THIOL AND DISULPHIDE DERIVATIVES OF CELLULOSE

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The acid catalyzed etherification of cellulose with 1-chloro-2,3-epoxypropane yielded 3-chloro-2-hydroxypropylcellulose ($S_{Cl} \leqq 0{\cdot}67$); the latter, by a known sequence of reactions through the thiosulphate derivative and after its reduction, gave 2-hydroxy-3-mercaptopropylcellulose (up to 0·43 mmol SH/g). Mercaptodeoxycelulose (up to 0·53 mmol SH/g) was prepared from the less reactive chlorodeoxycellulose by an analogous sequence of reactions. Bead mercaptodeoxycellulose is more advantageously obtained by using tosylate of bead cellulose; this procedure is also more advantageous than the preparation of bead 2-hydroxy-3-mercaptopropylcellulose. Disulphide derivatives of cellulose were prepared (*a*) by a quantitative reaction of thiol derivatives with 2,2′-dipyridyl disulphide and (*b*) by a reversible crosslinking of cellulose or carboxymethylcellulose with bifunctional disulphides. Disulphide derivatives of cellulose prepared by procedure (*b*) give after reduction thiol derivatives (up to 0·185 mmol SH/g), and further by employing procedure (*a*) yield 2-pyridyl disulphide derivatives.

Symmetrical, but mainly asymmetrical disulphide derivatives of polysaccharides are used in the immobilization of thiol enzymes and in the covalent chromatography of thiol enzymes/proteins and peptides. Thus, mixed disulphides derived from glutathionyl and 2-hydroxy-3-mercaptopropylagarose (Sepharose)[1,2] by a reaction with 2,2′-dipyridyl disulphide were mainly used in the isolation and purification of thiol enzymes (*e.g.*, papain, urease, phosphofructokinase, thiol-disulphide oxidoreductases, protein disulphide isomerase, glutathione-insulin transhydrogenase), thiol proteins (such as mercaptoalbumin, collagen, casein, copper thioneine, alpha-chains of hemoglobin) and thiol peptides (*e.g.* from ceruloplasmine, human colonic tumour glycoprotein)[3,4]. In our preceding paper[5] we demonstrated that the thiol enzymes could be immobilized also using disulphide derivatives of cellulose. So far, however, no attention has been concentrated on disulphide derivatives of cellulose in connection with their use in covalent chromatography.

Disulphide derivatives of cellulose may be obtained by reacting thiol celluloses with suitable disulphides or by binding disulphides onto the cellulose matrix. The former procedure (reaction of mercaptodeoxy- and 2-hydroxy-3-mercaptopropylcellulose with 2,2′-dipyridyl disulphide) was used in our preceding paper[5]. In this study we describe the improved procedures of preparation of both thiol derivatives.

In the case of 2-hydroxy-3-mercaptopropylcellulose the change consists in the substitution of fluoroborate catalysts with perchloric acid in the activation of cellulose

with 1-chloro-2,3-epoxypropane, similarly to starch[6,7], and also in a direct reaction between 3-chloro-2-hydroxypropylcellulose and thiosulphate (Scheme (*A*)).

$$\text{cel-OH} + \underset{\diagdown\,\text{O}\,\diagup}{\text{CH}_2\text{—CHCH}_2\text{Cl}} \xrightarrow{\text{H}^+} \underset{I}{\text{cel-OCH}_2\text{CH(OH)CH}_2\text{Cl}} \rightarrow$$

$$\xrightarrow{\text{Na}_2\text{S}_2\text{O}_3} \underset{II}{\text{cel-OCH}_2\text{CH(OH)CH}_2\text{S}_2\text{O}_3\text{Na}} \xrightarrow{\text{RSH}} \underset{III}{\text{cel-OCH}_2\text{CH(OH)CH}_2\text{SH}} \qquad (A)$$

Mercaptodeoxycellulose is prepared either through chlorodeoxycellulose[5] (Scheme (*B*)) or by a sequence of reactions similarly to starch[8] (Scheme (*C*))

$$\text{cel-OH} \xrightarrow{\text{SOCl}_2} \underset{IV}{\text{cel-Cl}} \xrightarrow{\text{Na}_2\text{S}_2\text{O}_3} \underset{V}{\text{cel-S}_2\text{O}_3\text{Na}} \xrightarrow{\text{RSH}} \underset{VI}{\text{cel-SH}} \qquad (B)$$

$$\text{cel-OH} \xrightarrow{\text{TsCl}} \underset{VII}{\text{cel-OTs}} \xrightarrow{\text{KS—CS—OC}_2\text{H}_5} \underset{VIII}{\text{cel-SCSOC}_2\text{H}_5} \xrightarrow{\text{NH}_4\text{OH}} \underset{VI}{\text{cel-SH}} \qquad (C)$$

Disulphide derivatives are prepared from thiol celluloses by a reaction with 2,2′-dipyridyl disulphide. Direct introduction of disulphide into the cellulose matrix may also be accomplished by a reaction of disulphides containing acylhydrazide or isocyanate groups. This procedure was used in the reversible crosslinking of oxidized cellulose with dithiodiacetohydrazide[9], or of cellulose with dithiodiethyl isocyanate[10].

$$\text{cel-OCH}_2\text{COOCH}_3 + \text{(H}_2\text{NNHCOCH}_2\text{S-)}_2 \rightarrow \underset{IX}{\text{cel-OCH}_2\text{CONHNHCOCH}_2\text{SSCH}_2\text{CONHNH}_2}$$

$$\text{cel-OCH}_2\text{CONHNHCOCH}_2\text{SSCH}_2\text{CONHNHCOCH}_2\text{O-cel}$$

$$\xrightarrow{(C_4H_9)_3P} \underset{X}{\text{cel-OCH}_2\text{CONHNHCOCH}_2\text{SH}} \qquad (D)$$

$$\text{cel-OH} + \text{(OCNCH}_2\text{S-)}_2 \rightarrow \underset{XI}{\text{cel-OCONHCH}_2\text{SSCH}_2\text{NHOCO-cel}} \xrightarrow{\text{RSH}}$$

$$\rightarrow \underset{XII}{\text{cel-OCONHCH}_2\text{SH}} \qquad (E)$$

In this work we tried to prepare symmetrical disulphide derivatives of cellulose by reacting carboxymethylcellulose methyl ester with dithiodiacetohydrazide (Scheme (*D*)), and cellulose with dithiodimethyl isocyanate (Scheme (*E*)).

In addition to the presence of functional groups, the geometric shape of sorbent particles is also important for chromatographic purposes. The obvious advantages of bead cellulose[11] as a carrier for the immobilization of enzymes[12] and affinity chromatography[13] compared with the traditional forms of cellulose (fibrous, powder, microcrystalline) have already been dealt with elsewhere. This is why bead cellulose was examined with special attention, also in connection with the use of its thiol and disulphide derivatives in covalent chromatography.*

EXPERIMENTAL

Initial Celluloses

Cellulose powder (*a*: Whatman, standard grade) crosslinked with 1-chloro-2,3-epoxypropane (*b*: $Q = 1{\cdot}5/1{\cdot}5$, $q = 10$, $v = 3{\cdot}6$ ml g^{-1}, ref.[14]), bead cellulose (*c*: lot C-538/1, Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, Prague), carboxymethylcellulose (*d*: Lovosa TS-20, North Bohemian Chemical Works, Lovosice). In the derivatives denoted with Roman numbers the type of initial cellulose is given by the corresponding letter (*a*—*d*) assigned to it.

2-Hydroxy-3-mercaptopropylcelluloses *IIIa*—*c*

To 2·25 g of dried cellulose (in the case of cellulose *c* drying was accomplished with acetone and ether[11] to a content of 5·6% water; 2·385 g of the material was used), 0·4 ml water (0·26 ml

TABLE I

Preparation and analysis of 3-chloro-2-hydroxypropylcelluloses (*S* denotes the degree of substitution, $S_{Cl} = 3{\cdot}0$ corresponds to 100% conversion)

Cellulose	Product	Temperature °C	Chlorine		
			%	mmol/g	S_{Cl}
Powder	*Ia*	95 ± 3	4·57	1·29	0·24
Crosslinked	*Ib*	80 ± 2	10·60	2·99	0·67
Bead	*Ic*	90 ± 2	6·03	1·70	0·33

* During the editing of this paper, a novel procedure of preparation of the thiol derivative of cellulose and its application in the chromatography of mercurated polynucleotides have been reported — Feist P. L., Danna K. J.: Biochemistry *20*, 4243 (1981).

in the case of cellulose *c*) and 4·4 ml 1-chloro-2,3-epoxypropane were added, the mixture was stirred at room temperature 5 min, and 50 μl of 60% perchloric acid was added in parts; the suspension was maintained with occasional stirring at these temperatures for 3 h (Table I). The products, *i.e.* 3-chloro-2-hydroxypropyl derivatives (*Ia—c*) were washed with water and acetone (*Ic* was washed with acetone and water); their characteristics are given in Table I.

0·5 g *I* (in the case of *Ic* 1·45 g of the compound was first slowly washed with 100 ml of 5·7M-$Na_2S_2O_3$ and the liquid was removed by suction) was suspended in an aqueous solution of sodium thiosulphate (Table II), and the suspension was maintained at 100°C with occasional stirring. The products, 2-hydroxy-3-thiosulphatopropyl derivatives (*IIa—c*) were washed with water and acetone (*IIc* only with water); the characteristics are given in Table II.

TABLE II

Reaction between 3-chloro-2-hydroxypropylcelluloses *Ia—c* (0·5 g) and sodium thiosulphate to yield 2-hydroxy-3-thiosulphatopropylcelluloses *IIa—c* (*S* denotes the degree of substitution)

Solution of $Na_2S_2O_3$		Time h	Product	Sulphur			Chlorine	
ml	c, M			%	mmol/g	$S_{S_2O_3Na}$	%	mmol/g
3·0	4·4	15	*IIa*	4·05	1·26	0·12	0	0
1·5	20·0	40[a]	*IIb*	8·74	2·73	0·30	3·61[b]	1·02
3·0[c]	5·7	20	*IIc*	7·79	2·43	0·26	0	0

[a] In the reaction *Ib* also times 20 and 60 h (Fig. 1) were chosen; [b] $S_{Cl} = 0·18$. [c] 1·45 g *Ic* filtered by suction was used.

TABLE III

Reduction of 2-hydroxy-3-thiosulphatopropylcelluloses *IIa—c* to 2-hydroxy-3-mercaptopropyl-celluloses *IIIa—c* (conversion, ξ, calculated from the content of functional groups — mmol/g)

Initial *II*	50 mM $Na_2B_4O_7$	$HS(CH_2)_2OH$	Product	S		SH	ξ, %	
g	ml	ml		%	mmol/g	mmol/g	*II*	*I*
0·44	2·7	0·37	*IIIa*	2·40	0·75	0·25	39·7	19·4
0·55[a]	5·3	0·92	*IIIb*	4·88	1·52	0·17	14·8	5·7
0·50[b]	5·1	0·90		5·84	1·82	0·19	14·0	6·4
0·50[c]	3·1	1·25		7·93	2·47	0·18	10·9	6·0
0·885	3·8	0·75	*IIIc*	5·06	1·58	0·43	35·4	25·3

[a—c] Preparation times of *IIb* (h): [a] 20, [b] 40, [c] 60 (*cf.* Table II).

2-Hydroxy-3-thiosulphatopropylcellulose *IIb* was also prepared using an oxirane derivative: 0·5 g *Ib* was stirred in 0·485M NaOH (5 ml) at room temperature for 1 h, washed with water, 2·3M solution of sodium thiosulphate in 0·5M phosphate buffer pH 6·3 (50 ml) and filtered with suction. After resuspending in a 2·3M solution of sodium thiosulphate in 0·5M phosphate buffer pH 6·3 (25 ml), the mixture was stirred at room temperature for 16 h, washed with water and acetone. The product contained 8·15% sulphur and 2·15% chloride.

Reduction of derivatives of II: 0·5 g *II* (in the case of *IIc*, the initial 0·885 g of the compound was first washed with 50 ml 50 mM-$Na_2B_4O_7$ and the liquid was removed by suction) was resuspended in 50 mM-$Na_2B_4O_7$ containing tributylphosphine (1% v/v), 2-mercaptoethanol was added (Table III), and pH was adjusted to 9; the suspension was stirred at room temperature 30 min, pH was maintained at 9. The products, 2-hydroxy-3-mercaptopropylcelluloses (*IIIa–c*), were washed with 1 mM disodium EDTA, water and acetone (product *IIIc* was filtered with suction after being washed with 1 mM disodium EDTA and water, and if needed, was stored in 0·1M acetic acid containing 1 mM disodium EDTA at 4°C).

II were also reduced with sodium borohydride in 1M 2-amino-2-hydroxymethyl-1,3-propanediol (pH 9) at room temperature for 1 h[15].

Mercaptodeoxycelluloses *VIa,c*

Chlorodeoxycellulose *IVa* was prepared by reacting activated cellulose powder with thionyl chloride[5] at 25°, 40° and 60°C (Table IV). Thiosulphatodeoxycellulose *Va* was than obtained by heating *IVa* (5 g) with 25 ml of an aqueous solution of sodium thiosulphate (Table IV) at 100°C for 40–216 h (Fig. 1). Derivative *Va* (0·5 g) was reduced to *VIa* with 2-mercaptoethanol in 50 mM-$Na_2B_4O_7$ containing 1 mM-EDTA, pH 7·7–7·9, 30 min at room temperature (Table V). In some cases the reduction was carried out also with thioglycolic acid or dithiothreitol[2,5], or with sodium borohydride at 50°C for 3 h (ref.[6]).

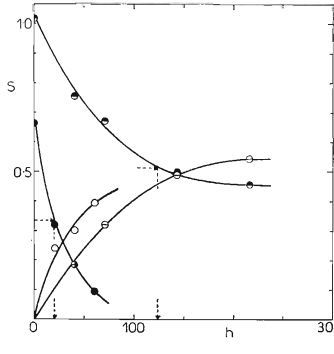

FIG. 1

Time dependence of the reaction between 3-chloro-2-hydroxypropylcellulose *Ib* and 20M-$Na_2S_2O_3$, and between chlorodeoxycellulose *IVa* and 12·5M-$Na_2S_2O_3$. Incorporation of sulphur in *IIb* (○) and *Va* (◒) and decrease of chlorine from *Ib* (●) and *IVa* (◓) are expressed through the corresponding degrees of substitution ($S_{S_2O_3}$, S_{Cl}); $\tau_{0,5}$ is time within which the samples "lost" 50% chlorine.

Mercaptodeoxycellulose based on bead cellulose (*VIc*) was obtained by reactions according to Scheme C: 25 g of bead regenerated cellulose (water content 83%) is stirred at room temperature with 34·5 ml acetone for 30 min; the liquid phase (12·5 ml), is removed, acetone (12·5 ml) is added, stirring continues for 30 min, and the liquid phase (12·5 ml) is removed again. After that, a 50% solution of sodium hydroxide (2·30 ml) is added, the mixture is stirred 1 h, cooled to 0°C, pure *p*-toluenesulphonyl chloride (5·02 g) is eventually added in parts, and stirring continues for another 1·5 h without cooling. Tosylcellulose *VIIc* is freed from the liquid fraction by suction, washed with acetone, water, and is transferred into ethanol (the analytical sample contains 2·69% sulphur in the dry residue, $S_{Ts} = 0{\cdot}16$). Product is stirred and heated to boil for 16 h with potassium xanthogenate (1·62 g) in ethanol (30 ml). S-Cellulose-O-ethyl dithiocarbonate *VIIIc* is washed with water, and then transferred into 5% ammonia (25 ml); the mixture is left to stand with occasional stirring for 24 h. Product *VIc* is washed with water, acidified with 0·1M-HCl and washed with water. It contained 2·93% sulphur.

Carboxymethylcellulose Crosslinked with Dithiodiacetohydrazide (*IXd*) and its Reduction

A suspension of carboxymethylcellulose methyl ester (0·5 g) in a 1·43M solution of dithiodiacetohydrazide[9] in 80% hot aqueous methanol (5 ml) was refluxed for 2 h. Product *IXd* (0·43 g) was washed with ethanol, water and acetone; it contained 0·96% sulphur. It was reduced with a 2% (v/v) solution of tributylphosphine in 90% methanol[10] (20 ml) with refluxing for 6 h. Product *Xd* (0·38 g) was washed with water, 50% ethanol, ethanol and acetone; it contained 0·68% sulphur.

Cellulose Crosslinked with Dithiodimethyl Isocyanate (*XIa*) and its Reduction

Before use (24 h(, the WAN activated[5] cellulose powder was removed from benzene by suction, washed, and dimethylformamide was poured over it. After the liquid was filtered off with suction, 1·8 g cellulose (0·5 g of dry cellulose) was mixed with a solution of 2·72 g dithiodimethyl isocyanate[10] in 12·5 ml dimethylformamide, the suspension was maintained at 80°C for 20 h, product *XIa* (2·27 g) was washed with dimethylformamide and extracted with hot acetone; it contained 26·13% sulphur and 12·55% nitrogen. Compound *XIa* (1 g) was reduced with 2-mercaptoethanol (2 ml) in 50 mM $Na_2B_4O_7$ (5 ml), pH 9, containing 1% (v/v) tributylphosphine, at room

Table IV

Preparation conditions and analysis of chlorodeoxycellulose *IVa* and thiosulphatodeoxycellulose *Va*. The chlorination of cellulose powder was carried out at the molar ratio thionyl chloride: anhydroglucose unit = 10·89; $S_{Cl} = 3{\cdot}0$ corresponds to 100% conversion. For the following reaction *IVa* (0·5 g) with $Na_2S_2O_3$ (25 ml of solution, 100°C, 40 h), the initial compound indicated on the same line was used

Temperature °C	Chlorine in *IVa*			$Na_2S_2O_3$ c, M	Sulphur in *Va*		Chlorine in *Va*	
	%	mmol/g	S_{Cl}		%	mmol/g	%	mmol/g
25	4·77	1·35	0·23	3·25	5·44	1·70	1·71	0·48
40	11·40	3·22	0·58	7·74	5·89	1·84	8·60	2·27
60	18·40	5·20	1·02	12·5	6·25	1·95	14·20	4·01

temperature for 8 h. Product *XIIa* (0·40 g) was washed with 1 mM disodium EDTA, water and acetone; it contained 21·06% sulphur.

2-Pyridyl Disulphide Derivatives of Cellulose

Thiol derivatives of cellulose were suspended in solutions of 2,2′-dipyridyl disulphide in a mixture acetone (60%) — aqueous 50 mM-$NaHCO_3$ (40%) containing 1 mM-EDTA, and stirred at room temperature for one hour. The concentration of 2,2′-dipyridyl disulphide was chosen so as to provide a fivefold molar excess of disulphide with respect to the thiol groups of cellulose. Prior to reaction, the thiol derivatives of bead cellulose were "activated" with 2-mercaptoethanol by employing the same procedure as in the reduction of thiosulphate derivatives. The products were washed with 60% acetone and acetone, the 2-pyridyldisulphidic derivatives of bead cellulose were washed with 60% acetone and water and stored at 4°C.

Analytical Methods

The degrees of substitution (*S*) of cellulose derivatives *I*—*XII* were calculated using the microdetermination of chlorine, sulphur and nitrogen. The content of SH groups was determined by a reaction with 5,5′-dithiobis(2-nitrobenzoic acid) using a 0·1M phosphate buffer, pH 7·6, containing 1 mM-EDTA and 1 mM 5,5′-dithiobis(2-nitrobenzoic acid); after reaction lasting three hours at room temperature, polythiol (1—5 mg dry mass) was removed, and absorbance at 412 nm was measured. The content of SH groups was calculated using $\varepsilon = 13\,600\ \text{M}^{-1}\ \text{cm}^{-1}$. A similar procedure was employed in the determination of the 2-pyridyl disulphide residues in the respective cellulose derivatives: 5—10 mg (dry mass) of the disulphide cellulose derivative was added to 5 ml of a 0·1M phosphate buffer containing 1 mM-EDTA and 20 mM cysteine, stirred at room temperature for 3 h, after which cellulose was removed and absorbance of the solution was measured at 343 nm. The concentration of 2-thiopyridone was calculated using $\varepsilon = 8\,080\ \text{M}^{-1}\ .\ \text{cm}^{-1}$ (ref.[16]). In some cases 2-mercaptoethanol or sodium sulphide was used instead of cysteine. At least two measurements were performed with each sample.

Degree of Substitution and Conversion

The degree of substitution for chloro (S_{Cl}), thiosulphato ($S_{S_2O_3Na}$) and tosyl derivatives (S_{Ts}) of cellulose was calculated using the equation[14] $S = 162Y_w/(100W - Y_w W_1)$, where 162 is the weight equivalent of an anhydroglucose unit, Y_w is the chlorine or sulphur content (in %) according to analysis, W are the weight equivalents of Cl, S or S_2 (in *V*), and W_1 are the weight equivalents of 3-chloro-2-hydroxypropyl, 2-hydroxy-3-thiosulphatopropyl, chloro (in *IVa*), tosyl (in *IVc*) and thiosulphato substituents minus 1 and 17, respectively.

Conversion (%) was calculated as a hundredfold ratio of the degree of substitution of the product and initial compound. In chloro, thiosulphato and tosyl derivatives the calculated *S* values were substituted, in other derivatives the content of functional groups (mol/g), was used for this purpose.

RESULTS AND DISCUSSION

2-Hydroxy-3-mercaptopropylcelluloses

3-Chloro-2-hydroxypropylcelluloses were prepared by the acid catalyzed (perchloric acid) etherification of cellulose with 1-chloro-2,3-epoxypropane. Cellulose powder, crosslinked cellulose powder and bead cellulose were used under the reaction condit-

ions (with the exception of temperature) given in ref.[7] for sample D in Table 2. The reactions proceeded differently from those observed with starch[6] and crosslinked starch[7]. If, namely, the etherifications occurred at temperatures (100–120°C) reported in preceding papers[6,7], they resulted in darkening and mass losses (powder, bead cellulose) or in complete dissolution of the product in acetone (crosslinked cellulose). Moreover, in bead cellulose there was complete decomposition of the macroporous structure. The highest temperature at which these unfavourable phenomena do not occur any more differs for different types of cellulose (Table I). The lowest conversions (8%) and at the same time the lowest yields were obtained with cellulose powder. However, unlike experiments with starch[6], no higher substituted soluble fractions were isolated in this case. On the contrary, in the acid catalyzed etherification the crosslinked cellulose powder is more reactive than cellulose powder (Table I). A similar result was obtained also if these celluloses were etherified with *p*-nitrobenzyl chloride in an alkaline solution[14]. This finding has been assigned to an increased number of accessible OH groups of cellulose crosslinked with 1-chloro-2,3-epoxypropane responsible for the improvement of cellulose reactivity with regard to some type of etherification reactions. We believe that such explanation is acceptable also for the increased reactivity of bead cellulose compared with cellulose powder (Table I).

An analogous derivative of crosslinked cellulose is the one most approaching 3-chloro-2-hydroxypropyl crosslinked starch (13·13% chlorine; 34% mass increase) (ref.[7], Table 2) by both conversion and yield (Table I). However, with respect to the temperatures used crosslinked cellulose is much more reactive. Similarly to crosslinked starch (The Hubinger Co., Keokuk, Iowa), cellulose crosslinked with 1-chloro-2,3-epoxypropane possessing the highest degree of crosslinking was used[14]. The degree of crosslinking of crosslinked starch remains unknown, however, which makes it impossible to decide if differences in reactivity in the etherification should be attributed to differences in the structure of native glucanes or to the degree of their crosslinking.

Unlike the preceding study[5], 2-hydroxy-3-thiosulphatopropyl derivatives were prepared by direct substitution of chlorine. It was mainly with the 3-chloro-2-hydroxypropyl derivative of bead cellulose that such procedure gave much better results (78·8% conversion and complete removal of chlorine) (Table II). In an analogous derivative of crosslinked cellulose (reaction time 40 h) at lower conversion (44·8%) part of chlorine also remained (26·9%). By extending the reaction time with thiosulphate for the latter derivative the sulphur content can be raised and the chlorine content reduced (Fig. 1), but after reduction there is no rise in the content of the SH groups (Table III). The preparation of 2-hydroxy-3-thiosulphatopropylcellulose from *Ib via* the oxiran derivative[5] leads to a product with a comparatively high sulphur content (8·15%) and low chlorine content (2·15%), but only 161 μmol SH/g are obtained after reduction.

Tributylphosphine is a more effective protective agent for thiols in aqueous solutions in the pH range 7–9 than EDTA (ref.[17]). Due to this, thiosulphate derivatives can be reduced with thiols also at pH 9. Reduction under such conditions leads up to a 40% conversion of 2-hydroxy-3-thiosulphatopropyl derivatives (Table III) compared with 15·9% in the preceding paper[5]. Favourable results were also obtained with bead cellulose; the overall conversion from 3-chloro to 3-mercapto-2-hydroxypropylcellulose (25·3%) was the highest of all. On the contrary, the effectiveness of reduction seems to be weakened by the "deactivation" of the SH groups of cellulose; changes in the sulphur content during the reduction are almost stoichiometric in most cases (Tables II, III). Reduction with sodium borohydride is not suitable, it gives only traces of SH groups in the case of sample *IIb* prepared from *Ib* by a 40 h reaction.

If sodium hydrogen sulphide was used in the preparation of the mercapto derivative from chlorohydroxypropyl crosslinked cellulose[18], a 19% conversion was reached with respect to the sulphur content; somewhat better results were obtained in a procedure *via* the alkylation of thiourea and decomposition of the arising alkylisothiuronium salt with sodium hydroxide (22·6% conversion). Not in one case, however, was the content of SH groups determined. Conversion obtained with such an evaluation procedure ranged between 50·8 and 90·9% (Table II).

Mercaptodeoxycellulose

Still higher degrees of substitution than those reached in the preparation of 3-chloro-2-hydroxypropyl crosslinked cellulose may be obtained in the preparation of chloro-

TABLE V

Reduction of thiosulphatodeoxycellulose *Va* (0·5 g) to mercaptodeoxycellulose *VIa* (conversion, ξ, calculated from the content of functional groups — mmol/g)

50 mM-$Na_2B_4O_7$ ml	$HS(CH_2)_2OH$ ml	S		SH mmol/g	ξ, %	
		%	mmol/g		*IV*	*V*
3·0	0·48	3·22	1·00	0·43	31·9	50·6
2·5[a]	1·00	5·16	1·61	0·44	8·4	28·6
10·0[b]	1·50	6·99	2·18	0·53	10·2	24·5

[a] Reduction of *Va* prepared from *IVa via* a 70 h reaction with 25 ml of 12·5M-$Na_2S_2O_3$ at pH in the range 7·7–7·9 according to ref.[5]. [b] Reduction of *Va* prepared from *IVa via* a 143 h reaction with 25 ml of 12·5M-$Na_2S_2O_3$ at pH 9, with tributylphosphine (1% v/v) added instead of 1 mM-EDTA (*cf.* Experimental).

deoxy cellulose powder (Table IV). Sufficiently long reaction times are then needed if effective substitution of chlorine with thiosulphate groups is to be achieved (Fig. 1); *e.g.*, *VIa*, prepared by reduction of *Va* after a preceding reaction of *IVa* with $Na_2S_2O_3$ during 143 h contains 530 μmol SH/g (Table V), *i.e.* by 51·4% more than in the case of the sample reported in the preceding paper[5]. Similarly to 2-hydroxy-3-thiosulphatopropyl cellulose, also with the initial thiosulphatodeoxycellulose mentioned above reduction with sodium borohydride appeared to be less suitable. Although the sulphur content changed almost stoichiometrically, the product contained only 65·2 μmol SH/g.

The increased reactivity of chlorine due to the α-halohydrine grouping is also indicated if one compares the kinetics of the reaction between 3-chloro-2-hydroxypropyl *Ib* and chlorodeoxycellulose *IVa* with sodium thiosulphate (Fig. 1). Within the time (124 h) when one half (50%) of chlorine is consumed from chlorodeoxycellulose and replaced with 46·9% thiosulphate groups,within an analogous time interval – ($\tau_{0.5}$) – (20 h) only 29·2% thiosulphate groups are incorporated in 3-chloro-2-hydroxypropylcellulose.

p-Tosylcellulose also is a more reactive alkylating derivative than chlorodeoxycellulose. For the preparation of spherical cellulose a procedure has been modified in which aqueous sodium hydroxide is used as the base in tosylation instead of the usual pyridine[19]. An advantage of this variant is that wet cellulose might be used.

TABLE VI

Characterization of 2-pyridyl disulphide derivatives of celluloses (c_p — content of 2-pyridyl disulphide groups)

Initial compound	[—SH] μmol/g	c_p μmol/g
IIIa	252·3 ± 14·3	233·9 ± 1·2
IIIb[a]	167·2 ± 6·7	104·9 ± 1·7
IIIc	428·0 ± 10·4	423·6 ± 0·3
VIa[b]	437·3 ± 23·8	440·6 ± 11·5
VIc	55·6 ± 7·5	271·5 ± 23·0
Xd	185·5 ± 5·8	78·2 ± 3·1
XIIa	60·2 ± 2·7	61·7 ± 7·0

[a] Sulphur content 4·88% (*cf.* Table III). [b] Prepared from *IVa via* a reaction with 25 ml of 12·5M--$Na_2S_2O_3$ (70 h) followed by reduction of *Va* with 1·0 ml of HS $(CH_2)_2OH$ in 2·5 ml of 50 mM -$Na_2B_4O_7$.

In this way drying is avoided which in the case of bead cellulose is mostly accompanied by an undesired drop in porosity. Regenerated cellulose is sufficiently reactive, and at the molar ratio toluenesulphonyl chloride: cellulose = 1, conversion up to 25% is reached already after 1·5 h. An intermediate product in the preparation of thiolcellulose is S-cellulose-O-ethyldithiocarbonate formed from tosylate by a reaction with potassium xanthogenate. This procedure, suggested for the preparation of low-molecular weight thiols[20], has also been employed with sugars[21] and starch[8]. Also with cellulose the reaction must be conducted for a sufficiently long time if high conversion is to be reached (at least 16 h). Hydrolysis to thiol can be accomplished under very mild conditions by action with aqueous ammonia. Tosylate was also used in an attempt to prepare thiol *via* isothiuronium salt[22] obtained by a reaction with thiourea (after boiling for 8 h in ethanol the conversion of tosylate is 36%). The isothiuronium salt is hydrolyzed by heating with sodium hydrogen sulphide similarly to[23]. According to the IR spectra, the thiol derivatives prepared by employing both procedures contain only traces of unreacted tosyl groups.

Symmetrical and Mixed Disulphide Derivatives of Celluloses

Symmetrical disulphide derivatives of celluloses were prepared using dithiodiacetohydrazide and dithiodimethyl isocyanate. The first of the disulphides reacted with carboxymethyl cellulose methyl ester as a monofunctional agent; although the product of this reaction (0·96% sulphur) contained after reduction only 62% of sulphur in the form of SH groups (185·5 μmol SH/g), these groups represented up to 88% of the incorporated sulphur (0·68% S). Conversion of the reaction between cellulose and dithiodimethyl isocyanate was comparatively high (39·5%), but the resulting product was not uniform.

In addition to symmetrical derivatives, also mixed disulphide derivatives of celluloses were prepared, by a reaction between thiol derivatives of celluloses (*III*, *VI*, *X* and *XII*) and 2,2′-dipyridyl disulphide followed by reaction with cystein. Both reactions were quantitative, data on the concentration of 2-thiopyridone released from 2-pyridyldisulphide derivatives are in good agreement with those on the content of SH-groups determined with 5,5′-dithiobis (2-nitrobenzoic acid) (Table VI). Similar results are also obtained by the reduction of 2-pyridyldisulphide derivatives with 2-mercaptoethanol or sodium sulphide, *e.g.* in the case of cellulose derivative *IIIa* reduction with 2-mercaptoethanol released 266·3 μmol of 2-thiopyridone groups/g, while reduction with sulphide released 277·6 μmol/g.

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