THE PREPARATION OF O-(FORMYLMETHYL)CELLULOSE

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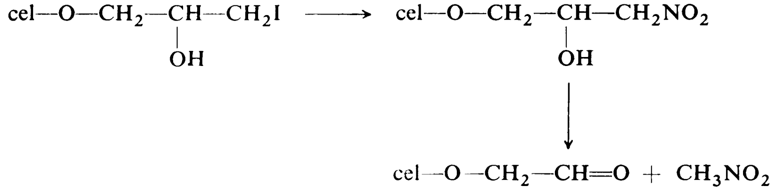
A new aldehyde derivative of cellulose, O-(formylmethyl)cellulose was prepared by three methods. The first one is based on the base-catalysed elimination of nitromethane from O-(3-nitro-2-hydroxypropyl)cellulose obtained by the reaction of sodium nitrite in dimethylformamide with O-(3-iodo-2-hydroxypropyl)cellulose. The second process is a mild acid hydrolysis of O-(2,2-diethoxyethyl)cellulose formed through the alkylation of dry alkali cellulose by bromoacetaldehyde diethyl acetal. The principle of the third procedure is a periodate oxidation of O-(2,3-dihydroxypropyl) cellulose, prepared from an O-(3-chloro-2-hydroxypropyl) derivative in an aqueous alkali solution. The procedure yields a mixed aldehydic derivative — O-(formylmethyl)-2,3-dialdehydocellulose. The application of these reactions to bead cellulose preserves its porosity as well as the spheric particle-shape even at the degree of substitution by formylmethyl groups up to 0·2.

The aldehydic cellulose derivatives may be divided into two groups. Those belonging to the first group have the aldehydic function which was formed *via* the oxidation of some carbon atom in the glucose chain, 2,3-dialdehydocellulose[1] and 6-aldehydocellulose[2-4] being typical examples. The second group of aldehydic derivatives is characterised by the aldehydic group attached to the cellulosic chain *via* a spacer which makes this reactive functional group more accessible to chemical transformations on the cellulose polymer. The benzaldehyde derivatives of cellulose[5] are representatives of this group. They are prepared by the reaction of benzaldehyde 4-isothiocyanate with cellulose amino derivatives. From the viewpoint of chromatographic column use the best flow properties are exhibited by the spherical macroporous cellulose. When the most simple aldehydic derivative, 2,3-dialdehydocellulose, is prepared from the bead cellulose then the spherical particles are destroyed already at the oxidation degree 0·05 – 0·1 leading thus to the loss of useful properties. For this reason, we focused our attention to the preparation of another type of aldehydic derivative belonging to the second group – O-(formylmethyl)cellulose, using three different procedures.

In the first case (Scheme 1) the starting material was O-(3-iodo-2-hydroxypropyl)-cellulose[6]. Its reaction with sodium nitrite in dimethylformamide (DMF) gave O-(3-nitro-2-hydroxypropyl)cellulose in 40% conversion of 3-iodo-2-hydroxypropyl to 3-nitro-2-hydroxypropyl groups.

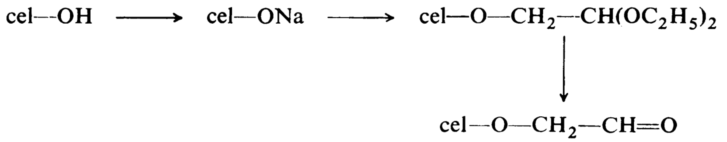


Scheme 1

This method is a modification of the preparation of methyl 2,3,4-tri-O-acetyl-6-deoxy-6-nitro-α-D-glucopyranoside from a corresponding iodo derivative[7].

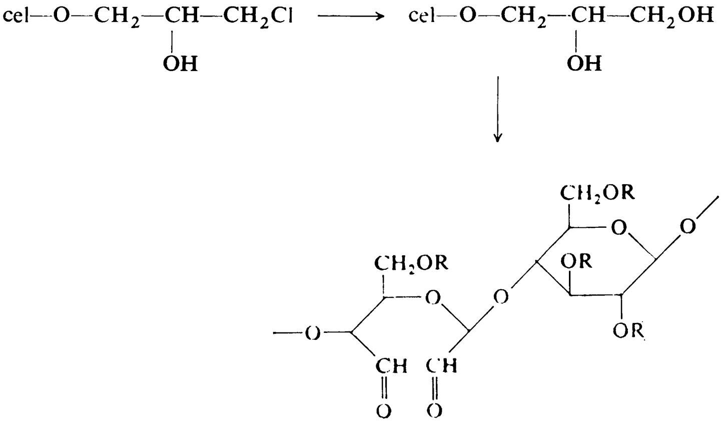
The prepared product was identified by infrared spectroscopy, the band absorptions at 1 550 and 1 382 cm^{-1} being characteristic for the aliphatic nitro groups. The O-(3-nitro-2-hydroxypropyl)cellulose is, in fact, an ether of a three carbon 1-deoxy-1-nitroalditol and cellulose; it might be obtained from the glycolaldehyde ether of cellulose and nitromethane. For this reason O-(3-nitro-2-hydroxypropyl)cellulose yields, upon the action of aqueous sodium hydroxide, nitromethane and an ether of cellulose and glycolaldehyde, O-(formylmethyl)cellulose. The liberated nitromethane was detected in the reaction mixture by comparing the ultraviolet spectra with the standard. The base catalysed nitromethane elimination, which is in fact a reverse reaction of the nitromethane synthesis, proceeds in this case up to 65% conversion. One third of nitrogen remains bonded in the aldehydic derivative even after a several-fold extension of the reaction time. This is due probably to the structure of the starting O-(3-iodo-2-hydroxypropyl) derivative prepared from O-(3-chloro-2-hydroxypropyl)cellulose[6]. When the hydroxyl hydrogen of 3-chloro-2-hydroxypropyl group is substituted by another 3-chloro-2-hydroxypropyl moiety then, after above mentioned transforming, the parent three carbon chain even with nitro group is stable *i.e.* is not able to lose a nitromethane. A direct conversion of O-(3-chloro-2-hydroxypropyl) derivative to O-(3-nitro-2-hydroxypropyl)derivative does not proceed in the presence of alkali nitrite. As the free aldehydic group is sensitive to oxidation by *e.g.* atmospheric oxygen, the intermediate product, 3-nitro-2-hydroxypropyl derivative, is most suitable for the storage. The aldehyde can then easily be prepared prior to use. The aldehyde group content was determined from the nitrogen content upon the transformation of aldehyde to 4-nitrophenylhydrazone of O-(formylmethyl)cellulose. The aldehydic group concentration determining factor is, in this case, the content of 3-iodo-2-hydroxypropyl groups in the starting substrate. The properties of the bead cellulose are preserved in the preparation containing up to 18% of iodine[6] which corresponds to the substitution degree of 0·3 with regard to 3-iodo-2-hydroxypropyl groups. At given conversions a final substitution degree with regard to formylmethyl groups corresponds to the maximum value of 0·1. The aldehyde derivative thus prepared still preserves the spherical shape of particles.

The second method (Scheme 2) represented the Williams synthesis of ethers from the water-free bead alkali cellulose and bromoacetaldehyde diethyl acetal in dioxane in the presence of sodium iodide.



SCHEME 2

The water-free alkali cellulose was prepared by the dehydration of bead material by absolute methanol and by action of methanolic sodium methanolate followed by evaporating the solvent *in vacuo*. The optimum evaporating time was found to be around one hour; when shorter or longer drying was applied then the conversions to final product were lower or nil. The masking group from the O-(2,2-diethoxyethyl)-cellulose intermediate was removed by acid hydrolysis and in the final product, O-(formylmethyl)cellulose, the achieved substitution degree by formylmethyl groups was up to 0·2. During the hydrolysis there is a simultaneous decrease of an average polymerization degree of cellulose by 100–150 units but the final bead O-(formylmethyl)cellulose preserves the particle shape.



$R = CH_2CH{=}O$ or H

SCHEME 3

When using the third method (Scheme 3), O-(3-chloro-2-hydroxypropyl)cellulose[6] is the starting material. The latter is in the first step transformed to O-(2,3-dihydroxypropyl)cellulose by the four-fold molar excess of sodium hydroxide with regard to chlorine concentration. In the second step this product is oxidized by an equimolar amount of sodium periodate with regard to 2,3-dihydroxypropyl groups. The concentration of 2,3-dihydroxypropyl groups was tentatively defined as that of 2,3-epoxypropyl groups which was obtained from O-(3-chloro-2-hydroxypropyl)cellulose by the action 1·5 molar excess of sodium hydroxide. When a derivative containing 2,3-dihydroxypropyl groups to a degree of substitution 0·13 was subjected to oxidation using 0·13 molar amount of $NaIO_4$ with regard to cellulose then, after 24 hours of reaction, an aldehyde derivative was obtained containing 0·20 mol of aldehydic groups per 1 mol of glucose units. Under identical conditions a derivative containing 0·15 mol of aldehydic groups per 1 mol of glucose units was prepared from non-substituted spherical cellulose. It follows that alpha-glycol groups in the side 2,3-dihydroxypropyl chains are oxidised more readily than the glucose units of the cellulose chain. This is plausible both from the viewpoint of accessibility and sterical properties of these groups. The mixed derivative prepared in this way (O-(formylmethyl)-2,3-dialdehydocellulose) preserves also its spherical particle shape.

New aldehyde derivative, when considered from the point of view of the primary structure, has more accesible aldehydic groups than corresponding 2,3-dialdehyde derivative since the aldehydic groups are bonded to the cellulose chain *via* oxymethylene bridges. The O-(formylmethyl) derivative of bead cellulose prepared by any of the three above mentioned methods is capable of maintaining a perfect spherical shape of particles at approximatively equal concentration of aldehydic groups, this being not the case of 2,3-dialdehydocellulose. This phenomenon may be accounted for by the formation of hemiacetal crosslinks between the aldehydic groups and the hydroxyls of the adjacent O-(formylmethyl)cellulose chains.

In the first and third cases, *i.e.* when the starting derivative was O-(3-halogene-2-hydroxypropyl)cellulose there exists another crosslinking possibility by forming the 2-hydroxy-1,3-propylene bonds in alkaline medium. Both these possibilities of crosslinking are out of place in 2,3-dialdehyde derivative. Here, the geminal aldehydic groups are intramolecularly stabilised *e.g.* by forming the hemialdal with 1,4-dioxepane ring.

The selection of an analytical method for the determination of aldehydic groups in aldehydic derivatives prepared by the above presented procedures based on the transformation to corresponding 4-nitrophenylhydrazones is justified for the following reasons. 4-Nitrophenylhydrazine reacts unequivocally even with the dialdehyde structure[8] (its consumption is 2 mol per one mol of dialdehyde, this being a principal difference from other amines or hydrazines which mostly do not react unambiguously and frequently yield an equimolar condensation product with the dialdehyde, possessing the perhydro-1,4-oxazepine structure). When comparing 4-nitrophenylhydrazine and tryptophane derivatives of bead 2,3-dialdehydocellulose it is found that even in the tryptophane derivative there is also almost an equimolar ratio of components. Thus in 2,3-dialdehydocellulose only about a half of aldehydic groups are effectively used for bonding the amino compounds. The use of O-(formylmethyl)cellulose for the attachment of amino compounds and proteins as well as the selection of methods for their bonding to this derivative will be a subject of our further work.

EXPERIMENTAL

The bead cellulose (dry matter 16·1%, water regain 5·22 g/g of dry matter) from Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, Prague, was used throughout this work. The degrees of substitution (S) in various cellulose derivatives were calculated according to the relation $S = 162/(100\,M_X/X - M_s\cdot)$, where M_X is the atomic weight and X is the wt-% of the element being determined while $M_s\cdot$ represents the molecular weight of a substituent. The elemental analyses were carried out on a Perkin–Elmer 240 automatic analyser. Average polymerization degrees (P) of the cellulose and its derivatives were determined, after dissolving the sample in cuoxame, by the standard viscometric method[9]. Infrared spectra were measured on a Perkin–Elmer 457 spectrometer. A Specord UV VIS Jena was used for taking UV spectra. The carbonyl content in aldehyde derivatives of cellulose was determined by elemental analysis of nitrogen upon their transformation to 4-nitrophenylhydrazones. The latter were prepared by reacting a part of the sample (0·3 g) which was washed with ethanol (5 × 2 ml) with 4-nitrophenylhydrazine (0·1 g) in 5 ml of ethanol. The mixture was heated at 80°C for 4 hours and the solid matter was then extracted with acetone. O-(3-Chloro-2-hydroxypropyl)cellulose[6] was transformed to O-(2,3-epoxypropyl) cellulose and the concentration of 2,3-epoxypropyl groups in this derivative was determined through the reaction with sodium thiosulphate[10].

O-(3-Nitro-2-hydroxypropyl)cellulose

Bead O-(3-iodo-2-hydroxypropyl)cellulose[6] (2·5 g, 19·5% dry matter, 17·4% iodine, S 0·30 with regard to 3-iodo-2-hydroxypropyl groups) was washed with methanol and dimethylformamide (each time 3 × 5 ml). After sucking off the excess of solvent from the material, 10 ml of dimethylformamide, 1 g of sodium nitrite and 1·5 g of floroglucine were added and the mixture was stirred at ambient temperature in a closed vessel under nitrogen for 48 hours. Then 20 ml of water was added, stirred and the solid was filtered, washed with methanol, acetone (each time 5 × 10 ml) and finally with water until negative reaction on nitrite. Light brown spherical O-(3-nitro-2-hydroxypropyl)cellulose was obtained in the yield of 2·4 g (16·8% dry matter, 0·8% nitrogen, 0·8% iodine, S 0·10 based on 3-nitro-2-hydroxypropyl). The infrared spectrum of the product exhibits absorptions at 1 550 and 1 382 cm^{-1} that are typical for the presence of aliphatic nitro substituents.

O-(Formylmethyl)cellulose from O-(3-Nitro-2-hydroxypropyl) Derivative

The prepared bead O-(3-nitro-2-hydroxypropyl)cellulose was sucked off and 2 g of this material were mixed with 10 ml of 1M aqueous sodium hydroxide. This mixture was slowly stirred at ambient temperature under nitrogen for 1 hour. The solid phase was filtered off and washed with water until neutral. White, spherical O-(formylmethyl)cellulose was obtained in the yield of 1·9 g (15·0% dry matter, 0·3% nitrogen). The material was insoluble in water and common organic solvents. The reaction with 4-nitrophenylhydrazine gave 4-nitrophenylhydrazone of O-(formylmethyl)cellulose, containing 2·0% of nitrogen. When the residual nitrogen in the aldehyde derivative is subtracted then this amount corresponds to the value of 0·07 for the substitution degree based on formylmethyl groups.

O-(2,2-Diethoxyethyl)cellulose

Bead celulose (15·1% dry matter) was sucked off and 8 g of this material was succesively washed with methanol, absolute methanol and 10% methanolic sodium methanolate (each time 3 × 15 ml). After sucking off the liquid under dry nitrogen the sample was dried in a dessicator (3 kPa) at room temperature for 1 hour. Then 7·3 g of bromoacetaldehyde diethyl acetal was added together with absolute 1,4-dioxane (5 ml) and dry sodium iodide (1 g) and the mixture was refluxed for 5 hours.

The solid phase was filtered off, washed with methanol (5 × 15 ml) and finally with water until negative reaction on halides. White bead O-(2,2-diethoxyethyl)cellulose (14·5% dry matter) was obtained in the yield of 8·7 g.

O-(Formylmethyl)cellulose from O-(2,2-Diethoxyethyl) Derivative

The mixture of O-(2,2-diethoxyethyl)cellulose (5 g) with 0·05M of hydrochloric acid (15 ml) was heated at 100°C for 20 minutes. The solid phase was filtered off and washed with water until neutral White bead O-(formylmethyl)cellulose (14·3% dry matter) was obtained in the yield of 4·5 g. Its reaction with 4-nitrophenylhydrazine gave a corresponding 4-nitrophenylhydrazone containing 3·9% of nitrogen which indicates the value of 0·18 for the substitution degree S with regard to formylmethyl groups. In blank experiments where non-substituted bead cellulose, $P = 320$, or "hydrolysed" one, $P = 208$ (0·05M hydrochloric acid, 100°C, 20 min) was used, there was no detectable amount of nitrogen found to be present.

O-(2,3-Dihydroxypropyl)cellulose

Bead O-(3-chloro-2-hydroxypropyl)cellulose[6] (1 g, 16·0% dry matter, 4·9% chlorine, 51% of available 2,3-epoxypropyl groups[10]) in 10 ml of 0·09M sodium hydroxide was stirred ambient temperature under nitrogen for 4 hours. The solid phase was filtered off and washed with water until neutral. White bead O-(2,3-dihydroxypropyl)cellulose was obtained (1 g, 16% dry matter, S 0·13 on 2,3-dihydroxypropyl groups).

O-(Formylmethyl)-2,3-dialdehydocellulose

The suspension of O-(2,3-dihydroxypropyl)cellulose (1 g) in 0·05M solution of sodium periodate (2·35 ml) was stirred in dark at ambient temperature under nitrogen for 24 hours. The bead product was filtered off and washed with water (5 × 5 ml). In this way 1 g of white O-(formylmethyl)-2,3-dialdehydocellulose (15·6% dry matter) was obtained. The reaction with 4-nitrophenylhydrazine yielded 4-nitrophenylhydrazone derivative containing 4·3% of nitrogen which corresponds to 0·20 mol of aldehydic groups per 1 mol of glucose units. An identical experiment with non-substituted bead cellulose gave di(4-nitrophenylhydrazone) of 2,3-dialdehydocellulose comprising 3·5% of nitrogen, *i.e.* 2,3-dialdehydocellulose contained 0·15 mol of aldehydic groups per 1 mol of glucose units.

Tryptophane Derivative of 2,3-Dialdehydocellulose

The mixture of spherical cellulose (1·35 g, 15·1% dry matter) with 0·05M solution of sodium periodate (25 ml) was stirred in dark at room temperature for 2 hours. The solid phase was filtered off and washed with water (5 × 5 ml). 2,3-Dialdehydocellulose (14·7% dry matter) was obtained in amount of 1·2 g. It contained 0·17 mol of aldehydic groups per 1 mol of glucose units (38% nitrogen found in the 4-nitrophenylhydrazone derivative).

The mixture of thus prepared 2,3-dialdehydocellulose (1 g) with 0·125M tryptophane solution (8 ml) in 0·05M acetate buffer (pH = 5) was stirred at ambient temperature for 1 hour. After adding sodium cyanoborohydride (0·08 g) the stirring was continued for another 4 hour. The solid phase upon filtering off, washing with water, 1M acetic acid, 1M sodium chloride and with water again (each time 5 × 5 ml), represents the reduced tryptophane derivative of 2,3-dialdehydocellulose containing 0·10 mol of tryptophane substituents per 1 mol of glucose units (1·5% nitrogen).

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