THE STRUCTURE OF REAGENT RESIDUES IN COTTON CELLULOSE TREATED WITH DIVINYL SULFONE

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ABSTRACT

Authentic, *simple* 2-, 3-, and 6-*O*-substituted and simple crosslinked derivatives of D-glucose having only one residue of reagent per substituent or crosslink were prepared by the reaction of protected D-glucoses with divinyl sulfone, a difunctional reagent. *Complex* substituted and complex crosslinked derivatives of D-glucose having more than one residue of reagent per substituent or crosslink were prepared by reaction of a protected D-glucose with an excess of bis(2-hydroxyethyl) sulfone. Cotton cellulose was modified by treatment with divinyl sulfone in 4.0M sodium hydroxide. After hydrolysis of the modified cellulose to substituted and crosslinked D-glucoses, the identity of the components on paper chromatograms was established by comparison with authentic samples. Standard curves for the quantitative evaluation of the substituted and crosslinked D-glucoses and D-glucose were prepared. The mole fraction of reagent residues in the form of simple substituents, simple crosslinks, and complex structures was estimated to be 0.20, 0.72, and 0.08, respectively. The substituent linkages and crosslinkages to the D-glucose residues were found mainly to involve O-6.

INTRODUCTION

Crosslinking reactions of cotton cellulose are of considerable importance as they are the basis for the preparation of durable-press fabrics. Abend *et al.*[1] published a study of the reaction of divinyl sulfone with cotton cellulose that constitutes the first direct proof of the formation of a linkage between two D-glucose residues in different cellulose chains; a structural component consisting of residues of D-glucose and of divinyl sulfone in the ratio of 2:1 was isolated. Neither quantitative measurement, nor determination of the site of attachment, of the reagent residues to D-glucose residues was made. More recently, Roberts and Rowland[2] employed gas–liquid chromatography (g.l.c.) and electrophoresis to isolate, identify, and measure quantitatively the crosslink structures from the reaction of bis(2-chloroethyl)-methylamine with cotton cellulose.

Although g.l.c. lends itself to the determination of the simple substituted D-glucoses formed by condensation with methyl vinyl sulfone[3] or of the monofunctional attachment of divinyl sulfone residues to cotton fabrics, other structural units resulting from the difunctional reaction of divinyl sulfone with cotton or simple derivatives of D-glucose were found to be subject to thermal breakdown at the temperatures needed for their elution. Resolution of the structural components from the reaction of divinyl sulfone with cotton or simple derivatives of D-glucose was, however, attained by a paper-chromatographic procedure, and this method was, therefore, used for quantitative evaluation. Direct densitometric measurement of components was conducted according to a method reported earlier[4].

This paper describes the preparation of simple substituted D-glucoses and crosslinked D-glucoses, each containing a single residue of the reagent, corresponding to the structures expected in cotton cellulose crosslinked with divinyl sulfone. The authentic derivatives that were synthesized were used (*1*) to identify the structures present in the chemically modified cotton cellulose, and (*2*), for preparation of calibration curves in the quantitative evaluation of the structural components isolated from the chemically modified cotton cellulose.

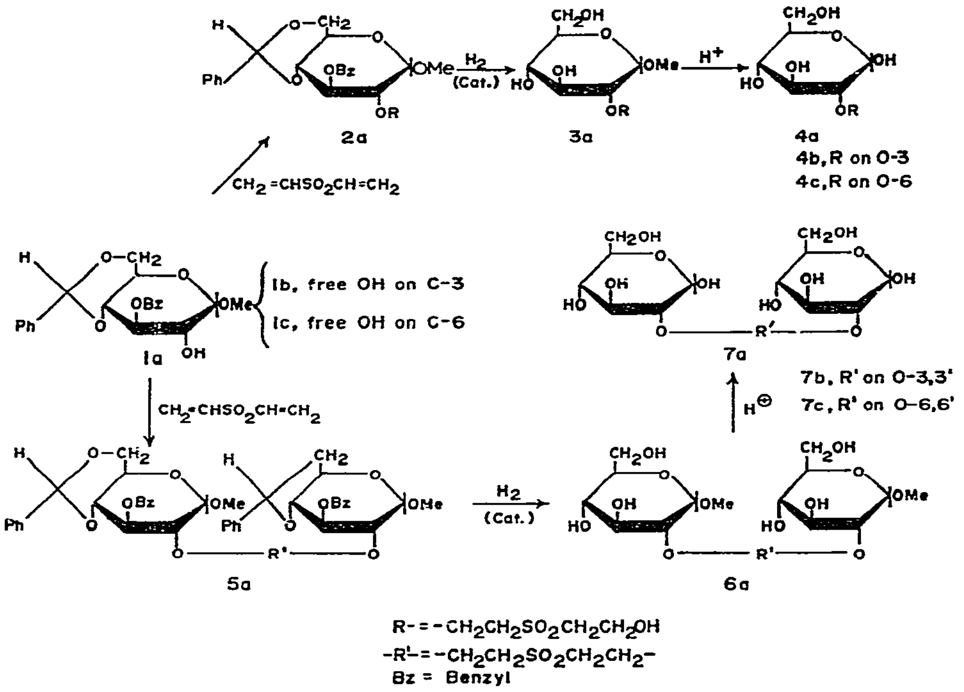
The fabric sample was prepared by treating cotton cellulose with divinyl sulfone in 4.0M aqueous sodium hydroxide. The sample of treated fabric was then hydrolyzed, and the amounts of simple substituted, simple crosslinked, and complex products were determined. In addition, the site of linkages between the reagent and D-glucose was determined both for the simple substituted and the simple crosslinked D-glucoses.

RESULTS AND DISCUSSION

Preparation and identification of substituted and crosslinked D-*glucoses*. — For identification of the reagent residues in cotton cellulose modified with divinyl sulfone, the modified cellulose was hydrolyzed to a mixture of D-glucose, substituted D-glucoses, and crosslinked D-glucoses; the last two types of components were then identified as follows. A series of authentic derivatives of D-glucose was prepared under conditions designed to yield only the *simple* substituted D-glucoses (containing only one residue of reagent as the substituent) and *simple* crosslinked D-glucoses (containing only one residue of reagent as the crosslink). These compounds then served as a basis of comparison (through g.l.c. and paper chromatography) with the components of the hydrolyzates from the modified cottons.

The synthesis of the authentic derivatives having linkages at O-2 of the D-glucopyranosyl residue is illustrated in Scheme 1. These D-glucose derivatives were prepared from protected D-glucoses (**1**) under anhydrous conditions. The derivatives were obtained as mixtures of unsubstituted D-glucose, substituted D-glucose (**4**), and the crosslinked D-glucose (**7**). The individual components were obtained in purified form by a preparative-scale, chromatographic technique. The identification of these derivatives was based on the method of preparation, the g.l.c. analyses, and the paper-chromatographic behavior, together with the analyses for sulfur and the molecular weights determined on **4a** and **7a**.

The 2-, 3-, and 6-*O*-substituted D-glucoses in the hydrolyzates were identified by comparison of their retention times in g.l.c. (see Table I) with those of the com-



Scheme 1. Steps in the preparation of a mixture of **4a** and **7a** from the reaction of **1a** with divinyl sulfone. Included also are the numerical identifications of the starting materials and products for the 3- and 6-*O* isomers.

TABLE I

RETENTION VALUES IN G.L.C. AND R_G VALUES IN PAPER CHROMATOGRAPHY FOR DIVINYL SULFONE DERIVATIVES OF D-GLUCOSE AND RELATED COMPOUNDS

Substituted D-*glucoses*	*R.t. values (min)*	R_G *values*[a]
2-*O*-{2-[(2-Hydroxyethyl)sulfonyl]ethyl}-D-glucose	9.10, 9.50[b]	1.74
2-*O*-[2-(Methylsulfonyl)ethyl]-D-glucose	8.85, 9.25	
Bis-2-(2-deoxy-D-glucos-2-yl)ethyl sulfone	17.30	0.74
3-*O*-{2-[(2-Hydroxyethyl)sulfonyl]ethyl}-D-glucose	8.13, 9.15	1.26
3-*O*-[2-(Methylsulfonyl)ethyl]-D-glucose	7.75, 8.80	
Bis-2-(3-deoxy-D-glucos-3-yl)ethyl sulfone	17.85, 19.20, 20.80[b]	0.74
6-*O*-{2-[(2-Hydroxyethyl)sulfonyl]ethyl}-D-glucose	9.20, 10.25	1.25[c]
6-*O*-[2-(Methylsulfonyl)ethyl]-D-glucose	8.90, 9.15	
Bis-2-(6-deoxy-D-glucos-6-yl)ethyl sulfone	20.60	0.56
[D-Glucose		1.00]

[a] R_G values are equal to: $\dfrac{\text{Distance traveled by compound}}{\text{Distance traveled by D-glucose}}$. [b] Two peaks for the anomers of the simple substituted D-glucoses, and three peaks for the α,α', β,β', and α,β' anomers of the simple crosslinked D-glucoses are resolved by g.l.c. [c] Compound **4c** showed the presence of a second component at R_G 1.65. This component was also evident in g.l.c., appearing at r.t. 8.55 and 9.60 min. Hydrolysis under the conditions employed for the chemically modified cotton cellulose converts this component into **4c**. This second component is considered to be analogous to compound **4c**, but without the hydroxyl group on the substituent group: *i.e.*, 6-*O*-[2-(vinylsulfonyl)ethyl]-D-glucose.

parable methyl vinyl sulfone derivatives of D-glucose. The similarity in retention times is evidence that these derivatives are the simple substituted glucoses. The R_G values (by paper chromatography; see Table I) greater than that of glucose (R_G 1.0) is a further indication that these compounds are simple substituted glucoses, as a decrease in the solubility (in water) of glucose ethers compared to that of glucose results in higher R_F values[7]. Analyiss of **4a** showed a sulfur content of 11.07% (calc. 10.14%) and a molecular weight of 275 (calc. 316), confirming the identity of these simple substituted D-glucoses.

Retention times in g.l.c. of **7a**, **7b**, and **7c**, as well as the high temperatures required for elution, were consistent with expectation for the compounds of higher molecular weight. Their R_G values (by paper chromatography) were smaller than that of glucose, because of their larger molecular weight, providing additional evidence as to their identity. Analysis of **7a** showed a sulfur content of 6.96% (calc. 6.70%) and a molecular weight of 447 (calc. 478), also confirming their identity.

In order to assess the presence of *complex* substituted D-glucoses (having more than one residue of reagent in the substitutent group) and of complex crosslinked D-glucoses among the structural residues of cotton cellulose modified with divinyl sulfone, it was necessary to prepare a known mixture containing the complex substituted and crosslinked D-glucoses. For this purpose, a protected D-glucose containing a single free hydroxyl group was chosen; **1b** was treated with a fourfold excess of bis(2-hydroxyethyl) sulfone (BHES), and the mixture of products was hydrolyzed to afford a mixture of the substituted and crosslinked D-glucoses.

The M_G values given in Table II for seven components detected in an electrophoretogram of the borate complexes of the hydrolysis products constitute evidence for the existence of the complex structures. Three of the components on the electrophoretogram have been identified by comparison of their M_G values with those of authentic compounds. One had the same M_G value as that of glucose; another had the M_G value of **4b**; and another, the M_G value of **7b**. The four remaining com-

TABLE II

ELECTROPHORETIC MOBILITIES OF DIVINYL SULFONE DERIVATIVES OF D-GLUCOSE SUBSTITUTED AT O-3, AND THE ASSIGNMENT OF TENTATIVE STRUCTURES TO THE DERIVATIVES

Spot No.	M_G *values*[a]	*Structure of compound*[b]
1	0.45	G–ESE–O–ESE–O–HSE
2	0.52	G–ESE–O–HSE
3	0.58	G–ESE–O–ESE–O–ESE–G
4 (**4b**)	0.68	G–HSE
5	0.80	G–ESE–O–ESE–G
6 (**7b**)	0.85	G–ESE–G
7 (D-glucose)	1.00	G

[a] M_G values are equal to the distance traveled by the compound relative to that traveled by D-glucose. [b] G = D-glucose, ESE = 2-(ethylsulfonyl)ethyl, HSE = 2-[(2-hydroxyethyl)sulfonyl]ethyl, and O = oxygen.

ponents may be substituted D-glucoses containing more than one residue of reagent and crosslinked D-glucoses having more than one residue of reagent constituting the crosslink. Structures are tentatively assigned in Table II on the basis of the M_G values, which are dependent on the formation of a borate complex with the hydroxyl groups present[8]. Substituent groups were present only at O-3; therefore, the differences between the M_G values of the derivatives are attributed to the different percentages of D-glucose in the derivatives. The positions occupied in this series by the authentic samples are consistent with this premise. These complex structures appear on paper chromatograms as zones extending upwards from R_G 0.50.

Identity of the reagent residues in the treated fabrics. — A depiction of paper chromatograms showing typical separations of components in fermented hydrolyzates of treated cotton fabrics is shown in Fig. 1. Three spots appear in chromatograms 1, 2, and 3, and their R_G values (from the bottom to the top of chromatogram 1) are identical with those of authentic 7c, D-glucose, and 4c. There is no evidence in these chromatograms for spots having R_G values corresponding to those for 7a, 7b, or 4a; were 4b present, it would occupy the same spot as 4c.

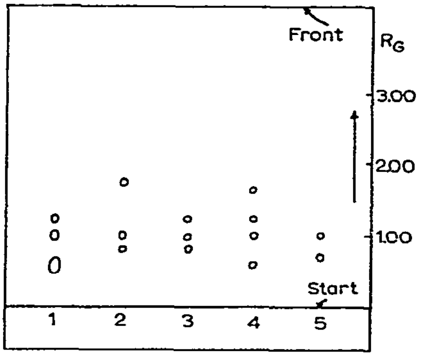


Fig. 1. Depiction of a chromatogram of D-glucose, 4b, and 7b on Whatman No. 3 MM paper, developed with 4:1:5 (v/v) butyl alcohol–acetic acid–water and detected with aniline hydrogen phosphate spray reagent. Distance of development, 15 cm; compositions: 1, the product of reaction between divinyl sulfone and cotton cellulose after hydrolysis and fermentation. 2, R_G 0.74, 7a; R_G 1.00, D-glucose; R_G 1.74, 4a; 3, R_G 0.74, 7b; R_G 1.00, D-glucose; R_G 1.26, 4b; 4, R_G 0.56, 7c; R_G 1.00, D-glucose; R_G 1.25, 4c; R_G 1.65, 6-*O*-[2-(vinylsulfonyl)ethyl]-D-glucose; 5, yeast by-products.

From the results of preceding studies involving methyl vinyl sulfone[9,10], a monofunctional analog of divinyl sulfone, it was estimated that the distribution of substituted D-glucoses that results from reaction conditions similar to those employed with divinyl sulfone is ~16, 4, and 80% at O-2, O-3, and O-6. If the two functional groups of divinyl sulfone have the same relative reactivities as that of methyl vinyl sulfone toward the hydroxyl groups on C-2, C-3, and C-6, the major and detectable components in the present study would be expected to be: among the simple substituted D-glucoses, mainly 4c, minor 4a, and traces of 4b; among the simple crosslinked D-glucoses, mainly 7c, minor 2,6′-*O*-crosslinked D-glucose, and traces of the other possibilities.

The paper-chromatographic results on fermented hydrolyzates of cotton treated with divinyl sulfone were in essential agreement with those expected from the results with methyl vinyl sulfone, except that no indications of spots for **4a** and 2,6′-*O*-crosslinked D-glucoses were observed. The conclusion is that distribution of linkages from divinyl sulfone to D-glucose residues is very similar to that between methyl vinyl sulfone and D-glucose residues; if there is a real difference, it appears to consist in fewer 2-*O*-linkages for the products from divinyl sulfone.

Quantitative evaluation of reagent residues in the treated fabric. — Densitometric measurements of authentic samples of D-glucose, **4**, and **7** at the concentrations used in the determination of the individual components of the hydrolyzates showed straight-line relationships between optical density (as measured in cm^2 from a recorder graph) and weight of component (μg) introduced onto the chromatographic paper. This relationship is shown by the curves of Fig. 2.

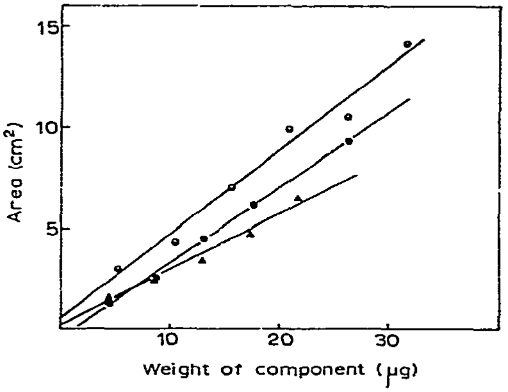


Fig. 2. Relationship of area to weight for **4**, **7**, and D-glucose; ○, D-glucose; ●, **7**; △, **4**.

To obtain quantitative information on the proportions of reagent residues in the form of **4**, **7**, and complex structures in the divinyl sulfone-treated cotton cellulose, solutions of the fermented hydrolyzates were spotted on paper and the chromatograms were scanned. The sample showed very little evidence of complex structures (*i.e.*, substituted or crosslinked D-glucoses composed of more than one residue of reagent). From the chromatograms, it was estimated that simple substituted D-glucoses accounted for 13 nmoles of reagent residues and simple crosslinked glucoses accounted for 46 nmoles of reagent residues. Since 64 nmoles of reagent residues were contained in the sample of hydrolyzate under analysis (based on the sulfur content), it may be estimated that 5 nmoles of reagent residues were in the form of complex structures. On this basis, the mole fractions of reagent residues in the form of **4**, **7**, and complex structures are 0.20, 0.72, and 0.08, respectively.

Hence, the structural components of cotton cellulose modified by treatment with divinyl sulfone in 4.0M sodium hydroxide consist of simple substituents, simple crosslinks, and complex structures in which the mole fraction of reagent residues is in the ratios 0.20:0.72:0.08. The substituent linkages and crosslinkages to the D-glucose residues are mainly at O-6; this is in agreement with the preponderance of the O-6-linkage resulting from the reaction of methyl vinyl sulfone with cotton in 4.0M sodium hydroxide[9,10].

EXPERIMENTAL

Gas–liquid chromatography. — The gas–liquid chromatograms were obtained with an F & M model 1609 instrument* equipped with a flame-ionization detector. The stainless-steel column (2 ft × 0.25 in. O.D.) was packed with 3% OV-1 (liquid phase) on a solid support of acid-washed, dichlorodimethylsilane-treated Chromosorb W (80–100 mesh) from Johns–Manville Corporation. In order to elute all of the components from the column, it was necessary to program the temperature from 190 to 300° at 10°/min. Chromatography was used for the characterization of the sugar derivatives as their trimethylsilyl ethers. Retention times (r.t.) are expressed in minutes.

Paper chromatography. — Paper chromatography on Whatman No. 3 MM paper proved to be effective for the separation from each other of D-glucose, substituted D-glucoses formed by the reaction of divinyl sulfone with one hydroxyl group of a single D-glucose residue, and crosslinked D-glucoses formed by the reaction of divinyl sulfone with one hydroxyl group of each of two D-glucose residues. Aqueous solutions (~1% concentration in the individual D-glucoses) were spotted 2 cm from one edge of the paper, and the spots (~3 mm diameter) were made as uniform as possible. Ascending chromatography was used for the development of the chromatogram with 4:1:5 (v/v) butyl alcohol–acetic acid–water. After development (15 cm) and overnight drying under ambient conditions, the chromatograms were sprayed twice on each side with aniline phosphate reagent (7 g of aniline and 18 g of phosphoric acid in 100 ml of water-saturated butyl alcohol) and heated for 10 min at 105–107°. An authentic sample of α-D-glucose (reagent grade, J. T. Baker Co.) was used for identifying the unsubstituted D-glucose in the mixtures. For purposes of identification, the distances traveled by the D-glucose derivatives were measured relative to that traveled by the D-glucose sample, and are recorded as R_G values. The identities of the derivatives were verified by g.l.c.

Quantitation of the chromatograms was performed directly on the paper chromatograms by measurement with a densitometer (Photovolt Corporation, Model 530) equipped with an automatic scanning stage and recorder. The peak areas from the recorder plots were measured by the increment method. Calibration curves were prepared for each of the authentic compounds as follows: aliquots of aqueous solutions of the compounds, containing from 4 to 30 μg of compound, were applied at the origin on the paper. After development, spraying, and densitometric scanning,

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*Mention of a company or product by the U. S. Department of Agriculture does not imply approval or recommendation of the company or product to the exclusion of other products that may also be suitable.

the areas under the curves were measured. Straight lines were fitted to plots of the area under each curve *vs.* μg of that specific component.

Samples of the concentrate from hydrolysis and fermentation of the divinyl sulfone-treated cotton celluloses were so chosen for paper-chromatographic analyses that the concentration of each component lay in the range of concentration most appropriate for accurate analysis (*i.e.*, within the linear range of the standard curve). Aqueous solutions (~2%) of the hydrolyzates were prepared. Aliquots (4 μl) were spotted on the chromatograms for the development and densitometric scanning.

Correction of the densitometric scans for contributions due to the byproducts from the fermentation with yeast was made by scanning an equivalent amount of the fermentation byproducts from a sample containing only D-glucose plus yeast. The peak areas from the recorder plots for the fermentation byproducts were subtracted from the peak areas on the chromatograms of the samples of divinyl sulfone-treated cotton cellulose.

Electrophoretic separation of components consisting of divinyl sulfone derivatives of D-*glucose*. — Aqueous solutions (~2%) of hydrolyzates were prepared, and samples were spotted on Whatman No. 3 MM paper about 9 in. from one end of the strip of paper (27 × 9 in.) with a platinum-wire loop (diameter ~2 mm). The paper was carefully wetted with 0.05M sodium borate by dipping the paper in the buffer solution, starting from the ends of the paper, and then blotting to allow the two buffer-solution "fronts" to meet by capillary action at the line where the sample spots were located. A voltage of ~1.90 kV was applied to the paper for 2 h. The electrophoretogram was allowed to dry overnight under ambient conditions; it was then sprayed with the aniline phosphate reagent, and heated for 10 min at 110°.

2-O-Substituted (**4a**) *and 2,2'-O-crosslinked* (**7a**) D-*glucoses*. — Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α,β-D-glucoside (**1a**) (m.p. 178–180°, 4.0 g, 10.74 mmoles), prepared by the method of Roberts and Rowland[5], was dissolved in 20 ml of dry *N,N*-dimethylformamide (DMF), and 0.23 g of tetramethylguanidine (TMG) was added as a catalyst. Divinyl sulfone (DVS) (1.59 g, 13.45 mmoles) was added, and the solution was stirred for 1 h at room temperature. The mixture was diluted with ~100 ml of distilled water, M sulfuric acid (8 ml) was added, the suspension was filtered, and the insoluble material was washed with water and dissolved in diethyl ether. The solution was washed with three 25-ml portions of distilled water, and evaporated (rotary evaporator) to dryness. The residue was dissolved in absolute ethanol (200 ml), M sulfuric acid (8 ml) and 10% palladium-on-carbon (0.5 g) were added, and the solution was shaken with hydrogen under a slight pressure at room temperature until uptake of hydrogen ceased (~72 h). The catalyst was then filtered off, and the filtrate was concentrated (rotary evaporator) to ~20 ml. M Sulfuric acid (100 ml) was added, and the solution was boiled under reflux (nitrogen atmosphere) for 16 h to hydrolyze off the methyl groups. The sulfuric acid was neutralized with barium hydroxide, the suspension was filtered, and the filtrate was evaporated under diminished pressure, and freeze-dried, yielding 2.39 g of material. Paper-chromatographic analysis showed the presence of only three components, R_G 1.0, 1.74, and 0.74.

Analysis by g.l.c. confirmed that the component having R_G 1.0 was D-glucose. The component having R_G 1.74 had g.l.c. retention times of 9.5 and 11.0 min, comparable to those of the anomers of the 2-*O*-monosubstituted methyl vinyl sulfone derivatives of D-glucose. The component having R_G 0.74 is considered to be **7a**, the only other product expected from this reaction; the R_G value is consistent with that expected, as a solvent system that moves **4a** a greater distance than D-glucose would not be expected to move the crosslinked derivative so far. Analysis by g.l.c. of a fraction containing only the crosslinked derivative (R_G 0.74) showed peaks corresponding to unsubstituted D-glucose, **4a**, and a substance eluted at 17.3 min. The last substance, eluted after the highest temperature of the column had been reached, corresponds to **7a**. This mixture of compounds is the result of thermal breakdown of **7a** that occurs on g.l.c. at the temperature (300°) necessary for elution.

3-O-Substituted (**4b**) *and 3,3'-O-crosslinked* (**7b**) D-*glucoses*. — 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**1b**) was recrystallized from hexane to m.p. 110°; 2.5 g (9.63 mmoles) was dissolved in 20 ml of dry DMF, TMG (0.2 g) and divinyl sulfone (725 mg, 6.14 mmoles) were added, and the mixture was stirred for 24 h at room temperature. The mixture was diluted with distilled water (50 ml), and made neutral with M sulfuric acid (2 ml); the suspension was extracted with diethyl ether (250 ml) and the aqueous layer (*A*) was set aside. The extract was washed with three 15-ml portions of distilled water, and evaporated to a thick syrup which was dissolved in acetone and kept for 72 h, affording crystals. The mother liquor was removed with a pipet, the crystals were washed several times with 1–2 ml of acetone, and the washings were added to the mother liquor. A third fraction was obtained from the aqueous layer *A*; this was filtered, and the solid was washed with water, and dissolved in acetone. The three fractions, namely (*1*) the ether-soluble fraction crystallized from acetone, (*2*) the ether-soluble fraction that did not crystallize from acetone, and (*3*) the fraction insoluble in ether and in the aqueous acid, were hydrolyzed individually by refluxing overnight in M sulfuric acid. Each hydrolyzate was made neutral with barium hydroxide, concentrated under diminished pressure, and freeze-dried. Analysis of the three fractions by g.l.c. showed that fractions *1* and *2* consisted of a mixture of unsubstituted α,β-D-glucoses (r.t. 3.5 and 4.25 min), **4b** (r.t. 8.13 and 9.15 min), and **7b** (r.t. 17.85, 19.20, and 20.80 min). A mixture of unsubstituted α,β-D-glucoses and **4b** was found in fraction *3*. Paper chromatography of fraction *1* showed mainly **7b** (R_G 0.74), indicating that the three components found by g.l.c. were artifacts caused by thermal breakdown of the crosslinked derivative at the temperature (300°) required for elution. Paper chromatography of fraction *2* showed a mixture of unsubstituted D-glucose (R_G 1.0), **4b** (R_G 1.26), and **7b** (R_G 0.74). Paper chromatography showed that fraction *3* contained unsubstituted D-glucose and **4b**, a result confirmed by g.l.c. analysis. The total yield of the products of hydrolysis was 1.45 g.

6-O-Substituted (**4c**) *and 6,6'-O-crosslinked* (**7c**) D-*glucoses*. — 3,5-*O*-Benzylidene-1,2-*O*-isopropylidene-α-D-glucofuranose (**1c**) (5 g, 16.2 mmoles) was dissolved in dry DMF (20 ml), and TMG (0.35 g) and divinyl sulfone (1.0 g, 8.5 mmoles) were added. The mixture was stirred for 40 h at room temperature, and distilled water (50 ml) and M sulfuric acid (8 ml) were added. The mixture was extracted with diethyl ether (200 ml), and the aqueous layer (*B*) was set aside. The extract was washed with three 15-ml portions of water, and evaporated to a light-colored syrup, fraction *1*. Layer *B* contained an ether-insoluble precipitate formed when the water and acid were added; this fraction (*2*) was washed with water by decantation, and dissolved in acetone, and the solution was decolorized with carbon and filtered through a Celite mat. The filtrate was evaporated to a dark-colored syrup, which was dissolved in ~50 ml of cyclohexane and the minimal volume of acetone. The solution was kept overnight, and the resulting, clear solution was decanted from a dark residue, and evaporated to a light-colored syrup. The ether-soluble product (fraction *1*) and the ether-insoluble product (purified fraction *2*) were separately hydrolyzed by refluxing overnight in M sulfuric acid. Each hydrolyzate was made neutral with barium hydroxide, and the filtrate was concentrated under diminished pressure, and freeze-dried. Paper-chromatographic analysis of the hydrolyzates was performed, and the components in the spots were identified by g.l.c. Both in fractions *1* and *2*, unsubstituted D-glucose (R_G 1.00), **4c** (R_G 1.25 and 1.65), and **7c** (R_G 0.56) were found to be present. The yield of hydrolysis product was 2.25 g.

Preparation of complex substituted and crosslinked D-*glucoses*. — The objective of this reaction was to produce more-complex substituted D-glucoses having a substituent chain composed of more than one reagent residue, and more-complex crosslinked D-glucoses having a crosslink chain consisting of more than one reagent residue, in addition to the simple substituted D-glucoses and simple crosslinked D-glucoses already described. The reaction was therefore conducted with a 4:1 mole ratio of sulfone to protected D-glucose. Compound **1c** (1.0 g, 3.8 mmoles) was mixed with bis(2-hydroxyethyl) sulfone (BHES) (1.06 g, 6.9 mmoles), sufficient water was added to wet the reaction mixture, sodium carbonate (~0.2 g) was added as a catalyst, and the mixture was stirred and then dried in a vacuum oven at 50–55°/0.5 torr. The dry mixture was then heated for 5 min at 150°, and the isopropylidene groups were hydrolyzed off by refluxing overnight in M sulfuric acid. The acid was neutralized with barium hydroxide, the suspension was filtered, and the filtrate was concentrated under diminished pressure to ~10 ml, and freeze-dried. The yield of hydrolysis products was 1.48 g; sulfur content 12.15%, corresponding to ~1.2 residues of BHES per residue of D-glucose.

Isolation of the substituted and crosslinked D-*glucose derivatives*. — The preparative-scale, paper-chromatographic method of Frush[6] was employed for the isolation of the D-glucose derivatives described. An aliquot (containing about 200 mg) of a 20% solution of the hydrolysis product was applied as a narrow band ~1.5 in. from one edge of the thick chromatographic paper (Whatman No. 17); the sample was loaded onto both sides of the paper. A wick (prepared from Whatman No. 3 MM paper) was hand-stitched to the thick paper, forming a stirrup by which the chromatogram could be suspended in a chromatographic development chamber, and development was conducted by descending chromatography with 4:1:5 (v/v) butyl alcohol–acetic acid–water. After development, a "print" was obtained as described by Frush[6] by placing the wet chromatogram on a clean, large piece of plate glass, placing a sheet of Whatman No. 1 paper over the still-wet chromatogram, and drawing a series of straight lines down the length of the chromatogram in the direction of development by use of a narrow, plastic wheel. This process transferred a small amount of material from the chromatogram to the thin paper. The "print" was sprayed with aniline hydrogen phosphate reagent, and heated for 5–10 min at 110°, and the bands containing the D-glucose derivatives were located. The bands were lightly sketched on the air-dried chromatogram with a graphite pencil, and separated by cutting with scissors, and the compound was eluted from each strip of paper with distilled water. Each fraction eluted was concentrated under diminished pressure, freeze-dried, and purified by dissolving in dry pyridine, treating with decolorizing carbon, and filtering. The filtrate was evaporated to dryness, and the residue was dissolved in distilled water and freeze-dried to remove the last traces of pyridine.

Modification of cotton cellulose with divinyl sulfone. — De-sized, scoured, and bleached print-cloth weighing ~3.3 oz/yd^2 and having a thread count of 68 × 72 was employed in this study. The sample was prepared by treating a piece of fabric (12 × 18 in.) with an aqueous solution (11%) of divinyl sulfone to a weight increase ("wet pickup") of ~100%; the wet fabric was then soaked in 4.0M sodium hydroxide (2 liters) for 1 h, washed for 45 min in running tap-water, dried in a forced-draft oven at 90–100°, and allowed to equilibrate in room air. The treated fabric contained 0.96% of sulfur.

Hydrolysis of the chemically modified cotton cellulose, and concentration of the substituted and crosslinked D-*glucoses.* — The sample of fabric modified with divinyl sulfone was ground to pass a 20-mesh screen. The chopped fibers were hydrolyzed according to the method of Rowland *et al.*[3] with 72% sulfuric acid, which was diluted in subsequent steps. The resulting solution of D-glucose, substituted D-glucoses, and crosslinked D-glucoses was made neutral with barium hydroxide, the suspension was filtered, and the filtrate was concentrated under diminished pressure to about 150 ml.

The unsubstituted D-glucose, which, on paper chromatograms, masked the substituted D-glucoses so that analysis was impossible, was removed by fermentation; the pH of the hydrolyzate was lowered to 5–6 with glacial acetic acid, bakers' yeast (1 g) was added, and fermentation was allowed to proceed for 72 h at 30–35°. The suspension was concentrated, and filtered, and the filtrate was evaporated under diminished pressure, and freeze-dried. The sulfur content of the product was 2.55%.

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