intensity of the two slowest moving zones was read by means of the densitometer and was found to be 1:1.5 (for the zones of R_{TMG} 0.575:0.76).

The slowest moving zone was isolated as a syrup by preparative thin layer chromatography on microcrystalline cellulose[5] and was crystallized as 2,4-di-*O*-methyl-D-galactose monohydrate from 95% ethanol: m.p. 93–95° (lit.[11] m.p. 103°); X-ray powder diffraction data[12] (identical with those of an authentic specimen), 11.11 (m), 9.25 (s, 1), 7.38 (w), 5.86 (m), 5.56 (m), 4.97 (m), 4.27 (s, 2), 4.17 (w), 3.98 (m), 3.62 (s, 3), 3.16 (s), 3.01 (m), 2.93 (m), 2.75 (w), 2.60 (m), 2.52 (w), 2.45 (w), 2.30 (w), 2.28 (m), 2.24 (w), 2.18 (w), 2.10 (w), 2.05 (m).

Further identification was afforded by preparation of 2,4-di-*O*-methyl-*N*-phenyl-D-galactosylamine,[11] m.p. 210–211° (lit.[11] m.p. 216°).

The zone of intermediate mobility was isolated as a syrup in the same manner and was characterized as 2,4,6-tri-*O*-methyl-D-galactose by preparation of its crystalline *N*-phenylglycosylamine: m.p. 168–169° (lit.[13] m.p. 170°); X-ray powder diffraction data[12] (identical with an authentic specimen[14]), 13.73 (m), 11.76 (s, 3), 9.75 (m), 8.24 (s, 2), 6.93 (s), 6.40 (vw), 5.69 (m), 5.16 (w), 4.85 (w), 4.38 (m), 4.16 (s, 1).

The material in the fastest moving zone was isolated as a syrup in the same manner and was identified as 2,3,5-tri-*O*-methyl-L-arabinoamide after oxidation with bromine water and amide formation[15]: m.p. 133–135° (lit.[15] m.p. 138°); $[\alpha]^{28}D$ +18° (*c* 1.3, water) (lit.[15] $[\alpha]^{28}D$ +18°); X-ray powder diffraction data[12] (identical with those of an authentic specimen[16]), 8.71 (s, 1), 7.67 (w), 6.34 (m), 6.00 (s, 2), 5.60 (vw), 5.01 (s, 3), 4.39 (w), 4.19 (s), 3.78 (m), 3.61 (m), 3.43 (w), 3.25 (w), 2.80 (vw), 2.74 (w), 2.52 (w).

Conversion of the Arabinogalactan to Its Component Galactan. Methylation Analysis of the Galactan.—The arabinogalactan (8.3 g.) was dissolved in 500 ml. of hot 2.5% sulfuric acid and refluxed for 20 min., cooled, and dialyzed. The dialysate was concentrated to 300 ml. and poured into 1.5 l. of chilled ethanol. The solids were isolated by decantation and evaporated under reduced pressure with dimethyl sulfoxide (300 ml.). The material was then methylated twice with dimethyl sulfate and sodium hydroxide as described for the methylation of the arabinogalactan. The isolated product from the second methylation was then methylated in dimethyl sulfoxide with methyl iodide and barium oxide according to Kuhn and Trischman.[6] The product was a white solid of 35% methoxyl content (calcd. 45.5%, 77% of theory).

An amount of 300 mg. of the above methylated galactan was refluxed for 22 hr. with 4% methanolic hydrogen chloride. The cooled solution was neutralized with Duolite A-4 ion-exchange resin which was removed by filtration. The filtrate and washings were concentrated under reduced pressure to yield an amber colored syrup which was chromatographed on 0.5-mm. silica gel G plates using 7% methanol in benzene as developer. Six zones were indicated by sulfuric acid spraying and heating. R_f values and relative intensity were estimated visually (3 = highest): 0.073 (3), 0.169 (2), 0.190 (2), 0.210 (2), 0.392 (2), 0.450 (1).

The contents of the two fastest moving zones, which were not present in the methanolysate of the methylated arabinogalactan, were then identified. The syrup was loaded on 0.5-mm. plates and the zones were indicated on the edges with sulfuric acid. The two fastest moving zones were removed by scraping, were then allowed to stand in methanol for 10 hr., filtered, and concentrated several times to remove the adsorbent. The syrup obtained on solvent removal was dissolved in 1 *N* hydrochloric acid and refluxed for 24 hr. The resultant syrup obtained from each, after removal of the acid by codistillation with 2-propanol, was chromatographed by thin layer chromatography on microcrystalline cellulose[5] alongside authentic tetra-*O*-methyl-D-galactose. The main zone exhibited the same R_f as the authentic tetra-*O*-methyl-D-galactose.

Syrup from the combined zones was treated with aniline and processed as described above for the *N*-phenylglycosylamines of the other methyl ethers of D-galactose. Crystals were obtained on final solvent removal: yield 11 mg.; m.p. 193 (lit. m.p. 194°,[17] 196°[18]); X-ray powder diffraction data[12] (identical with those of an authentic specimen of tetra-*O*-methyl-*N*-phenyl-D-galactopyranosylamine), 8.54 (s, 1), 6.77 (m), 6.40 (w), 5.45 (m), 5.16 (w), 5.02 (w), 4.56 (s, 3), 4.25 (m), 4.04 (s, 2), 3.89 (w), 3.77 (m), 3.57 (w), 3.46 (vw), 3.29 (w), 3.16 (w), 3.08 (w), 2.96 (vw).

(11) F. Smith, *J. Chem. Soc.*, 1724 (1939).

(12) Interplanar spacing in Å., Cu Kα radiation; relative intensity, estimated visually: s, strong; m, medium; w, weak; v, very. First three strongest lines are numbered (1 strongest).

(13) A. L. Clingman, J. R. Nunn, and A. M. Stephen, *J. Chem. Soc.*, 197 (1957).

(14) Kindly furnished by Dr. G. A. Adams, Ottawa, Ontario, Canada.

(15) J. I. Cunneen and F. Smith, *J. Chem. Soc.*, 1146 (1948).

(16) Kindly furnished by the late Professor F. Smith.

(17) F. Smith, *J. Am. Chem. Soc.*, **70**, 3249 (1948).

(18) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1482 (1939).