Note

Byssinosan, an aminopolysaccharide isolated from cotton dust

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There is considerable and widespread belief that cotton dust is the main factor responsible for the aetiology of byssinosis, an occupational disease affecting the respiratory function of textile workers[1]. Preliminary chemical analysis of acid-hydrolyzed dust-extract showed the presence of glucose, galactose, arabinose, mannose, ribose, and rhamnose[2]. More recently, Davenport and Paton[3] showed that the biological activity of cotton-dust extracts, as measured by the contraction of guinea-pig ileum, is due to an unknown substance of low molecular weight in admixture with small amounts of 5-hydroxytryptamine and a trace of histamine.

The present investigation is concerned with free sugars and with an aminopolysaccharide, isolated from the aqueous cotton-dust extract, to which we give the name byssinosan. Free sugars were separated by preparative, paper chromatography. Their amounts were determined colorimetrically and by microtitration. Of these sugars a glucose was found to be the major component, whereas a mannose and a fructose occurred in lesser proportions (Table I). It is almost certain that none of these sugars would play any role in the aetiology of byssinosis. However, D-mannose and D-fructose, in proportions equal to those present in the aqueous extract, exhibited contractor activity when tested on guinea-pig ileum (unpublished results).

TABLE I

SUGAR COMPONENTS OF COTTON DUST[a]

Fraction[b]	*Sugars present*					
	Glucose	*Galactose*	*Mannose*	*Fructose*	*2-Amino-2-deoxyglucose*	*2-Acetamido-2-deoxyglucose*
Free sugars[b], %	0.24 (0.21)		0.06 (0.07)	0.05 (0.06)		
Sugars in byssinosan, %	26.4 (25.4)	30.4 (31.0)	24.0 (26.3)		4.4	13.1

[a] For analytical methods, see Experimental section. Figures in parentheses give values determined by ferricyanide method; those not in parentheses were determined calorimetrically with a "Densigraph" (Arthur H., Thomas Co., Philadelphia, Pa., U. S. A.). [b] Total free sugars present (by difference) in dry cotton dust = 0.59%, total carbohydrate,determined by the anthrone method[7] = 1.12%.

Byssinosan was obtained from the aqueous extract by repeated precipitation with two volumes of acetone, followed by dialysis and reprecipitation. The byssinosan obtained after paper electrophoresis in borate buffer of pH 10 gave a single band, and gel filtration on Sephadex G-100 gave a single peak (Fig. 1). The i.r. spectrum of byssinosan showed a strong band at 1640 cm^{-1} characteristic of the acetamido group. The nitrogen content (1.7%) could result from one hexosamine unit per three hexose molecules[4]. The degree of polymerization as determined by the reducing power before hydrolysis, was 19, assuming one terminal, reducing end-group. However, the results of gel-filtration suggest a higher molecular weight.

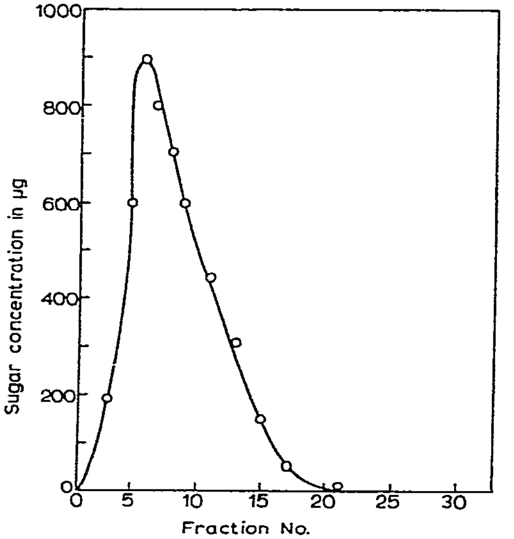

Fig. 1. Gel-filtration of byssinosan on a Sephadex G-100 column.

Acid hydrolysis of byssinosan, followed by chromatography, gave spots for five sugars, corresponding in their mobilities to standards of 2-amino-2-deoxy-D-glucose (originating from 2-acetamido-2-deoxy-D-glucose), D-glucose, D-galactose, D-mannose, and 2-acetamido-2-deoxy-D-glucose. Further identification of the sugars was made by comparing derivatives with authentic specimens. The percentage of each sugar was determined volumetrically and colorimetrically (Table I). The relatively low, apparent value for total amino sugar (17.5%, of 2-amino-2-deoxy-D-glucose plus 2-acetamido-2-deoxy-D-glucose) may result from interference of the other sugars present in the hydrolyzate of byssinosan[5], and the sugars, glucose, galactose, mannose, and 2-acetamido-2-deoxy-glucose may actually be present in the byssinosan molecule in approximately equimolar ratio.

EXPERIMENTAL

Source of material. — Airborne and settled cotton dust was collected from different stages of cotton processing, starting from the three stages of cleaning (ginning, afrita, and cotton-seed cleaning), and from the first stage in the preparation of cotton for spinning (cardrooms). All samples were obtained from Kafr-El-Dawar factories (near Alexandria). Airborne dust was collected by suction through a Büchner funnel. Quantities ranging from 0.1–0.5 g were collected during one h. Settled dust was easily collected from the top parts of the machines near the exhaust fans. The dust was kept in cellophane bags in a cold room until used.

Paper chromatography. — Descending paper chromatography on Whatman No. 1 paper for 48 h was used with the following solvent systems: A, butyl alcohol–ethanol–water (4:1:5, upper layer); B, ethyl acetate–pyridine–water (8:2:1); and C, butyl alcohol–pyridine–water (6:4:3). The chromatograms were subsequently sprayed with silver nitrate in acetone, followed by dilute alkali[6].

Separation of the sugars present in the crude dust-extract. — Cotton dust (3 g) was extracted with water (12.5 ml). The free sugars in this extract (1.5-ml amounts) were separated by preparative paper chromatography with solvent system A, with a guiding spot of a standard mixture of D-glucose, D-mannose, and D-fructose. After spraying the guide strips, the sugar bands from the dust were accordingly marked, cut off, eluted, and dried.

Identification of glucose, mannose, and fructose. — Spots from the previously eluted sugar bands from the dust were again chromatographed, by using solvent systems A and B together with standard spots (30 μg each) of D-glucose, D-mannose, and D-fructose. The free sugars from the dust gave, after spraying, spots identical with those given by reference sugars.

Determination of sugars in the dust extract and the in hydrolyzate of byssinosan Total carbohydrate in the dust extract was determined by the anthrone method[7] with D-glucose as a standard, and total reducing sugars were determined as follows: dust extract (0.25 ml, corresponding to 60 mg of dust) was estimated by the alkaline ferricyanide microtitration method of Jendrassik and Polgar[8] or colorimetrically, by measuring the density of the sugar spots revealed after spraying. Individual sugars separated by chromatography (corresponding to 720 mg of dust) were dissolved in water (1 ml), and calculated amounts (0.25 ml of each) were determined by the ferricyanide method or were chromatographed in solvent system A. After spraying, the density of the spots revealed was measured directly. The same methods were used for the determination of the component sugars of byssinosan. Total amino sugars were determined by a modified Elson–Morgan[9] method, and 2-acetamido-2-deoxy-D-glucose was determined by the method of Reissig *et al.*[10].

Byssinosan. — *Preparation.* Cotton dust (20-g amounts) was mixed with distilled water (100 ml) then kept overnight at 4°, after which time the material was squeezed through a muslin cloth. The filtrate was clarified by centrifugation at 3000 *g* for 15 min and the resulting clear, yellowish supernatant was decanted from a brown residue. This process of cold aqueous extraction was repeated three times (50 ml each). The combined aqueous extract (250 ml) was decolorized by adding charcoal (1 g) and stirring for 30 min. The extract was clarified by centrifugation followed by filtration, and the filtrate was dried under vacuum to give a residue of 1.1 g (5.04%). This residue was again treated with water (60 ml), and the insoluble part (0.135 g, 0.6%) was separated by centrifugation. Cold acetone (120 ml) was added to the supernatant and the mixture was kept overnight at 4°. The resulting fluffy precipitate was separated by centrifugation, washed twice with acetone and then with ether; yield 0.44 g (2.2%). The resulting product was dissolved in water (10 ml), freed from a small insoluble residue, and then dialyzed against distilled water for 4 days. The undialyzable material was clarified by centrifugation and dried under vacuum to give crude byssinosan (0.026 g, 0.13%). Pure byssinosan was obtained by dissolving the crude product in water (1 ml) and precipitating it by the addition of two volumes of cold acetone (11 mg, 0.05%).

Properties. Byssinosan was soluble in water and could be reprecipitated by alcohol or acetone; $[\alpha]_D$ +26.6° (*c* 1.54, water); ν_{max}^{KBr} 3400 (OH), 1640 (NHAc), 1430, and 1400 cm^{-1}. (Found: C, 43.8; H, 6.2; N, 1.7%; P and halogen absent. $C_{26}H_{45}NO_{21}$ requires C, 44.1; H, 6.5; N, 2.0%).

Isoagglutination inhibition activity. Byssinosan showed complete inhibition of anti-blood group A activity at a concentration of 25 μg (the amount of byssinosan in 0.1 ml used in the test; with 4 completely agglutinating doses[11]).

Gel-filtration. Byssinosan (9 mg) was dissolved in 0.2M sodium chloride (1.2 ml), and then applied to a previously packed column (50 × 1.5 cm) of Sephadex G-100 equilibrated with by 0.2M sodium chloride. Elution was effected by 0.2M sodium chloride and 3-ml fractions were collected. Total carbohydrate was determined for each fraction by the anthrone method, with D-glucose as a standard.

Paper electrophoresis. Electrophoresis was performed for 4 h on Whatman No. 1 paper in borate buffer, pH 10, at a voltage gradient of 3.5 V/cm and 10 mA. Byssinosan migrated 5 cm towards the anode and gave a purple colour with periodate–Schiff's reagent.

Acid hydrolysis of byssinosan. — Byssinosan (5 mg) was dissolved in water (1 ml), 0.125M sulphuric acid (1 ml) was added, and the solution was heated in a sealed ampoule for 18 h at 100°. The product of hydrolysis was neutralized with barium carbonate and the dried sugars were again dissolved in water (0.5 ml) and spots (0.07 ml) were chromatographed together with standard samples of D-galactose, D-glucose, D-mannose, 2-acetamido-2-deoxy-D-glucose and 2-amino-2-deoxy-D-glucose, with solvent system A. The monosaccharides liberated from byssinosan corresponded in their mobilities to those of the standards used.

Separation and identification of the sugar components of byssinosan. — The pooled hydrolyzate obtained from byssinosan was dried and separated into its component sugars by preparative paper chromatography with solvent system A. The sugars separated were tentatively identified by repeated chromatography in solvent system C. Further identification was made by preparation of the following derivatives (not definitively identified as D or L); galactose *N*-methylphenylhydrazone, m.p. 189°; mannose phenylhydrazone, m.p. 200°; and glucose diethyl dithioacetal[12], m.p. 127°. Melting points were not depressed by admixture with authentic specimens of the

D forms. 2-Acetamido-2-deoxyglucose had, m.p. 199°, alone or in admixture with an authentic specimen of the D form.

Degree of polymerization. — The degree of polymerization was determined on 10 mg of byssinosan by determining its reducing power[8].

Liberation of 2-amino-2-deoxyglucose from byssinosan. — Byssinosan (3 mg) was hydrolyzed by heating with 4M hydrochloric acid (1 ml) for 2 h at 100° in a sealed ampoule. The hydrolyzate was evaporated under diminished pressure, the residue was dissolved in water (0.5 ml) and aliquots (0.1 ml) each were chromatographed together with standards of 2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose by using solvent system A. Spraying with ninhydrin in ethanol or with silver nitrate in acetone showed that the amino sugar from byssinosan is indistinguishable from 2-amino-2-deoxy-D-glucose.

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