SYNTHESIS OF MONOSUBSTITUTED CYCLOHEXAAMYLOSES

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ABSTRACT

Eight crystalline, monosubstituted derivatives of cyclohexaamylose have been prepared. They are specifically substituted at C-6 of one of the α-D-glucopyranosyl residues of cyclohexaamylose. 6-*O*-*p*-Toluenesulphonyl-cyclohexaamylose was prepared from cyclohexaamylose and purified on a column of activated charcoal. 6-Azido-6-deoxy-, 6-chloro-6-deoxy-, 6-bromo-6-deoxy-, and 6-deoxy-6-iodo-cyclohexaamylose were obtained by nucleophilic displacements of the sulphonate group of the monotosyl-cyclohexaamylose. The monoazido and monoiodo derivatives were reduced to 6-amino-6-deoxy- and 6-deoxy-cyclohexaamylose respectively. 6-*O*-Trityl-cyclohexaamylose was also prepared from cyclohexaamylose.

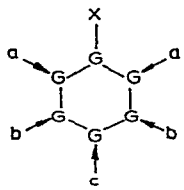
INTRODUCTION

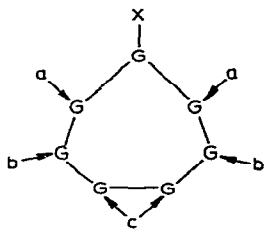
The synthesis of cyclohexaamylose derivatives has been hindered by difficulties in purifying these products. Preparations having a high degree of substitution (12 or 18), have been reviewed by French[1]. Subsequently, a dodeca-*O*-methylcyclohexaamylose has been prepared, but a controversy exists over the positions of the methyl ether groups. In one report these groups were assigned to O-2 and O-6 of the D-glucose residues[2], whereas in another[3] they were assigned to O-3 and O-6.

Lautsch and coworkers[4] were the first to attempt specific modification of one position in each D-glucose residue. They attempted to prepare hexakis(6-*O*-tosyl)- and hexakis(6-*O*-mesyl)-cyclohexaamyloses by using one equivalent of sulphonyl chloride per D-glucose residue. Recently Cramer and coworkers[5], using this method with a 50% excess of tosyl chloride (9:1 molar ratio), have claimed preparation of hexakis-(6-*O*-tosyl)-cyclohexaamylose. This material was subjected to nucleophilic displacement reactions to give products that gave satisfactory analyses for pentakis-(6-amino-6-deoxy)-cyclohexaamylose and dodeca-*O*-acetyl-pentakis(6-deoxy-6-iodo)-mono-(6-*O*-tosyl)-cyclohexaamylose[5]. It is doubtful that tosylation did occur exclusively at the primary hydroxyl groups in all residues as claimed by these workers, as tosylation of α-D-glucose[6], methyl α-D-glucopyranoside[7], and amylose[8,9] under comparable conditions causes some additional tosylation at secondary hydroxyl groups. Similarly, mesylation of methyl α-D-glucopyranoside, even with only one mole of mesyl chloride, gives a mixture of products that includes methyl 2,6-di-*O*-mesyl-α-D-glucopyranoside and methyl 2-*O*-mesyl-α-D-glucopyranoside[10]. Umezawa

and Tatsuta[11], using a 9:1 molar ratio found that a mixture of tosylated products resulted and that the pure hexakis(6-*O*-tosyl)-cyclohexaamylose had to be obtained by separation on a silica-gel column. Hexakis(6-azido-6-deoxy)-, hexakis(6-amino-6-deoxy)-, and hexakis(6-acetamido-6-deoxy)-cyclohexaamyloses were prepared from this pure hexakis(*p*-toluenesulphonate)[11]

Cramer and Mackensen[12] have attached various imidazole groups to the C-6 positions of cycloheptaamylose. The d.s. varied from two to four imidazole groups per molecule of cycloheptaamylose. It seems likely that these materials were mixtures having not only different degrees of substitution but also containing positional isomers as well, because a second, identical substituent at a C-6 position of cyclohexa- or cycloheptaamylose can be introduced at any one of 3 positions to produce 3 positional isomers.





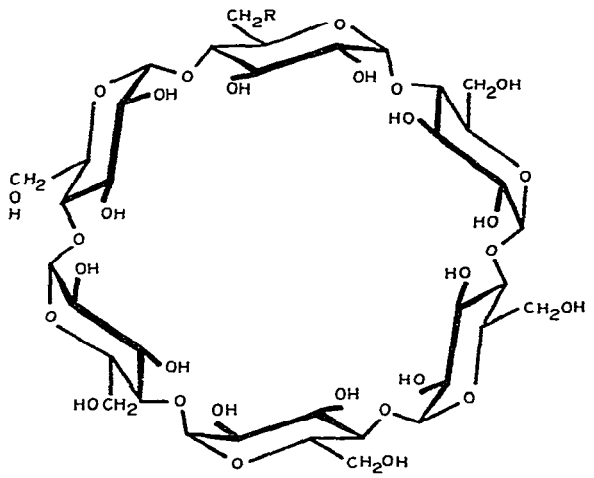
Other preparations of specifically modified cyclohexaamyloses have resulted from kinetic studies of cycloamyloses as model enzymes. The "enzyme–substrate" intermediates, cycloheptaamylose monophosphate[13] and cyclohexaamylose monobenzoate[14], as well as a spin-labelled cycloheptaamylose ester[15], have been identified and isolated. This approach has been used on a preparative scale to obtain the cyclohexaamylose ester of pyridine-2,5-dicarboxylic acid[16]. The position of attachment of the ester group has not been unambiguously established in any of these derivatives.

In the present report, the syntheses of eight monosubstituted cyclohexaamylose derivatives are described. All of these derivatives are specifically substituted at the C-6 position of one of the α-D-glucopyranosyl residues of the cyclohexaamylose molecule (**1**).

RESULTS AND DISCUSSION

Considering the difficulties experienced working with these compounds, the yields obtained were reasonable (20–46% from cyclohexaamylose).

6-*O*-*p*-Toluenesulphonyl-cyclohexaamylose (**3**) is the key intermediate in the synthesis of six of the cyclohexaamylose derivatives. Although the tosylation of cyclohexaamylose was conducted for only a short time, a mixture of starting material, mono-, and polytosyl derivatives resulted. Column chromatography on activated charcoal allowed the isolation of pure monotosyl-cyclohexaamylose in reasonable yield. The sulphonate group was then readily replaced by nucleophilic substitution with a variety of standard nucleophiles. In this way, the monochloro (**6**), monobromo (**7**), and monoiodo (**8**) derivatives were obtained. The monoamino (**5**) derivative was



1 R=OH 4 R=N_3 7 R=Br
2 R=OTr 5 R=NH_2 8 R=I
3 R=OTs 6 R=Cl 9 R=H

prepared from the monoazide (**4**). Solvolysis of the monotosyl-cycloamylose competes with the desired nucleophilic substitution when the reaction is carried out in water, giving rise to cyclohexaamylose. With good nucleophiles, such as iodide, there is virtually no solvolysis; whereas azide and bromide give small proportions of cyclohexaamylose (approximately 5% and 10% respectively by t.l.c.). In order to avoid formation of any cyclohexaamylose, the procedure finally adopted to prepare the monobromide (**7**) was to use dry *N,N*-dimethylformamide. With chloride, an even poorer nucleophile, it was essential to exclude water. An attempt to prepare the monochloride derivative (**6**) by heating monotosyl-cyclohexaamylose with 0.25% aqueous sodium chloride solution gave approximately 95% of cyclohexaamylose and only 5% monochloro-cyclohexaamylose, as detected by t.l.c When tetramethylammonium chloride in dry *N,N*-dimethylformamide as solvent was used, monochloro-cyclohexaamylose was obtained exclusively.

6-Deoxy-cyclohexaamylose (**9**) was formed by reduction of 6-deoxy-6-iodocyclohexaamylose with hydrogen over W-2 Raney nickel. A 4,6-dichloro-4,6-dideoxy-D-galactopyranoside derivative has been reduced to the corresponding 4,6-dideoxy sugar with hydrogen and W-4 Raney nickel[17]. By this method monochloro-cyclohexaamylose was reduced on a small scale to a compound that gave the same colour reaction with iodine and the same R_F value on t.l.c. (solvent A) as 6-deoxy-cyclohexaamylose prepared from the monoiodo derivative (**8**).

The use of chlorotriphenylmethane to alkylate specifically one C-6 hydroxyl position of cyclohexaamylose[18] is based on the fact that tritylation occurs preferentially at a primary hydroxyl group[19]. The formation of 6-*O*-trityl-cyclohexaamylose (**2**) provides another means of preparing monosubstituted cyclohexaamyloses. In an earlier investigation[18] monotrityl-cyclohexaamylose was fully acetylated, detritylated, and monotosylated, and then nucleophilic displacement of the sulphonate group was

effected by iodide. Deacetylation yielded 6-deoxy-6-iodo-cyclohexaamylose, which was finally reduced to 6-deoxy-cyclohexaamylose. The monoiodo-cyclohexaamylose produced by this method was identical by t.l.c. (solvent A) with that produced via the sulphonate **3**. The preparation of deoxy and iodo derivatives from the trityl ether involves more steps and the overall yields were low compared with the methods used in the present work.

An interesting property of the monohalo derivatives was their distinct gradation of solubility in water. The iodide was the least soluble, being only partially soluble in boiling water. While the bromide was intermediate in solubility, the chloride was readily soluble in warm water but reluctant to dissolve in cold water.

A well known characteristic of cycloamyloses is their ability to form complexes with widely different types of compounds[1,20,21]. This complexing property has been useful in several instances in the present investigations. Iodine has been known for a long time to give a coloured complex with cyclohexaamylose, and it was found that all the cyclohexaamylose derivatives prepared complexed with iodine vapour on t.l.c. plates to give characteristic colours that aided in identification. (Maltohexaose in high concentrations gave only a pale yellow colour with iodine on t.l.c. an its lower homologues gave even weaker reactions.) Organic liquids that form insoluble complexes have been used previously in the separation of cycloamyloses[1,22], and they were employed here in the purification of some of the cyclohexaamylose derivatives. The monoazide was separated from a small proportion of unidentified side product by precipitation with tetrachloroethane. The monotrityl ether was separated from a mixture of cyclohexaamylose and its ditrityl ether with cyclohexane. Complexes with organic liquids, and the soluble, yellow complex formed with iodine during the preparation of monoiodo-cyclohexaamylose, could be decomposed by heating in boiling water until the complexing agent had evaporated, yielding the free sugar.

The specifically modified cyclohexaamyloses described in this work should be of considerable use in a variety of ways. Cycloamyloses have created a good deal of interest as model enzyme-systems[14–16,21] and some of our derivatives will be useful as models for binding involving protonated centers (RNH_3^+) or improved nucleophilic centers (RNH_2). Specifically substituted cycloamyloses will be invaluable as substrates in studies on *Bacillus macerans* cyclodextrinase[23], and amylases from *B. macerans*[1], *B. polymyxa*[24], *Aspergillus oryzae*[25], and *Penicillum africanum*[25]. Such studies will lead to an improved understanding of the mode of action of these amylases. Specifically substituted cycloamyloses offer attractive intermediates for the synthesis of modified oligosaccharides, substances often available only by long and arduous synthetic routes.

EXPERIMENTAL

General methods. — Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Evaporations were conducted on a Buchi rotary evaporator at bath temperatures not exceeding 45°. Optical rotations were measured

on a Perkin–Elmer Model P22 spectropolarimater. Freeze drying was done at −40°/0.3 torr for approximately 20 h. *N,N*-Dimethylformamide was dried over calcium hydride, distilled, and kept over barium oxide. Thin-layer chromatography (t.l.c.) was performed on Silica gel G with solvents: A, 14:3:3 butanone–methanol–water; and B, 12:3:5 butanone–methanol–M acetic acid. Iodine vapour and 10% aqueous sulphuric acid spray followed by heating were used to detect the cyclohexaamylose derivatives on t.l.c. plates. *p*-Toluenesulphonates were observed as fluorescent spots under u.v. light after spraying with 2% diphenylamine in ethanol and exposing to u.v. radiation for several min. Ninhydrin (0.2%) in ethanol was used as a spray to detect the amine. The R_F values were measured relative to cyclohexaamylose (R_C) and unless otherwise stated refer to solvent A.

Water included in the central cavity of crystalline cycloamyloses is not easily removed[26,27], but it has been shown[26] that all the water can be removed from a cycloamylose by freeze drying. Therefore all samples for elemental analysis were freeze dried and quantitatively dried immediately before analysis.

Cyclohexaamylose (**1**). — This compound was prepared by the method of French, Pulley, and Whelan[22], on a smaller scale and without using *Bacillus subtilis* amylase to remove starchy residues. A culture of *Bacillus macerans* (American Type Culture Collection 7069) was the source of *B. macerans* amylase. An amylase activity of 0.5 to 1.5 Tilden and Hudson units[28] was used to prepare pure cyclohexaamylose (72 g) from potato starch (305 g).

6-O-Trityl-cyclohexaamylose (**2**). — To cyclohexaamylose (8.3 g) dissolved in pyridine (1650 ml), chlorotriphenylmethane (10.0 g) was added. The solution was heated for 1 h at 100° and then poured into water (1650 ml). Barium carbonate (16.5 g) was added and the solution was evaporated to about 100 ml. Ethanol (2 × 600 ml) was added to facilitate removal of residual pyridine and the mixture was evaporated to dryness. The residue was extracted twice with boiling water (2 × 650 ml). Each extract was cooled to room temperature before filtration. Cyclohexane (7.5 ml) was added to the combined extracts and the mixture was shaken thoroughly before filtration. The precipitate was washed with cold water (250 ml) and added to boiling water (600 ml). The water was kept boiling for exactly 20 min with continuous stirring. The mixture was cooled to room temperature and filtered. Cyclohexane (5 ml) was added to the filtrate and the foregoing procedure was repeated. The filtrate obtained was freeze dried to yield 6-*O*-trityl-cyclohexaamylose (3.42 g, *ca.* 95% pure; R_C 2.5, yellow-brown with iodine). A small portion of this material was purified twice by cyclohexane precipitation as described above and crystallized from water. Recrystallization from water gave **2** as crystals having m.p. 286–289°, $[\alpha]_D^{21}$ +128° (*c* 0.7, ethanol).

Anal. Calc. for $C_{55}H_{74}O_{30}$: C, 54.36; H, 6.14. Found: C, 54.16; H, 6.23.

6-O-p-Tolylsulphonyl-cyclohexaamylose (**3**). — Freeze-dried cyclohexaamylose (2.0 g) and *p*-toluenesulphonyl chloride (8.0 g) were dissolved in pyridine (400 ml). After 40 min at room temperature the reaction was stopped by addition of water. T.l.c. in solvent A showed that the product contained about 67% of monotosyl-

cyclohexaamylose (R_C 2.1, yellow-brown with iodine), about 33% of unreacted cyclohexaamylose (purple with iodine) and small amounts of more-highly substituted derivatives. Pyridine was removed by evaporation until a syrup was obtained. To this material water (100 ml) was added and the evaporation was continued. This process was repeated several times until no odor of pyridine could be detected. The aqueous solution (400 ml) was filtered and applied to a column (27 × 2.5 cm) of activated charcoal (50 g), Darco, G-60, 20–40 mesh (Matheson, Coleman, and Bell). The column was eluted with water (1.5 liters), followed by 30% ethanol (1 liter) and 25% 1-propanol (1 liter). All eluents were deaerated by evacuation with a water pump before use. Collection of the ethanolic eluate from the column allowed recovery of unreacted cyclohexaamylose. The 1-propanol eluate was collected in 100-ml fractions. Concentrated ammonia (*ca.* 2 ml) was added to each fraction and the solutions were evaporated to dryness. The purity of monotosyl-cyclohexaamylose was checked by t.l.c. (solvent A) and the appropriate fractions (dissolved in water) were combined, filtered through a Celite pad, and freeze dried to yield the monotosylated product **3** (1.07 g). A portion of this material was crystallized from 95% ethanol, m.p. 159–162° dec., $[\alpha]_D^{22}$ +111° (*c* 0.41, water).

Anal. Calc. for $C_{43}H_{66}O_{32}S$: C, 45.82; H, 5.90; S, 2.84. Found: C, 45.64; H, 5.69; S, 2.72.

6-Azido-6-deoxy-cyclohexaamylose (**4**). — Freeze-dried monotosyl-cyclohexaamylose (**3**, 0.90 g) and sodium azide (0.90 g) in water (100 ml) were heated for 90 min on a boiling water bath. T.l.c. (solvent B) showed that, as well as monoazido-cyclohexaamylose (*ca.* 95%), two side products (*ca.* 5%) were formed, one of which had the same R_F value as cyclohexaamylose. The solution was filtered and then evaporated to a low volume (3 ml) and 1,1,2,2-tetrachloroethane (*ca* 0.5 ml) was added. The tetrachloroethane complex was separated from the aqueous solution by centrifugation. The monoazido-cyclohexaamylose was obtained by heating the complex in boiling water. Evaporation of the aqueous solution yielded the product **4** (0.51 g). T.l.c. indicated that the product was pure except for cyclohexaamylose (*ca.* 5%). A small amount of this material was recrystallized three times from water to give **4** having m.p. 217° dec., $[\alpha]_D^{22}$ +128° (*c* 0.40, water). T.l.c. indicated this material (R_C 1.7, dark purple with iodine) was pure.

Anal. Calc. for $C_{36}H_{59}N_3O_{29}$: C, 43.33; H, 5.96; N, 4.21. Found: C, 43.12; H, 5.85; N, 4.07.

6-Amino-6-deoxy-cyclohexaamylose (**5**). — 95% Pure 6-azido-6-deoxy-cyclohexaamylose (0.51 g) was dissolved in water (100 ml) and reduced with palladium black (150 mg) at 14 lb.in^{-2} of hydrogen on a Parr hydrogenator for 10 h. After removal of the catalyst by filtration, the solution was evaporated to dryness (0.51 g). A small amount of the product (**5**) was obtained chromatographically pure (solvent B, R_C 0.8, brown with iodine) by recrystallization 3 times from water. m.p. 200° dec., $[\alpha]_D^{22}$ +117° (*c* 0.40, water).

Anal. Calc. for $C_{36}H_{61}NO_{29}$: C, 44.49; H, 6.33; N, 1.44. Found: C, 44.38; H, 6.39; N, 1.64.

6-Chloro-6-deoxy-cyclohexaamylose (**6**). — Freeze-dried **3** (200 mg) was dissolved in dry *N,N*-dimethylformamide (100 ml) and tetramethylammonium chloride was (500 mg) added. The suspension was stirred magnetically and heated for 90 min at 100°. T.l.c. (solvent A) showed that the starting material was converted entirely into one product. The solution was kept overnight at 5° and the white solid that formed was removed by filtration. The *N,N*-dimethylformamide was removed by vacuum distillation (0.05 mm) until only a small proportion remained, from which more white material precipitated, and this was removed by filtration. The remaining *N,N* dimethylformamide was removed by coevaporation with water under vacuum. An aqueous solution of the product was then passed through Dowex-50 (H^+) (10 ml) ion-exchange resin, followed by Dowex-3 (OH^-) (10 ml). The aqueous eluate was concentrated and gave colourless crystals (95 mg) of **6** having m.p. 205° dec., $[\alpha]_D^{22}$ +128° (*c* 0.38, pyridine). T.l.c. (R_C 1.8, purple with iodine) showed that these crystals were pure.

Anal. Calc. for $C_{36}H_{59}ClO_{29}$: C, 43.61; H, 6.00; Cl, 3.58. Found: C, 43.44; H, 6.11; Cl, 3.47.

6-Bromo-6-deoxy-cyclohexaamylose (**7**). — Freeze-dried **3** (0.71 g) and lithium bromide (2.84 g, oven-dried at 150°) in dry *N,N*-dimethylformamide were heated for 2 h at 100°. T.l.c. (solvent A) indicated that the reaction was complete in 1 h, giving only one product. *N,N*-Dimethylformamide was removed by vacuum distillation at 50° leaving ~3 ml of solution, to which water was added and the vacuum distillation was repeated to yield a syrup. The syrup was dissolved in water (150 ml) and passed through Dowex-50 (H^+) (100 ml) followed by Dowex-3 (OH^-) (100 ml). Concentration of the eluate gave a syrup that was dissolved in a minimum of hot water. Colourless crystals of **7** were formed on cooling, having m.p. 174° dec., $[\alpha]_D^{22}$ +122° (*c* 0.40, pyridine). T.l.c. (solvents A and B) showed that the crystals were pure [R_C (solvent A), 1.9, R_C (solvent B) 1.1, purple with iodine]. The mother liquor was freeze dried to give a total yield of 0.58 g of **7**.

Anal. Calc. for $C_{36}H_{59}BrO_{29}$: C, 41.74; H, 5.74; Br, 7.71. Found: C, 41.72; H, 5.67; Br, 7.85.

6-Deoxy-6-iodo-cyclohexaamylose (**8**). — An aqueous solution (100 ml) of **3** (0.54 g) and sodium iodide (2.16 g) was heated for 1 h on a boiling water bath. T.l.c. (solvent A) indicated that the sulphonate had reacted to give mainly one new compound and a small proportion (<5%) of material having the same R_F value as cyclohexaamylose. The reaction solution was concentrated to 2 ml and, on cooling, pale-yellow crystals (0.36 g) were formed, which were shown to be pure by t.l.c. (solvent A). The yellow colour was removed by dissolving the crystals in boiling water and heating the solution until it was colourless. On concentration to 1 ml, colourless crystals (0.27 g) were readily obtained (R_C 1.9, purple with iodine). Part of the product (**8**) was recrystallized twice from water, to give colourless crystals having m.p. 175° dec , $[\alpha]_D^{22}$ +106° (*c* 0.42, pyridine).

Anal. Calc. for $C_{36}H_{59}IO_{29}$: C, 39.93; H, 5.49; I, 11.72. Found: C, 39.75; H, 5.33; I, 11.57.

6-Deoxy-cyclohexaamylose (**9**). — Monoiodo-cyclohexaamylose (**8**) (450 gm) was dissolved in 1:9 pyridine–water (100 ml) and W-2 Raney nickel (*ca.* 0.5 g) was added. The reduction was carried out at 14 $lb.in^{-2}$ of hydrogen for 6 h. T.l.c. (solvent A) showed that the reaction was complete. The catalyst was filtered off with a Celite pad, and the filtrate was evaporated to 20 ml. Water (80 ml) was added and the evaporation repeated. The solution was then made up to 100 ml with water and passed through Dowex-3 (OH^-) (5 ml). On evaporation of the eluate to dryness, a colourless glass (400 mg) was obtained. T.l.c. (solvent A) showed this material to be pure except for a trace (<5%) of very slow-migrating material, possibly a pyridinium salt of cyclohexaamylose. In order to obtain pure 6-deoxy-cyclohexaamylose, some of this material (230 mg) in water (50 ml) was applied to a charcoal column [10 g, 16.0 × 1.5 cm; see preparation of monotosyl-cyclohexaamylose (**3**)] The column was washed successively with water (500 ml), 10% ethanol (100 ml), and 25% ethanol (300 ml). Fractions (100 ml) of the eluate with 25% ethanol were collected. T.l.c. (solvents A and B) showed that the first two fractions contained pure deoxy-cyclohexaamylose. On evaporation to dryness colourless material (190 mg) was obtained [R_C (solvent A) 1.3, R_C (solvent B) 1.1, purple with iodine]. This material was crystallized from water by adding ethanol dropwise until the critical concentration was reached. Colourless crystals (150 mg) were obtained that could be recrystallized from water to give the product **9** having m.p. 287–289°, $[\alpha]_D^{22}$ +119° (*c* 0.40, water).

Anal. Calc. for $C_{36}H_{60}O_{29}$: C, 45.19; H, 6.32. Found: C, 44.81; H, 6.42.

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