resulting solution held at 46–50°. After nine days the rotation was nearly constant (+2.71° in a 1.5-dm. tube at 20°)[21] and acid was then removed with Duolite A-4. Concentration *in vacuo* gave a crystalline residue; from ethanol–pentane the product (330 mg., 74%) crystallized as fine needles melting at 150–156°. Recrystallization from ethanol–pentane afforded pure material melting at 156–157° and rotating +54.2° in chloroform (*c* 0.88).

Anal. Calcd. for $C_{17}H_{24}O_7$: C, 59.99; H, 7.11. Found: C, 59.97; H, 7.27.

Methyl 4,6-*O*-Benzylidene-2-*O*-mesitoyl-α-D-glucopyranoside.—A solution of 2.1 g. of mesitoyl chloride in 10 ml. of dry pyridine was cooled to 0° and treated dropwise with a solution of 2.1 g. of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside[22] in 10 ml. of dry pyridine. The solution was slowly warmed to room temperature and left at 22° overnight. Ten drops of water was added and 30 min. later the mixture was diluted with methylene chloride. The solution was extracted with cold 3 *N* sulfuric acid, aqueous sodium bicarbonate and, finally, dried with sodium sulfate. After concentration there was obtained a solid residue; crystallization from warm methanol gave 3.3 g. of crude crystalline material. Recrystallization from absolute ethanol afforded mesitoic anhydride; addition of pentane to the ethanolic mother liquor led to the formation of fine needles (1.5 g., 47%) melting at 193–196°. Recrystallization from ethanol–pentane gave pure methyl 4,6-*O*-benzylidene-2-*O*-mesitoyl-α-D-glucopyranoside melting at 195–196° and rotating +95.0° in chloroform (*c* 1.0).

Anal. Calcd. for $C_{24}H_{28}O_7$: C, 67.27; H, 6.59. Found: C, 67.48; H, 6.79.

Methyl 2-*O*-Mesitoyl-α-D-glucopyranoside.—The benzylidene derivative (1.07 g.) was reduced in a mixture of 30 ml. of methanol and 10 ml. of ethanol in the presence of palladium black (0.5 g.) at room temperature and pressure. The reaction being completed in 50 min., the catalyst was removed and the solution concentrated to a sirup which crystallized spontaneously. From ethanol–pentane 844 mg. (99%) of crude product was obtained in two crops. Recrystallization from the same solvent mixture gave pure material melting at 164–165° and rotating +119° in chloroform (*c* 1.1).

Anal. Calcd. for $C_{17}H_{24}O_7$: C, 59.99; H, 7.11. Found: C, 60.20; H, 7.21.

A sample of the product was dissolved in glacial acetic acid and treated with lead tetraacetate in the usual fashion. Analysis of aliquots at 2, 8 and 22 days showed the consumption of 0.134, 0.64 and 1.05 moles of oxidant.

Acknowledgment.—Microanalysis were carried out in the Institutes' Microanalytical Laboratory under the direction of Dr. W. C. Alford.

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(21) A rapid, initial levomutarotation was followed by a gradual dextromutarotation. In another run, made with 1% methanolic hydrogen chloride at 20°, the reaction was halted after the initial phase of the mutarotation was complete. A crystalline product, melting at 165–166° and rotating −13° in chloroform (*c* 0.47), was obtained in 92% yield. Combustion analysis (calcd. for $C_{17}H_{24}O_7$: C, 59.99; H, 7.11. Found: C, 59.99; H, 7.19) and the formation of formaldehyde (albeit in low yield) during oxidation with lead tetraacetate suggest that this substance is a methyl 2-*O*-mesitoyl-D-glucofuranoside.

(22) We are indebted to Dr. James W. Pratt of this Laboratory for our supply of this substance.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Cacao Polysaccharides[1]

BY ROY L. WHISTLER, EDWARD MASAK, JR., AND R. A. PLUNKETT

RECEIVED DECEMBER 12, 1955

Two distinct hot-water-soluble polysaccharides are extractable from mature caracas cacao fruit husk and seed. Preliminary work indicates the husk polysaccharide to be composed mainly of L-rhamnose, L-arabinose, D-galactose and D-mannose plus small amounts of glucose, xylose and an unidentified pentose. The seed polysaccharide contains the same major components but in different proportions.

Much of the previous characterization of the cacao bean, *Theobroma cacao*, the commercial source of cocoa and chocolate, has been confined to the ethanol-extractable sugars before[2,3] and after[4,5] fermentation and processing. The alcohol-extractable sugars are found to be D-glucose, D-fructose and D-galactose together with oligosaccharides composed of these monosaccharide units. However, little information has been reported on the ethanol-insoluble mucilages. The mucilage content of the bean is said to be 0.88% before commercial fermentation and 4.3% after fermentation.[6] The husk, which accounts for 76% of the fruit, is reported to contain as high as 8% mucilage and gums[3] before fermentation.

To obtain further information about these mucilages, the hot-water-soluble polysaccharides present in the mature Costa Rica Caracas cacao fruit husk and seed were extracted in yields of 2.0 and 0.3%, respectively, of the total dry fruit. Some differences between the polysaccharides are shown in Table I. The two polysaccharides are precipitated from solution at different potassium chloride concentrations. Quantitative paper chromatography indicates that the husk polysaccharide contains rhamnose, galactose, arabinose and mannose with a trace amount of glucose in the ratio of 11.5:11.5:4:3 while the polysaccharide isolated from the seed contains the same sugar units in the ratio of 3:2:2.5:1. The short gum-forming proper-

TABLE I

PROPERTIES OF POLYSACCHARIDES

Properties	Seed polysaccharide	Husk polysaccharide
$[\alpha]^{25}D$	+106°	+114°
Intrinsic viscosity	6.73	10.12
*p*H, 1% solution	6.85	6.35
Ash, natural	8.3	8.2
Ash, after dialysis	0.2	0.3
Phosphate test	+	+
Sulfate test	−	−
Uronic acid	−	−
Nitrogen, %	3.68	0.557

(1) Journal Paper No. 858 of the Purdue University Agricultural Experiment Station.

(2) E. M. Chatt, "Cocoa," Interscience Publishers, Inc., New York, N. Y., 1954, p. 89.

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ties of the polysaccharides indicate branched structures. Upon partial hydrolysis of the polysaccharides, arabinose is always the first hydrolysis product observed while rhamnose is the last. This suggests main chains of L-rhamnose units and side chains of L-arabinose units with D-mannose and D-galactose distributed within this structure.

Experimental

Fruit.—Freshly picked, mature, whole, paraffin-coated Caracas cacao fruit were shipped air-express from Turrialba, Costa Rica. Upon arrival eight days later, the paraffin was removed from the intact fruit. The tissues were separated and extracted.

Extraction of Husk and Seed Polysaccharides.—After preliminary extraction of the husks with ethanol and acetone,[7] water-soluble mucilages were extracted by heating a water suspension (50 ml./g.) in an autoclave at a pressure of 15 lb./sq. inch for 30 minutes. After filtration of insoluble material, the filtrate was poured into two volumes of 95% ethanol, the precipitated water-soluble polysaccharide was dried by washing with ethanol, and placed over calcium chloride in a vacuum desiccator.[7]

The mucilaginous seed coat was removed from the seeds by shaking them with water for 2 hours at room temperature. The seed polysaccharide was extracted in a manner similar to that described for the husk polysaccharide.

Fractionation of the Polysaccharides.—Attempts to fractionally precipitate with absolute ethanol were made.[8] To a one per cent. husk-polysaccharide solution maintained at 25°, increments of absolute ethanol were added with rapid stirring. After each increment the mixture was stirred for 10 minutes, and then centrifuged at 804 times gravity for 10 minutes. Precipitates were collected, oven-dried and weighed. The husk polysaccharide was entirely precipitated in the range of 54.5–62.0% ethanol content by volume.

Under similar conditions the seed polysaccharide was precipitated within the range of 64.8–70.0% ethanol. Within these narrow ranges no fractionation of either polysaccharide was observed.

Potassium Chloride Precipitation.—Potassium chloride has been used to fractionate hydrocolloids such as carrageenan.[9] One per cent. solutions of the husk and seed polysaccharides were precipitated by a 1.0 *M* potassium chloride solution in a manner similar to the ethanol fractional precipitation method. Each successive precipitate was collected, dried and weighed. It was found that 16.5% of the husk polysaccharide was precipitated by a total potassium chloride molarity of 0.60 while no precipitation of seed polysaccharide occurred up to 0.83 *M* potassium chloride concentration.

Hydrolysis Products.—Separate samples of husk polysaccharide were hydrolyzed at 100° for 8 hours with 1 *N* sulfuric acid or with 1 *N* hydrochloric acid solutions. The hydrolyzates were neutralized by barium carbonate or silver oxide, respectively. Hydrolysis products were compared with known compounds by chromatography using the solvent systems of ethyl acetate, pyridine and water (10:4:3) and (8:2:1); butanol, propanol and water (2:5:3); and ethyl acetate, acetic acid, formic acid and water (18:3:1:4). Alkaline silver nitrate[10] and *p*-anisidine hydrochloride were used as spray reagents. The hydrolyzate consisted of rhamnose, arabinose, glucose, galactose and a chromatographically slow-moving spot. This spot was identified as a phosphate ion by a modification of Gutzeit's method.[11] The polysaccharide gave a positive ammonium molybdate test for phosphorus. Uronic acid was not present as indicated by paper chromatography using brom cresol spray reagent or by measuring the carbon dioxide evolved during decarboxylation of the polysaccharide with 12% hydrochloric acid.[12] A negative test with naphthoresorcinol indicated the absence of ketoses.[13] Sulfate was not found in the polysaccharide. Nitrogen was determined by micro-Kjeldahl analysis. The starch-hydrolyzing enzyme, β-amylase, did not hydrolyze the polysaccharide.

Test results similar to those for the husk polysaccharide were given by the seed polysaccharide.

Husk and seed polysaccharides were hydrolyzed with 2% sulfuric acid at 43°. Periodically, 2-ml. aliquot portions were withdrawn, neutralized with barium carbonate, filtered, and analyzed by paper chromatography. This analysis gave the order of release of the different monosaccharides as shown in Table II.

TABLE II

ORDER OF MONOSACCHARIDE RELEASE UPON ACID HYDROLYSIS

Time, hours	Seed polysaccharide	Husk polysaccharide
0.5	Arabinose	Arabinose
1.5	Mannose	Galactose
5	Galactose	
8	Rhamnose and phosphate	Mannose and phosphate
12		Rhamnose

Identification of Constituent Sugars.—Husk polysaccharide was hydrolyzed with 5% sulfuric acid in one case and with 43% hydrochloric acid in another case. An amount of 3.8 g. of sugar sirup was obtained in this manner. This sirup was resolved on a column of cellulose[14] (7.0 cm. diam., 82 cm. long) using ethyl acetate–acetic acid–formic acid–water (18:3:1:4, vol. ratio before mixing) as the mobile phase. The effluent was collected on an automatic fraction collector and after examination on paper chromatograms was grouped into six separate fractions. Wherever possible only fractions containing single constituents were combined. On the basis of paper chromatographic evidence the fractions collected were, in the order of elution, (A) rhamnose, 246 mg.; (B) an unknown pentose saccharide which gave a pink color with the aniline acid phthalate spray reagent, 86 mg.; (C) arabinose plus a trace of xylose, 321 mg.; (D) mannose, 170 mg.; (E) galactose plus a trace of glucose, 238 mg.; and (F) galactose, 558 mg.

L-Rhamnose was identified in fraction A by crystallization from a thick aqueous sirup as α-L-rhamnose monohydrate, m.p. 89–90°, m.m.p. 90–93°, $[\alpha]^{25}D$ +8.3°, (*c* 0.344, water); accepted values, m.p. 93–94°, $[\alpha]^{20}D$ +8.2°.

L-Arabinose was identified in fraction C as its insoluble α-benzyl-α-phenylhydrazone, white plates from 50% ethanol, m.p. 173–174°, m.m.p. 170–174°, $[\alpha]^{25}D$ −9.5° (*c* 0.23, chloroform); accepted values, m.p. 174°, $[\alpha]^{20}D$ −11.5°.

D-Galactose was identified in fraction F by crystallization from a thin acidified (acetic acid) aqueous sirup as α-D-galactose, m.p. 160–161°, $[\alpha]^{25}D$ +81.2° (equil.); accepted values, m.p. 167°, $[\alpha]^{20}D$ +80.2°.

D-Mannose was characterized in fraction D as a dextrorotatory sirup, $[\alpha]^{25}D$ +20.6° (*c* 0.68, water); accepted value, $[\alpha]^{20}D$ +14.2°.

Neither xylose nor glucose was recovered in sufficient purity or quantity to permit definitive characterization.

The material of fraction B crystallized readily from a methanolic sirup at 25–30° even when impure. The crystalline compound melted at *ca.* 35°, resolidified at 60–70° and melted again at *ca.* 190°. This behavior is not characteristic of any of the unsubstituted pentoses. Insufficient material was available for further characterization.

Quantitative Chromatographic Analysis.—Two per cent. solutions of the husk and seed polysaccharides were hydrolyzed by 1 *N* sulfuric acid at 100° for 8 hours. After neutralization with barium carbonate, 50 λ of the filtered hydrolyzates were chromatographed[15] on Whatman No. 1 paper for 40 hours with ethyl acetate–pyridine–water (8:2:1).

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Monosaccharides were extracted by soaking and stirring appropriate sections in 5 ml. of water for 20 minutes and filtering the supernatant liquor into test-tubes. Papers were washed with 7 ml. of water and the washings combined with the supernatant liquors. This recovered 95–96.8% of the original material. Analysis with potassium ferricyanide[16] and comparison with known values indicated the composition of the polysaccharide (Table III).

Other Polysaccharides.—Water-soluble polysaccharides were isolated from the pulp, the mucus seed cover, and the seed placenta in yields of 0.17, 0.10 and 0.01% of the total fruit, respectively (dry weights). Hydrolysis of the polysaccharides produced the same monosaccharides as found in the husk and seed polysaccharide. In contrast to the seed polysaccharide, the placenta and mucus also contained traces of xylose.

Acknowledgments.—The authors wish to thank Mr. Arnold L. Erickson of the Inter-American Institute of Agricultural Sciences, Turrialba, Costa Rica, and Dr. L. Paul Oechsli, Director of Research of the American Cocoa Research Institute, Turrialba, Costa Rica, for supplying the cacao fruit.

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TABLE III

COMPOSITION OF POLYSACCHARIDES

Monosaccharides	Seed polysaccharides, %	Husk polysaccharides, %
Rhamnose	36.29	37.97
Arabinose	28.81	13.27
Mannose (plus trace glucose)	12.02	9.98
Galactose	22.86	38.77

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[CONTRIBUTION FROM THE DEPARTMENTS OF MEDICINE AND OPHTHALMOLOGY, COLUMBIA UNIVERSITY, COLLEGE OF PHYSICIANS & SURGEONS, AND THE PRESBYTERIAN HOSPITAL]

Fractionation of Gum Arabic by Chemical and Immunological Procedures[1]

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RECEIVED JANUARY 3, 1956

Gum arabic, recovered from its specific precipitate with Type II antipneumococcus serum, contains only one-third to one-fifth as much rhamnose as does the native gum. Gum arabic is therefore a mixture, and no single over-all structural formula can properly be given. Several attempts at chemical fractionation of the gum were less successful and less specific than the serological method. In the chemical fractions the ratio of D-glucuronic acid to L-rhamnose was remarkably constant, but was much higher in the precipitates with antibody because of the loss of L-rhamnose. The Type II-precipitable portion of the gum does not differ greatly from the remainder with respect to its content of L-arabinose, D-galactose and D-glucuronic acid. In accord with this is the failure of Type XIV antipneumococcus serum to fractionate the gum. Precipitation in this instance is due to D-galactose residues in the gum.

Although the composition of gum arabic (GA), like that of all natural gums, has been known to vary within certain limits, definite evidence of inhomogeneity was lacking until recently. Beiser, Kabat and Schor[3] precipitated an acid-degraded sample of the gum with Type II antipneumococcus (anti-Pn) horse serum, noted that only a small portion of the rhamnose in the sample was to be found in the specific precipitate, and concluded that the material consisted "of a mixture of at least two substances, one containing methylpentose and unrelated to the specific polysaccharide of Type II pneumococcus (S II) and the other lacking methylpentose and yet cross reacting with anti-S II." It will be recalled that S II consists of L-rhamnose, D-glucose and D-glucuronic acid,[3–5] while GA contains L-rhamnose, L-arabinose, D-galactose and D-glucuronic acid.[6] The cross reaction between GA and Type II anti-Pn serum[7] has now been studied quantitatively and shown[8] to be due mainly to multiple recurrences of D-glucuronic acid in the native and degraded gums. With larger quantities of specific precipitate than used by Beiser, *et al.*,[3] it was found that one-third to one-fifth of the rhamnose in the native gum and roughly two-thirds of the residual rhamnose in the degraded gum appears in the specific precipitate. Native GA, therefore, is also a mixture, and hitherto published formulas are uncertain to that extent.

Although the power of immunochemical techniques was thus once more demonstrated, it was considered advisable to attempt fractionation of native GA by chemical methods as well. These were less successful, but effected sufficient separation to show that GA is indeed a mixture of substances of rather similar composition.

Experimental

Materials and Methods.—The GA used was a commercial sample. It was dissolved in 0.9% sodium chloride solution up to concentrations of 200 mg. per ml., with neutralization as necessary and centrifugation to remove insoluble matter. Acid-degraded gum was prepared as in reference 7. The Type II anti-Pn horse serum was kindly supplied by the Bureau of Laboratories, New York City Department of Health, and the Type XIV anti-Pn horse serum by the Division of Laboratories, New York State Department of Health.

For the quantitative estimations of antibody nitrogen[9]

(1) Carried out under the Harkness Research Fund and a grant from the Commission on Acute Respiratory Diseases of the Armed Forces Epidemiological Board, Office of the Surgeon General, Department of the Army.

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