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Note

Preparation of 6-chloro-6-deoxyamyloses of various degrees of substitution: an alternative route to 6-aldehydoamylose*

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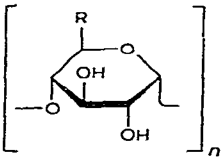
A prerequisite for specific oxidation at a primary hydroxymethyl group of a carbohydrate by the azide photolysis method[1–4] is the selective introduction of a suitable leaving-group at this primary position to allow the azide to be prepared. In previous work on the preparation of 6-aldehydo derivatives of D-glucopyranosides[1] and cellulose[4], the reagent methanesulfonyl chloride–*N*,*N*-dimethylformamide[5] was used with good success for specific introduction of chlorine at C-6 without the need for prior protection of the secondary hydroxyl groups.

This paper describes the preparation of 6-chloro-6-deoxyamyloses (**1**) of various degrees of substitution (d.s.) by use of this reagent, and their conversion by way of 6-azido-6-deoxyamylose (**2**) into 6-aldehydoamylose (**3**). In a previous report[2], 6-deoxy-6-*O*-*p*-tolylsulfonylamylose was used as the precursor for the azide **2** and, thence, to the aldehyde **3**. *p*-Toluenesulfonylation requires anhydrous conditions[6], and, at d.s. approaching 1.0, it is not completely selective for C-6.



1 R = CH_2Cl
2 R = CH_2N_3
3 R = CHO

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RESULTS AND DISCUSSION

Attempts to chlorinate, with methanesulfonyl chloride–*N*,*N*-dimethylformamide for 48 h at 62°, a granular form of potato amylose that had been soaked in *N*,*N*-dimethylformamide gave a product containing very little chlorine; evidently the reagent did not penetrate into the interior of the insoluble particles of amylose. Similar behavior has been noted[4] with cellulose that had not been pretreated to afford a fully accessible surface of the polymer.

The insoluble, granular amylose was brought into a reactive form by solubilizing it in water at 160° in an autoclave and then cooling and freeze-drying it. This reactive form of amylose underwent chlorination during 16 h at 62° to give a chlorinated, formylated product that was deformylated by treatment with aqueous sodium carbonate. 6-Chloro-6-deoxyamylose (**1**) was obtained as a water-insoluble, tan-colored powder having d.s. by chlorine of 0.8; it contained negligible proportions of sulfur and nitrogen, and gave 6-chloro-6-deoxy-D-glucose on hydrolysis.

By using a commercial, freeze-dried amylose preparation from which the lower molecular-weight portion had been leached out with *N*,*N*-dimethylformamide at 90°, it was possible, by chlorination for 16 h at 60°, to obtain (after deformylation) a white, water-soluble 6-chloro-6-deoxyamylose (**1**) of d.s. 0.45. Chlorination of the extracted amylose for 1 h at a higher temperature (90°) gave a white, water-insoluble **1** having d.s. 0.8–0.9. Chlorination of the *N*,*N*-dimethylformamide-soluble fraction of the freeze-dried amylose led to a dark-colored product.

For converting the 6-chloro-6-deoxyamyloses (**1**) of various d.s. into the corresponding 6-azides (**2**), sodium azide was used in either *N*,*N*-dimethylformamide, water, or methyl sulfoxide as the solvent; water was satisfactory with water-soluble preparations of **1**. As in previous work[1–4], it was found that the d.s. by azide in the product was essentially the same as that in the precursor 6-substituted derivative. In the example described (see Experimental section), the water-soluble chloro derivative **1** of d.s. 0.45 was converted by sodium azide in water after 72 h at 100° into the azido derivative **2**. This was isolated, after dialysis, in 65% yield as a white, water-soluble product whose d.s. by azide was 0.45 (by nitrogen analysis); it contained very little residual chlorine.

In previous reports, the photolysis of azido polysaccharides was satisfactorily conducted with 4:1 benzene–2-methoxyethanol or 2-methoxyethanol as the medium. To explore the use of other solvents, the water-soluble 6-azido-6-deoxyamylose (**2**) of d.s. 0.45 was photolyzed, in water, with radiation of ~300 nm for 2.5 h, during which time the pH of the solution rose to 9.0. Acetic acid was used to complete the hydrolysis of the resultant "aldimine", and the resultant 6-aldehydoamylose (**3**; strictly, an α-D-(1→4)-linked copolymer of D-glucose and D-*gluco*-hexodialdose residues) was isolated by dialysis; its i.r. spectrum indicated that azide was absent. A small proportion (~0.6%) of residual nitrogen in the product may have resulted from a side-reaction leading to formation of 6-amino groups. The yield of **3** was only 55%, indicating that losses caused by depolymerization had occurred. The copper number[4] of the product was 64.8, and its d.s. by aldehyde, as determined by the borodeuteride reduction–mass spectrometry procedure[3], was 0.21; this lower value for **3** in relation to the precursor **2** suggests that the initial chloro derivative **1** was derivatized more extensively in some regions of the chain than others, and that the derived aldehyde **3** underwent selective fragmentation, and loss as dialyzable fragments, in the more-highly substituted regions of the chain. It has been established from extensive studies[7] on 6-aldehydocellulose that presence of the 6-aldehydo groups permits rapid depolymerization by β-elimination under very mild conditions of pH and temperature. It is noteworthy that **3** showed weak u.v. absorption at ~250 nm that increased markedly upon addition of sodium hydroxide; such absorption is indicative of a 4,5-unsaturated aldehyde system present in a sugar fragment[1,8].

The chlorination procedure described here is considered to offer advantages of improved selectivity, procedural convenience, and lower cost, in comparison with sulfonylation procedures for introducing a leaving group at C-6.

EXPERIMENTAL

Materials. — Potato amylose (Type 1, Sigma Chemical Corporation, St. Louis, Missouri) was used as supplied, in either a granular (densely aggregated) or a freeze-dried form. Pure-grade *N*,*N*-dimethylformamide (min. 99%, b.p. 152–155°) was used for preparing swollen amylose, and the reagent-grade solvent was used for chlorination reactions. The methanesulfonyl chloride used was reagent grade (min. 95%).

General methods. — Solutions were evaporated under diminished pressure below 45°. T.l.c. was performed on Silica Gel G (Merck) with 4:1 ethyl acetate–methanol as developer and 50% sulfuric acid as indicator. Dialysis was conducted against running tap-water for at least 16 h. Viscosity measurements were made at 25° on 0.4% solutions in methyl sulfoxide, and results are expressed as relative viscosities. Samples were irradiated in a Rayonet Model RPR-208 preparative photochemical reactor (The Southern New England Ultraviolet Company, Middletown, Conn.), equipped with lamps producing maximal radiation at 300 nm.

6-Chloro-6-deoxyamylose (**1**). — *A. From granular amylose.* A suspension of granular amylose (6 g) in water (80 ml) was autoclaved for 2 h at 160° to solubilize the amylose, and the solution was cooled and then freeze-dried. To a stirred suspension of this activated amylose (6 g) in *N*,*N*-dimethylformamide (400 ml) was added methanesulfonyl chloride (50 ml) during 0.5 h at 60°, with stirring. The mixture was stirred for 16 h at 62°, and the resultant, dark mixture was cooled and poured, with stirring, into water (1 liter). The resultant precipitate was collected by centrifugation; the i.r. spectrum of a dried sample showed ν_{max} 1730 cm^{-1} (formyl). The solid was stirred with 2% aqueous sodium carbonate for 16 h at ~25°, and the suspension was then dialyzed. The non-dialyzable solid was centrifuged off and freeze-dried, to give **1** as a tan solid; yield 2.4 g (40%), relative viscosity 1.04 (Found: Cl, 15.9; S, 0.2; N, 0.6%. $C_6H_9O_4(OH)_{0.2}Cl_{0.8}$ requires Cl, 16.1%).

The supernatant liquor from the precipitation of **1** was concentrated to ~200 ml and then dialyzed, to afford an additional 1.2 g of non-dialyzable product. Hydrolysis of this product with 72% sulfuric acid[4] and subsequent neutralization with barium hydroxide gave, by t.l.c. evidence, an approximately equimolar mixture of glucose and 6-chloro-6-deoxyglucose.

In an experiment for evaluating the reactivity of non-autoclaved amylose, granular amylose (1 g) was soaked in *N*,*N*-dimethylformamide (100 ml) for 1 h on a steam bath. The suspension was filtered, and the solid remaining was stirred with *N*,*N*-dimethylformamide (100 ml) and methanesulfonyl chloride (20 ml) for 48 h at 62°. The suspension was poured into water (hood), and the resultant precipitate was centrifuged off. A portion was washed with ethanol, dried, and then hydrolyzed in 72% sulfuric acid as already described[4]. T.l.c. of the hydrolyzate, alongside a comparison sample of authentic 6-chloro-6-deoxy-D-glucose, indicated that little of the latter was present.

B. From freeze-dried amylose. The freeze-dried amylose (4 g) was swollen in *N*,*N*-dimethylformamide (100 ml) for 2 h on a steam bath. The insoluble residue was filtered off, and washed with more *N*,*N*-dimethylformamide. The wet, insoluble mass was stirred, as in the preceding experiment, with *N*,*N*-dimethylformamide (300 ml) at 60° while methanesulfonyl chloride (20 ml) was added dropwise. The yellow reaction-mixture, which contained some solid, was poured into water, and the precipitate was filtered off. This solid product was stirred with 2% aqueous sodium carbonate for 16 h, during which time it dissolved. The solution was concentrated to 200 ml, and dialyzed, and the non-dialyzable product was freeze-dried; yield 2.0 g (52%) of **1** as a water-soluble, white solid having a relative viscosity of 1.50 (relative viscosity of the freeze-dried amylose, 1.45) (Found: Cl, 9.1%. $C_6H_9O_4(OH)_{0.55}Cl_{0.45}$ requires Cl, 9.4%).

The filtrate from the swollen amylose was evaporated to give a syrup (0.5 g). Chlorination of this product in *N*,*N*-dimethylformamide (150 ml) with methanesulfonyl chloride (10 ml) for 16 h at 60° gave a dark solution. The product, isolated by pouring the solution into water, followed by saponification of formic ester groups to give a dark solid, was found to be 6-chlorinated to ~80% (by the evidence of t.l.c. on a sample hydrolyzed with sulfuric acid).

In an effort to secure **1** of high d.s. by chlorine, freeze-dried amylose (6 g) was swollen as just described, filtered off to remove soluble oligomers, and then chlorinated in *N*,*N*-dimethylformamide (300 ml) with methanesulfonyl chloride (30 ml) for 1 h at 90°. The resultant, yellow solution was poured into water, and the precipitate that resulted was centrifuged off. A solution of this product in methyl sulfoxide (100 ml) containing concentrated aqueous ammonium hydroxide (5 ml) was kept for 1 h at ~25°, and then dialyzed. The non-dialyzable solid was centrifuged off, and freeze-dried, to afford **1** as a white solid; yield 4.7 g (82%), relative viscosity 1.09; d.s. by chlorine (as estimated visually by t.l.c. after hydrolysis) ~0.9.

6-Azido-6-deoxyamylose (**2**). — In a typical preparation, a solution of the chloro derivative **1** having d.s. 0.45 (2.0 g) in water (25 ml) containing sodium azide (10 g) was kept in a stoppered flask for 72 h at 100° and then dialyzed, and the dialyzate was freeze-dried to afford **2** as a white, water-soluble solid; yield 1.3 g (65%), relative viscosity 2.14, ν_{max}^{KBr} 2070 cm^{-1} (N_3) [Found: N, 11.3; Cl, 0.73%. $C_6H_9O_4(OH)_{0.5}$-$Cl_{0.05}(N_3)_{0.45}$ requires Cl, 1.05; N, 11.0%].

In the reaction at various d.s. by chlorine, other solvents used were *N,N*-dimethylformamide, water, and methyl sulfoxide. The organic solvents allowed much more rapid conversion into the azide. Water was not a satisfactory medium for use with water-insoluble samples of **1** of high d.s.

6-Aldehydoamylose (**3**). — A solution of the azide **2** (300 mg, d.s. 0.45) in water (100 ml) was sealed in a polyethylene bag containing a magnetic stirring-bar, and photolyzed for 2.5 h with stirring. The pH of the solution (9.2) was brought to 4 with acetic acid, and the solution was stirred for 2 h at ~25° and then dialyzed. The solution was freeze-dried, to afford **3** as a pale-tan solid; yield 160 mg (53%), $\nu_{max}^{H_2O}$ 250 nm (weak, intensified on adding sodium hydroxide), copper number[4] 64.8; N, 0.59. The product dissolved in water to give a colorless solution.

To a solution of **3** (50 mg) from this experiment, in water (3 ml), was added sodium borodeuteride (50 mg). After 1 day, the same amount of reductant was added, and the solution was kept for a further 2 days. The solution was dialyzed, and the non-dialyzable product (30 mg) was hydrolyzed with 72% sulfuric acid (darkening). Conversion of the hydrolysis product into 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose-*6-d*, and analysis thereof by g.l.c.–mass spectrometry as already described[3,4], indicated an overall d.s. by deuterium of 0.21 in the non-dialyzable portion of the reduced **3**.

Photolysis of other samples of **2** at various d.s. could also be conducted satisfactorily in 2-methoxyethanol. Use of benzene in the medium gave an oily side-product[4] that could be removed by extraction with ethyl acetate.

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