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# REACTION OF CYCLODEXTRINS WITH PROPYLENE OXIDE OR WITH GLYCIDOL: ANALYSIS OF PRODUCT DISTRIBUTION

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ABSTRACT

Reaction of cyclomalto-hexaose, -heptaose, or -octaose with propylene oxide in strong aqueous alkali gave products in which distribution of the degrees of substitution was relatively narrow and nearly symmetrical, and increased with the average degree of substitution. When an equimolar mixture of cyclomalto-hexaose, heptaose, or -octaose was used, the average degrees of substitution of all three carbohydrates were close to each other. These findings indicate that the reactivities of the hydroxyl groups of cyclomalto-hexaose, -heptaose, or -octaose, and of all their (2-hydroxypropyl) ethers formed in the reactions, are quite similar. Reaction of cyclomaltoheptaose with glycidol also yielded a product having a narrow distribution of degree of substitution, but which was slightly skewed towards the higher degrees. Thus, as it proceeds, this etherification leads to products having higher reactivity towards the epoxide.

## INTRODUCTION

Some carbohydrates have crystal lattices that are highly energy-favored and consequently these compounds are not very soluble in water. Conversion of chemical individuum into a multicomponent mixture is an effective method by which to prevent crystallization; this conversion also often raises the solubility in water. Biomedical applications usually require good water solubility and thus, conversion of individual carbohydrates into a multicomponent mixture may be of advantage, but the process must yield products that can be thoroughly characterized. These aspects are now addressed for the products of reactions of propylene oxide with cyclomalto-hexaose, -heptaose, or -octaose. Additionally, we were interested in the relative reactivity of hydroxyl groups in these cyclic hexa-, hepta-, or octamers of D-glucose.

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RESULTS

Cyclodextrins (cycloamyloses) reacted in strong aqueous alkali with propylene oxide present in excess. Strongly alkaline conditions enabled the reaction to proceed in a homogeneous aqueous phase. The products were purified by extractions with solvents and by dialysis.

The average degrees of substitution (d.s.) were calculated from a ratio of signals in nuclear magnetic resonance spectra: the region at ~1 p.p.m. was used to quantitate methyl groups of the 2-hydroxypropyl substituent, and the signals of anomeric protons, at 5.0–4.8 p.p.m., were used for cycloamylose residues. Degrees of substitution are expressed as the average number of substituents per cycloamylose molecule.

Plasma-desorption mass spectrometry was used to measure both the average degree of substitution and the distribution of the degree of substitution. In this "soft" ionization method, no derivatization is needed; ions formed from the molecule of carbohydrate with adventitous sodium cation are formed directly in the

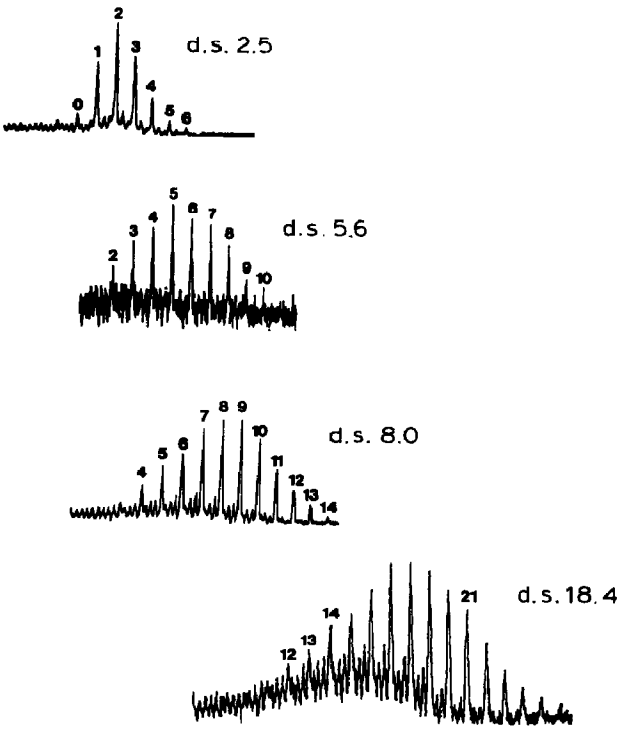


Fig. 1. Mass spectra of products of the reaction of cyclomaltoheptaose with propylene oxide. Numbers above a peak indicate the pertinent degree of substitution (d.s.); the average d.s. of the preparation is noted on the side. The "hump" of peaks at lower masses, seen best for d.s. 18.4, is probably due to accumulation of the fragment ions and metastable ions. For example, the two peaks between each major ion correspond respectively to loss of one and two molecules of water.

source part of the spectrometer through the effects of fission fragments of californium-252. In the spectra (see Fig. 1), the numbers above the peaks denote the number of 2-hydroxypropyl substituents per cycloamylose residue; the intensity of the peak is proportional to the relative amount of species of that degree of substitution in the preparation. Of course, all isomers of the same molecular weight are measured together. Thus, for example, even the monosubstituted species consists of six compounds, corresponding to the substitutions at O-2, -3, or -6 with the substituent containing a chiral carbon atom.

The spectra of preparations based on cyclomaltoheptaose are depicted in Fig. 1. The purification processes may result in some fractionation of the products, but as the yields were comparable and acceptable from the preparative point of view, any such effects on distribution should be minor. It is obvious that the reaction conditions chosen yielded preparations having rather narrow distributions of degree of substitution, the half-width of which increases with the average degree of substitution. Furthermore, all the distributions of the degree of substitution are without noticeable discontinuities and are quite symmetrical. These features are in agreement with cursory data previously published[1].

In this report, a point was made to prepare and evaluate preparations in which the important degrees of substitution figure prominently. The spectrum of the preparation with an average degree of substitution of 2.5 shows the initial reactant, cyclomaltoheptaose, together with its mono- and di-substitution products; obviously, there is no noticeable distinction of reactivities. Preparations with average degrees of substitution of 5.6 and 8.0 demonstrate the same for hepta-substitution, *i.e.*, the number of primary hydroxyl groups. Lastly, the preparation with an average degree of substitution of 18.4 documents the same for the number of 21, which is the total of all hydroxyl groups in cyclomaltoheptaose. It should be noted that the hydroxyl group of the 2-hydroxypropyl ethers may react further with propylene oxide, and that is what obviously happens, because there is no dis-

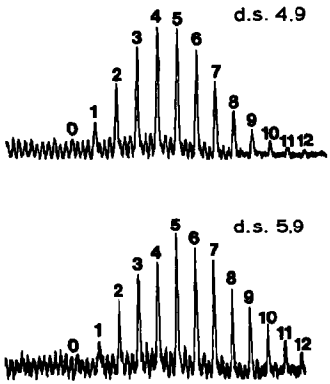


Fig. 2. Mass spectra of the products of reaction of cyclomaltohexaose (above) and cyclomalto-octaose (below) with propylene oxide. For explanation of notation, see legend to Fig. 1.

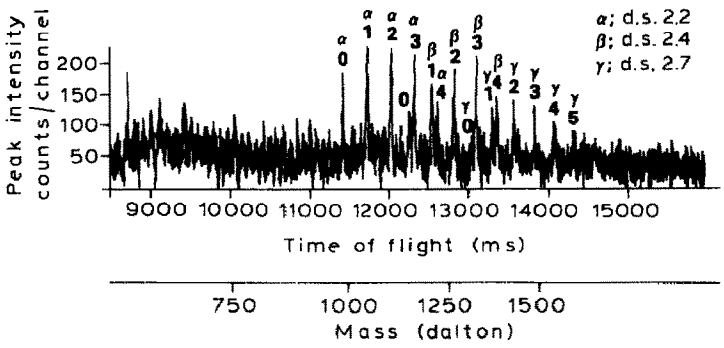


Fig. 3. Mass spectrum of the reaction product of an equimolecular mixture of cyclomalto-hexaose, heptaose, and octaose with propylene oxide. For explanation of notation see legend to Fig. 1; letters $\alpha$, $\beta$, and $\gamma$ are used to denote derivatives of cyclomalto-hexaose, -heptaose, and -octaose, respectively.

continuity at the degree of substitution of 21. A similar situation, *i.e.*, lack of discontinuities, was found for preparations derived from cyclomaltohexaose having an average degree of substitution of 4.9 and cyclomaltooctaose having an average degree of substitution of 5.9 (see Fig. 2).

To estimate the relative reactivities of cyclomalto-hexaose, -heptaose, or -octaose towards propylene oxide, an equimolecular mixture of these three carbohydrates was treated with an excess of propylene oxide and the product was isolated. Although the signals were weak, the resolution of the mass spectrometer was sufficient to allow complete analysis of the components of this product, and the results, together with an interpretation of them, are given in Fig. 3. Obviously, all three cycloamyloses have quite similar reactivity: average degrees of substitution were calculated as 2.2, 2.4, and 2.7 for cyclomalto-hexaose, -heptaose, and -octaose, respectively. Also, there were no discontinuities in envelopes of the degrees of substitution of any of these carbohydrates.

Cyclomaltoheptaose was furthermore allowed to react under the same conditions with a hydroxy derivative of propylene oxide, namely, glycidol. The distribution of the degrees of substitution in the product is shown in Fig. 4, and the average degree of substitution was 3.7. Again there is no discontinuity at any degree of substitution, but the distribution is quite obviously skewed towards the higher degrees of substitution. The ratio of areas under the curves of distribution to the

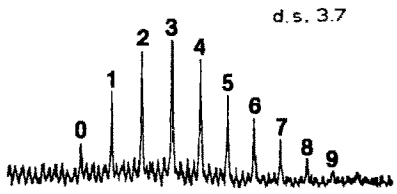


Fig. 4. Mass spectrum of the reaction product of cyclomaltoheptaose with glycidol. For explanation of notation, see legend to Fig. 1.

right of those to the left of the maximum can be used to estimate the degree of asymmetry. For products of all reactions in which propylene oxide was used (see Figs. 1 and 2) the average of these ratios is 0.99 with a standard deviation of 0.13; the corresponding ratio for the product of the glycidol reaction is 1.41.

## DISCUSSION

The rate of alcoholysis of epoxides is expected to decrease from primary to secondary alcohols and this empirical rule applies well to carbohydrates. The primary alcohol group, on C-6 of aldohexoses, was the main coupling site with 1,4-butanediol diglycidyl ether[2], and a study of a model compound, methyl α-D-glucopyranoside, established that at least 90% of the total reaction occurs at that group. The reaction products of cellulose or guaran with propylene oxide also testify to higher reactivity of primary over secondary hydroxyl groups[3–5].

The preparation of numerous derivatives of cycloamyloses was studied and reviewed with the conclusion that both the differences in reactivity of hydroxyl groups and the formation of inclusion complexes play a role there[6–10]. Good yields of individual products [*e.g.*, heptakis-(2,6-di-*O*-methyl)cyclomaltoheptaose] suggest that substitutions are relatively selective and that consequently the distribution of products is not given by simple statistics alone.

The present results on the reaction of cycloamyloses with propylene oxide suggest uniformity of reaction rates of the starting reaction and of all the sequential reactions. The distribution of products in this reaction resembles, the simple distribution curves of statistics. The formation of inclusion complexes cannot have a distinctive role in the present case as all three cycloamyloses, which have quite different cavities and thus also different abilities to form inclusion complexes, react with equal ease.

This reasoning was strengthened on comparing the profiles of substitution of cyclomaltoheptaose with propylene oxide (Fig. 1) and with glycidol (Fig. 4). The reaction with propylene oxide does not change the total number of hydroxyl groups in the molecule and thus, from a structural point, the process can be likened to the growth of an octopus, in which the number of arms does not change. This growth must be quite uniform because the distribution of the degree of substitution is highly symmetrical. The reaction with glycidol results in a local doubling of the number of hydroxyl groups by substitution, a process likened to the growth of tree branches in another synthetic context[11]. The distribution of the degree of substitution in that case is skewed towards the higher numbers, indicating clearly that reactivities of products increase with the progress of the reaction.

## EXPERIMENTAL

Nuclear magnetic resonance spectra were recorded with a JEOL PMX-60SI instrument for solutions (10% w/v) of compounds in deuterium oxide, with sodium

4,4-dimethyl-4-silapentane-1-sulfonate as standard. Mass spectra were recorded with a Cf-252 plasma-desorption mass spectrometer constructed for NHLBI by Dr. R. D. Macfarlane of Texas A and M University (College Station, TX). For the reaction products of cycloamyloses with propylene oxide, accumulation times of ~240 min were satisfactory; for the corresponding products with glycidol, 720 min was necessary. For dialysis, tubing of regenerated cellulose (Union Carbide; 4.77 cm diam. × 0.05 mm thick when dry) was used; tubing was pretreated by boiling in water for 30 min and washing thoroughly. For data on hydration and solubility of cycloamyloses, compare ref. 12 and 13.

*O-(2-Hydroxypropyl)cyclomaltoheptaose.* — (*a*) Sodium hydroxide (61.7 g, 1.54 mol) was dissolved in distilled water (300 mL), and cyclomaltoheptaose (200 g of commercial product, corresponding to 173.2 g of anhydrous compound; 153 mmol) was added. The suspension was stirred at 50–60° until the crystals dissolved. The flask was immersed in an ice bath, and propylene oxide (45 mL, 37.4 g, 0.64 mol) was added dropwise while stirring under propylene oxide reflux (Dry Ice–acetone condenser) during 3 h. The solution was stirred for 19 h at room temperature, made neutral with concentrated HCl solution (~128 mL), and concentrated *in vacuo* to about half volume. The resulting syrup was stirred with ethanol (2 L) for 30 min, the precipitated sodium chloride was filtered off and washed with ethanol, and all filtrates were combined, and evaporated; the residue was dissolved in water (200 mL), and the solution dialyzed for 5 h at 0°. The solution of the retained fraction was freeze-dried and then the white solid resulting was suspended in acetone (1 L), the suspension was stirred for 24 h, and then filtered. Washing of the precipitate with acetone was repeated. The final precipitate was dried *in vacuo*, dissolved in water (400 mL), and the solution freeze-dried, affording 183 g (94%) of product; $^1$H-n.m.r. data: $\delta$ 4.88–4.83 (broad s, anomeric protons, 1 H), 3.68–3.23 (m, cycloamylose skeleton protons and $CH_3$–C*H*OH–$CH_2$, 7.07 H), and 1.00 (d, *J* 6 Hz, $CH_3$, 1.08 H); calculated average degree of substitution, 2.5.

(*b*) The procedure was the same as in (*a*), but 153 mmol of cyclomaltoheptaose and 104 mL (1.49 mol) of propylene oxide were used; yield: 187 (84%); $^1$H-n.m.r. data: $\delta$ 5.02–4.74 (bd, anomeric protons, 1 H), 3.82–3.38 (m, cycloamylose skeleton protons and $CH_3$–C*H*OH–$CH_2$, 8.40 H), and 1.02 (d, *J* 6 Hz, $CH_3$, 2.40 H); calculated average degree of substitution, 5.6.

(*c*) The procedure was the same as in (*a*), but 153 mmol of cyclomaltoheptaose and 150 mL (2.14 mol) of propylene oxide were used; yield: 180 g (74%); $^1$H-n.m.r. data: $\delta$ 4.94–4.85 (bs, anomeric protons, 1 H), 3.73–3.24 (m, cycloamylose skeleton protons and $CH_3$–C*H*OH–$CH_2$, 9.44 H), and 1.00 (d, *J* 6 Hz, $CH_3$, 3.42 H); calculated average degree of substitution, 8.0.

(*d*) The procedure was the same as in (*a*) but only 8.8 mmol of cyclomaltoheptaose dissolved in water (300 mL) containing 1.54 mol of NaOH was used, together with 100 mL (1.43 mol) of propylene oxide. In this case, the product was soluble in acetone, and was precipitated from a solution therein by addition of cyclohexane; yield: 22.5 g (109%); $^1$H-n.m.r. data: $\delta$ 5.08–4.82 (bs, anomeric pro-

tons, 1 H), 3.95–3.04 (m, cycloamylose skeleton protons and $CH_3$–C*H*OH–C$H_2$, 15.0 H), and 1.00 (d, *J* 6 Hz, C$H_3$, 8.80 H); calculated average degree of substitution, 20.8. The average degree of substitution calculated from the mass spectrum was 18.4.

O-*(2-Hydroxypropyl)cyclomaltohexaose.* — The procedure was the same as in (*a*) but 153 mmol of cyclomaltohexaose and 92.6 mL (1.32 mol) of propylene oxide were used, yielding 196 g (99%) of product; $^1$H-n.m.r. data: $\delta$ 4.93–4.82 (bs, anomeric protons, 1 H), 3.65–3.21 (m, cycloamylose skeleton protons and $CH_3$–C*H*OH–C$H_2$, 8.7 H), and 0.98 (d, *J* 6 Hz, C$H_3$, 2.66 H); calculated average degree of substitution, 5.4. The average degree of substitution calculated from the mass spectrum was 4.9.

O-*(2-Hydroxypropyl)cyclomalto-octaose.* — The procedure was the same as in (*a*), but 153 mmol of cyclomalto-octaose and 84.2 mL (1.20 mol) of propylene oxide were used, yielding 204 g (84%) of product; $^1$H-n.m.r. data: $\delta$ 5.23–5.06 (bs, anomeric protons, 1 H), 3.98–3.28 (m, cycloamylose skeleton protons and $CH_3$–C*H*OH–C$H_2$, 7.90 H), and 1.12 (d, *J* 6 Hz, C$H_3$, 1.95 H); calculated average degree of substitution, 5.1. The average degree of substitution calculated from the mass spectrum was 5.9.

*Reaction of a mixture of cyclomalto-hexaose, -heptaose, and -octaose with propylene oxide.* — The procedure was the same as in (*a*) but 1 mmol each of cyclomalto-hexaose, -heptaose, and -octaose were used, co-dissolved in water (5.1 mL) containing 26.25 mmol of NaOH, and 0.76 mL (10.8 mmol) of propylene oxide was added; the yield of product was 2.7 g (70%); $^1$H-n.m.r. data: $\delta$ 5.09–4.93 (bs, anomeric protons, 1 H), 3.68–3.42 (m, cycloamylose skeleton protons and $CH_3$–C*H*OH–C$H_2$, 7.09 H), and 1.08 (d, *J* 6 Hz, C$H_3$, 1.08 H); calculated average degree of substitution, 2.5.

O-*(2,3-Dihydroxypropyl)cyclomaltoheptaose.* — Sodium hydroxide (31 g, 775 mmol) was dissolved in distilled water (150 mL), and cyclomaltoheptaose (100 g, 76 mmol) was added. The suspension was stirred at 50–60° until the crystals dissolved. Glycidol (37 mL, 41.4 g, 0.55 mol) was added dropwise while the mixture was stirred at room temperature. After 24 h, the mixture was made neutral with concentrated hydrochloric acid, and the solution dialyzed for 24 h at 0°. The retained fraction was freeze-dried. The residue was washed twice with acetone (500 mL), dried, dissolved in water (200 mL), and again freeze-dried, yielding 97 g (90%) of the product, a white powder that sintered upon exposure to humidity. The average degree of substitution calculated from the mass spectrum was 3.7.

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