POLYSACCHARIDES OF THE COCOA POD HUSK

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Cocoa pod husk from Ghana was extracted successively with hot water, hot 0·5% ammonium oxalate, cold 10% potassium hydroxide, and hot 20% sodium hydroxide, and the various polysaccharide fractions isolated after dialysis. The hot-water-soluble fraction was examined by hydrolysis with 50% formic acid, quantitative determination of the component sugars, and characterisation of the sugars by preparation of crystalline derivatives. The major part of this fraction is a pectic material containing D-galacturonic acid, D-galactose, L-rhamnose and L-arabinose, but is contaminated with several other polysaccharides, notably a glucan and a mannan. The ammonium oxalate fraction is also a pectin but in a depolymerised form, while the main component sugar of the alkaline extracts is xylose. The insoluble husk residue is probably chiefly cellulose.

Introduction

Cocoa and chocolate are made from the seeds of the cacao tree (*Theobroma cacao*). The fruits of the cacao tree are brilliantly coloured pods containing some 30–40 seeds per pod and embedded in a whitish mucilage. The seeds are extracted from the pod husk, fermented, and dried in the tropics, when they are then known commercially as 'cocoa beans'. The beans are about the size of almonds and have a thin skin or 'shell' which averages about 12% of their weight. On arrival at the cocoa factory, the beans are cleaned and roasted, which makes both the shell and interior crisp and brittle. The beans are then lightly crushed to reduce the shell to flakes and the interior to solid angular fragments known as 'cocoa nibs', which are separated by sieving and winnowing. The resultant nib is the fatty, commercially valuable material from which cocoa products are manufactured.

While the nib,[1] and to a lesser extent the shell,[2] have been the subject of intensive research, the husk of the cocoa pod has received little attention. This is due to the fact that the husk, which contains about 85% of water, is not transported to chocolate factories and is often not even collected at a central point on the individual farms where the cocoa is grown. It has been estimated[3] that the annual world crop of one million tons of cocoa produces about ten million tons of pod husks as by-product, and the only uses suggested for the pods are as a fertiliser and animal feedstuff. The purpose of the present study was to obtain some information about the general nature of the polysaccharides of the pod husk.

Dittmar[4] showed that cocoa pod husks contained crude protein (5·7–9·7%), pectin (5·3–7·1%), nitrogen-free extract (44·2–51·3%), crude fibre (33·2–39·4%), and ash (8·8–10·2%). A hot-water-soluble polysaccharide, extracted from Caracas pod husks in 2% yield, was shown[5] to be composed mainly of L-rhamnose, L-arabinose, D-galactose, and D-mannose, together with small amounts of glucose, xylose, and an unidentified pentose; rather surprisingly, uronic acid was not detected.

Experimental

Materials and general methods

Sun-dried cocoa pod husks, collected in Tafo, Ghana, through the courtesy of Prof. C. B. Coulson, were broken into small pieces and milled through an 0·75 mm screen. The moisture content of the powder, and of all isolated fractions, was determined at 60°/0·01 mm over phosphoric oxide. All weights are on a dry-weight basis. Nitrogen content was 1·07%, determined by micro-Kjeldahl.

All evaporations of solutions were carried out in a rotary film evaporator under reduced pressure at temperature below 45°. Specific rotations were measured at 20° in water unless otherwise stated, the concentration (c) being in g/100 ml of solution; the results for sugar solutions are equilibrium values. Inherent viscosity, $\eta_{inh} = c^{-1} \ln (\eta_{\text{solution}}/\eta_{\text{solvent}})$ dl/g, where c = g of solute in 100 ml of solution, was measured at 25° in an Ostwald viscometer (M2/BS/U/M) in water. Infrared spectra were recorded in potassium chloride discs with a Perkin-Elmer 'Infracord' 137 spectrophotometer.

Extraction of polysaccharide fractions

The dried milled husk (94·21 g) was refluxed with ethanol–water (4:1 by vol., 3 l) for 3·5 h, the mixture centrifuged, and the brown supernatant containing material of low molecular weight was discarded. The residue (92·79 g) was extracted three times with water (2 l each time) at 100° for 3 h, centrifuged, washed with cold water (500 ml), ethanol and ether, and dried. The aqueous extracts were dialysed, concentrated to 1 l, and the dark-brown, viscous solution, containing 0·1% of sodium chloride, was added slowly with stirring to ethanol (4 l). The fibrous, brown precipitate A was washed with ethanol and ether and dried (10·78 g). The husk residue (81·98 g) was similarly extracted three times with 0·5% ammonium oxalate solution (2 l each time) at 100° for 2 h, centrifuged, washed with cold water (500 ml), ethanol and ether, and dried. The extracts were dialysed, concentrated to 100 ml, and precipitated with four volumes of ethanol to yield fraction B as a buff powder (7·76 g). The husk residue (69·04 g) was then extracted twice with 10% potassium hydroxide solution (2 l each time) at 20° for 2 h, washed with 0·1 N-acetic acid (1 l) and water (1 l), and isolated and dried as before. The alkaline extracts were neutralised with glacial acetic acid, exhaustively dialysed against running water, concentrated to 1 l, and a red-brown gel removed at the centrifuge. This gel was dehydrated with ethanol and ether to give fraction C1 (2·58 g). The supernatant was concentrated to 200 ml and added to five volumes of ethanol, when fraction C2 was obtained as a dark-red solid (7·04 g). The husk residue was extracted twice with 20%

sodium hydroxide solution (2 × 500 ml each time) at 75–85° for 5 h in an atmosphere of nitrogen, washed with 0·1 N-acetic acid (3 × 500 ml) and water (500 ml), and finally isolated as a light-brown powder E (30·35 g). The dark-brown extracts were neutralised with glacial acetic acid, dialysed against running water, concentrated, and precipitated with ethanol to give fraction D as a red-brown solid (11·1 g).

The fractions are described in Table II.

Determination of sugars in water-soluble polysaccharide fraction A

Fraction A (138 mg) was hydrolysed with 50% (v/v) formic acid (7 ml) for 18 h at 100° in a sealed tube; a dark insoluble residue (39 mg; 28·3%) was filtered off and washed with water, the filtrate and washings were concentrated to dryness, and water was distilled repeatedly from the syrup to remove excess of formic acid. The hydrolysate was a brown syrup (100 mg; 72·4%). When hydrolysis was carried out with N- or 2N-sulphuric acid, lower yields of syrup were obtained owing to destruction of galacturonic acid.

The sugars present in the hydrolysate were identified qualitatively by paper chromatography using the following solvent systems: (A) isopropanol–water (80:20 by vol.); (B) ethyl acetate–pyridine–water (8:2:1 by vol.); and (C) ethyl acetate–acetic acid–formic acid–water (18:3:1:4 by vol.). Sugars were detected mainly with aniline hydrogen phthalate,[6] and less frequently with *p*-anisidine hydrochloride[7] and silver nitrate–sodium hydroxide.[8] No single solvent system gave a complete separation of all sugars, but good separations were obtained by using a combination of the three solvents. R_{Rha} values for the various sugars present in the hydrolysate are shown in Table I.

Quantitative determination of the sugars was carried out with the same solvents on Whatman No. 1 papers in the manner described by Dubois & co-workers.[9] After being eluted from the individual sections of the chromatograms, the sugars were estimated colorimetrically with the phenol–sulphuric acid reagent.[9] The results are given in Table II.

TABLE I

$R_{Rhamnose}$ values for the sugars in fraction A

Sugar	Solvent A	Solvent B	Solvent C
Rhamnose	1·00	1·00	1·00
Xylose	0·86	0·65	0·63
Arabinose	0·77	0·49	0·52
Mannose	0·80	0·40	0·40
Glucose	0·56	0·28	0·26
Galactose	0·64	0·23	0·23
Galacturonic acid	0·16	—	0·23

Characterisation of the sugars in polysaccharide fraction A

For preparative purposes, the sugars in the hydrolysate were separated on several large sheets (18 × 22 in.) of Whatman 3 MM paper using the acid solvent (C). The unsprayed portions of the chromatograms were cut into sections containing the individual sugars, the sections extracted with water, and the filtered extracts concentrated. The syrups were purified by extraction with ethanol, the insoluble residue centrifuged, and the supernatant concentrated. The syrups were characterised as follows:

(1) D-*Galactose.*—The galactose syrup (120 mg) crystallised, and the crystals (110 mg) were washed with methanol, giving m.p. 162–163°, $[\alpha]_D$ + 79·8° (*c* 5·24). The sugar gave a phenylmethylhydrazone[10] (80% yield), m.p. 184–185° and mixed m.p. 185° with an authentic specimen of m.p. 186°.

(2) D-*Glucose.*—The syrup (32 mg) crystallised from methanol–ethanol (1:1, 2 ml) giving crystals (21 mg), m.p. 145–147°, $[\alpha]_D$ + 51·8° (*c* 1·97). It was converted into *N*-*p*-nitrophenyl-β-D-glucosylamine[11] (79% yield), m.p. 182° and mixed m.p. 183° with an authentic sample of m.p. 184–185°, $[\alpha]_D$-189° (final value, *c* 2·19 in pyridine) (lit.[11], $[\alpha]_D$-202°).

(3) L-*Rhamnose.*—The syrup (38 mg) crystallised from ethanol (2 ml) containing a trace of water as the monohydrate (31 mg), m.p. 95–96°, $[\alpha]_D$ + 9° (*c* 2·9). It

TABLE II

Extraction of polysaccharide fractions

Extracting liquid	Husk residue, % of original dry husk	Polysaccharide fraction	Yield, % of dry husk	$[\alpha]_D$	$\eta_{inh.}$	Sulphated ash, %	Principal sugars liberated on hydrolysis, %
Refluxing 80% ethanol	98·5	—					
Water at 100°	87·0	A	11·4	+ 84° (*c* 0·27)	19·8 (*c* 0·01)	22·7	D-Galacturonic acid, 26·8; D-galactose, 21·1; D-glucose, 8·9; L-rhamnose, 8·6; D-mannose, 7·0; L-arabinose, 4·7; D-xylose, 1·7
0·5% Ammonium oxalate at 100°	73·2	B	8·2	73 (*c* 0·27)	0·43 (*c* 0·27)	12·8	Galacturonic acid, galactose, rhamnose, arabinose
10% Potassium hydroxide at 20°		C1 C2	2·7 7·5				Xylose, galactose, mannose, galacturonic acid
20% Sodium hydroxide at 75–85°	32·2 (residue E)	D	11·8		0·15 (*c* 0·1)	22·3	Xylose, galactose, glucose, arabinose, mannose, galacturonic acid
		Residue E	32·2				Glucose, galactose, xylose, arabinose

was converted into tetra-*O*-acetyl-L-rhamnose diethyl dithioacetal[12] (82% yield), m.p. and mixed m.p. with authentic material 56–57°.

(4) D-*Mannose*.—The syrup (33 mg), which failed to crystallise, had $[\alpha]_D$ + 12° (*c* 3·27) and gave a phenylhydrazone[10] (75% yield), m.p. 194–197° and mixed m.p. 195–197° with authentic D-mannose phenylhydrazone of m.p. 196°.

(5) L-*Arabinose*.—The syrup (18 mg), which failed to crystallise, had $[\alpha]_D$ + 77° (*c* 1·8). Authentic L-arabinose had $[\alpha]_D$ + 104·5°. The syrup was converted into its crystalline toluene-*p*-sulphonyl-hydrazone[13] (66% yield), m.p. 148–149° and mixed m.p. 149–151° with an authentic specimen of m.p. 151–153°.

(6) D-*Xylose*.—The syrup (9 mg) failed to crystallise and showed $[\alpha]_D$ + 12° (*c* 0·9). It gave a di-*O*-benzylidene dimethyl acetal[14] (51% yield), m.p. and mixed m.p. with authentic material (m.p. 208°) 206–208°.

Isolation and identification of D-galacturonic acid from cocoa pod husk

The dried milled husk (6·85 g) was heated in a sealed tube at 100° for 18 h with 50% (v/v) formic acid (100 ml), the insoluble residue (3·88 g; 56·6%) filtered off, and the filtrate concentrated and distilled repeatedly with water to remove formic acid. The brown syrup (2·98 g; 43·4%) was dissolved in water, cations removed by passing the solution through an Amberlite resin IR-120-H^+ column (50 ml), and acidic material absorbed on Amberlite IR-4B-OH^- resin (50 ml). The neutral sugars were not retained and were recovered as a syrup (2·31 g; 33·7%) by concentrating the effluent. Acids were eluted with 0·5 N-aqueous ammonia, the column washed, the eluate deionised with IR-120-H^+ resin, and free acids recovered as a brown syrup (267 mg; 3·9%).

The galacturonic acid in this syrup (170 mg) was separated on 3 MM paper, with ethyl acetate–pyridine–acetic acid–water (5:5:1:3 by vol.)[15] as solvent, and purified by solution in water (2 ml) and addition of ethanol (10 ml). Insoluble material was filtered and the filtrate concentrated to give D-galacturonic acid as a syrup (84 mg), $[\alpha]_D$ + 52° (*c* 4·2) (lit.,[15] + 51°), which was characterised by oxidation with saturated bromine water (2 ml) at 20° for 7 days to galactaric (mucic) acid (85% yield), m.p. 212–213°, not depressed on admixture with authentic galactaric acid of m.p. 213–214°.

Results and discussion

Table II shows the results for the extraction of pod husk with different extracting liquids.

Only the hot-water-soluble polysaccharide fraction A was examined in detail. This was a brown fibrous material, isolated in 11% yield, which had a high inherent viscosity (19·8 dl/g) in water. The viscosity, however, was much reduced in 0·1 M-sodium chloride solution (9·8 dl/g), this behaviour being typical of a polyelectrolyte. Its infra-red spectrum showed a strong absorption band at 1730–1720 cm^{-1} which indicated the presence of a carboxylic acid. Hydrolysis of the fraction with 50% formic acid, followed by quantitative estimation and characterisation of the sugars, revealed D-galacturonic acid as the main component, together with smaller amounts of D-galactose, L-rhamnose, and L-arabinose. This combination of sugars is characteristic of the pectic group of polysaccharides.[16] However, the occurrence also of D-glucose, D-mannose and D-xylose clearly indicated that the pectic acid was contaminated with several other polysaccharides. Unsuccessful attempts were made to separate the pectic acid from the other polysaccharides by forming insoluble pectates with lead acetate and calcium chloride,[16] but in neither case was a fraction obtained which had a specific rotation approaching that (> + 200°) of a 'pure' pectic acid. All the sugars in fraction A, with the exception of galacturonic acid, were found by Whistler *et al.*[5] in Caracas pod husk polysaccharide.

The ammonium oxalate fraction B was also a pectic material, but it did not have the high viscosity of fraction A. None of the alkali-soluble extracts showed a band in the infra-red spectrum characteristic of carboxylic acid, and galacturonic acid was present in the hydrolysates only in small amounts. The main component sugar was xylose. A mannan was not a major component of the 20% sodium hydroxide extract. The insoluble residue E, amounting to 32% of the original husk, was probably chiefly cellulose, in agreement with the high crude fibre content of pod husk[4].

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