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Distribution of substituents in *O*-(2-hydroxypropyl) derivatives of cyclomalto-oligosaccharides (cyclodextrins): influence of increasing substitution, of the base used in the preparation, and of macrocyclic size

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ABSTRACT

Samples of *O*-(2-hydroxypropyl) derivatives of cyclomaltoheptaose (β-cyclodextrin) with increasing substitution were prepared by withdrawing aliquots at different times from a reaction mixture containing cyclomaltoheptaose and an excess of (*S*)-propylene oxide in 0.39M aqueous sodium hydroxide. The distributions of substituents between the different molecules and between the different α-D-glucopyranosyl residues in these samples were determined by mass spectrometry and methylation analysis, respectively. The solubilities of the samples and their association constants with phenolphthalein were also determined. The relative reactivities at O-2 and O-3 *versus* O-6, calculated using Spurlin's equations, decreased with increasing degree of substitution, probably because of steric hindrance. No significant differences were observed when different strong bases were used as promoters of the hydroxypropylation of cyclomaltoheptaose. The hydroxypropylation of cyclomaltohexaose (α-cyclodextrin) and cyclomalto-octaose (γ-cyclodextrin) was briefly investigated.

INTRODUCTION

Mixtures of *O*-(2-hydroxypropyl) derivatives of cyclomaltoheptaose are effective non-toxic solubilisers of lipophilic drugs, scheduled to enter practical use[1,2]. The composition of such mixtures and its effect on their properties is therefore a matter of practical importance, and some studies of the effect of the average degree of substitution upon the pharmaceutical usefulness have been carried out[3–5]. However, more information about the distribution of substituents in these mixtures and how it affects their properties is needed.

The distribution of substituents in *O*-(2-hydroxypropyl) derivatives of cyclomaltoheptaose, prepared by alkylation with propylene oxide in aqueous sodium hydroxide, has been investigated[6,7]. It was observed[6] that high and low concentrations of alkali favoured alkylation at O-6 and O-2, respectively, and that substitution at O-2 increased the reactivity at O-3. As expected, the percentage of the isomeric *O*-(2-hydroxy-1-

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methylethyl) derivatives was low, as also was the percentage of oligo(propylene glycol) ethers.

We now report further detailed studies of the hydroxypropylation of cyclomaltoheptaose.

RESULTS AND DISCUSSION

Cyclomaltoheptaose, in 0.39M aqueous sodium hydroxide, was treated at room temperature with an excess of (*S*)-propylene oxide, and the products were isolated at intervals. The distribution of molecules with different numbers of substituents was investigated by mass spectrometry, using ^{252}Cf plasma desorption and f.a.b. in positive and negative modes. As fragmentation was minimal on plasma desorption, these mass spectra were used to calculate the percentages of molecules with different numbers of substituents. In Fig. 1, the molar percentages of starting material and molecules with different degrees of substitution are plotted against the average degree of substitution (d.s.). The results show that the distribution is relatively narrow. The average d.s. of the cyclomaltoheptaose molecules was estimated from the heights of the peaks for the pseudomolecular ions. There is reasonably good agreement between the values obtained using the three different methods of mass spectrometry, but these values are

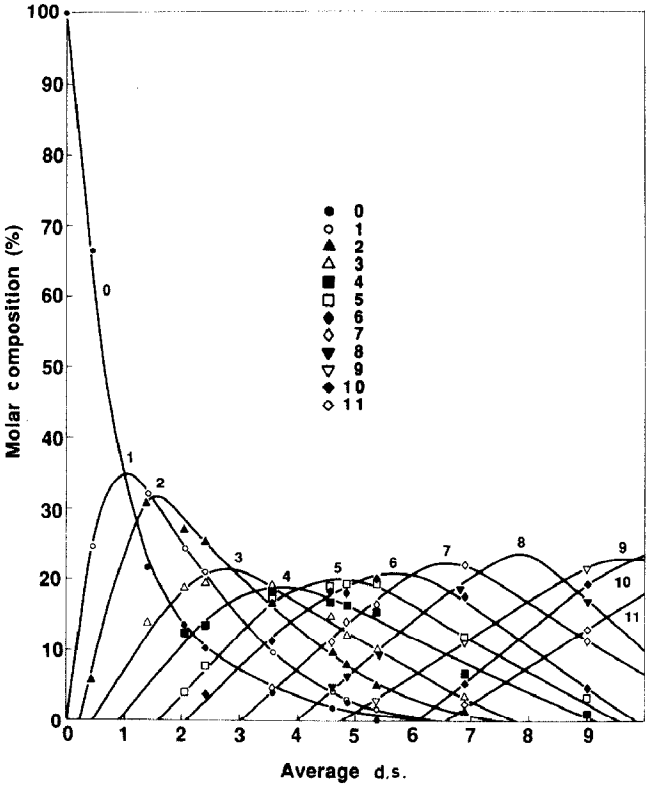

Fig. 1. Distribution of molecules with different numbers of 2-hydroxypropyl groups *versus* d.s.

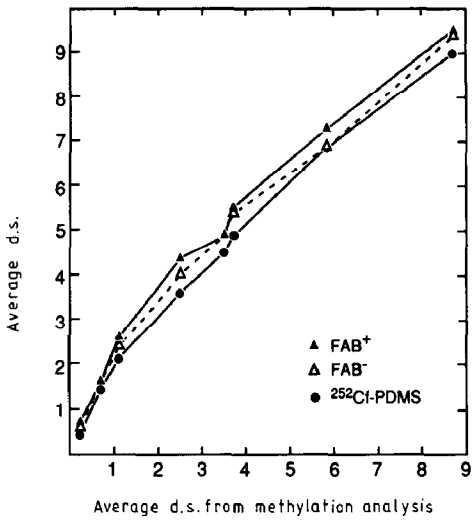

Fig. 2. D.s. determined by m.s. *versus* d.s. from methylation analysis.

higher than those obtained from methylation analysis (Fig. 2). The response factors for the different molecular species on mass spectrometry are not known, but may increase with increasing degree of alkylation, which would make the calculated d.s. values too high. Response factors were used in the methylation analysis, but these are uncertain. Minor components are overlooked in these analyses, which will render the calculated d.s. values too low. Determination of d.s. values by plasma-desorption m.s. is easily performed, and even if the values are not too accurate, they are useful for characterisation of different preparations.

The distribution of hydroxypropyl groups in the α-D-glucopyranosyl residues was determined by methylation analysis, which was performed as before[6]. Thus, the ethers were fully methylated, hydrolysed, reduced with sodium borohydride, and then acetylated, and the resulting mixture of 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-alkyl-D-glucitols, in which the alkyl group is either methyl or 2-methoxypropyl, was analysed by g.l.c. and g.l.c.–m.s. The molar proportions of the different ethers were estimated from g.l.c., using a flame-ionisation detector and relative molar response factors[8]. The results are summarised in Table I.

The relative reactivities at the different positions of the α-D-glucopyranosyl residues in cyclomaltoheptaose (Table I) were calculated using Spurlin's equations[9]. In these calculations, it is assumed that the relative reactivities at O-2 and O-6 (k_2 and k_6) are independent of substitution in other positions, but that the reactivity at O-3 (k_3) is changed (to k'_3) on alkylation at O-2. However, the values for k'_3 are not very accurate, in particular for samples with low d.s. values. It is evident from Table I that the values of k_2, k_3, and k'_3, relative to k_6, decrease with increasing d.s. Thus, the requirements for using Spurlin's equations are not fulfilled. The conformation of cyclomaltoheptaose is that of a truncated cone, with the secondary hydroxyl groups at the bottom, and the

TABLE I

Relative reactivities of the 2-, 3-, and 6-positions of cyclomaltoheptaose on hydroxypropylation, promoted by 0.39M sodium hydroxide, to different d.s., and the influence of d.s. on solubility and association constants

Sample	*Reaction time (h)*	*D.s.*[a]	*Ether*[b] *(mole %)*								*Rel. reactivity*[c]				*Solubility (%)*	K_{ass} *(mol*$^{-1}$*)*
			S_0	S_2	S_3	S_6	S_{23}	S_{26}	S_{36}	S_{236}	k_2	k_3	k'_3	k_6		
1	1.0	0.43	96.7	2.2	0.8	0.3	0.04	–	–	–	1	0.33	–	0.13	1.2	3.63×10^4
2	2.0	1.38	90.9	5.6	2.4	0.7	0.3	–	–	–	1	0.43	–	0.11	1.1	4.00×10^4
3	3.0	2.05	85.3	9.0	3.5	1.1	0.8	0.2	0.1	–	1	0.39	–	0.13	1.6	3.76×10^4
4	4.5	2.42	–	–	–	–	–	–	–	–	–	–	–	–	3.3	–
5	6.5	3.56	69.8	16.9	6.5	2.1	3.3	0.9	0.4	0.1	1	0.39	1.07	0.15	7.8	2.76×10^4
6	8.5	4.54	59.0	21.9	7.8	2.8	6.0	1.4	0.8	0.4	1	0.37	1.08	0.16	37.0	2.25×10^4
7	10.5	4.85	57.0	22.7	7.4	2.8	6.9	1.8	0.7	0.5	1	0.33	1.02	0.16	>50	2.13×10^4
8	12.5	5.35	–	–	–	–	–	–	–	–	–	–	–	–	>50	–
9	21.5	6.86	39.3	29.2	7.6	4.1	12.5	1.4	3.9	2.0	1	0.26	0.92	0.19	>50	1.13×10^4
10	84.0	9.01	22.2	27.3	5.5	6.3	18.6	9.9	2.8	7.5	1	0.25	0.75	0.30	>50	4.19×10^3

[a] Determined from plasma-desorption m.s. [b] S_2 = mole % of D-glucopyranosyl residues substituted at O-2, *etc.* [c] k_2 = the relative reactivity at O-2, *etc.*

primary groups at the top, of the cone. At the concentration of sodium hydroxide used in this experiment, the secondary hydroxyl groups are preferentially alkylated[6]. A reasonable explanation for the observed results is that, when the secondary ethers accumulate, the reactivity of the remaining free, secondary hydroxyl groups decreases due to steric hindrance, while the reactivity of the free primary hydroxyl groups is not significantly affected. A similar effect, due to crowding of primary alkyl groups, is expected when the alkylation is promoted by a high concentration of strong alkali, which favours alkylation at primary positions. In agreement with this view, the relative values of k_2, k_3, and k_6 for two derivatives with d.s. 1.7 and 5.3, prepared using 4M sodium hydroxide, were 1:0.43:2.1 and 1:0.40:1.5, respectively[6].

The solubilities of the different preparations in water, defined as the highest percentage of a sample that gives a clear solution at room temperature, were determined (Table I and Fig. 3). At low d.s. values, this solubility was even lower than for

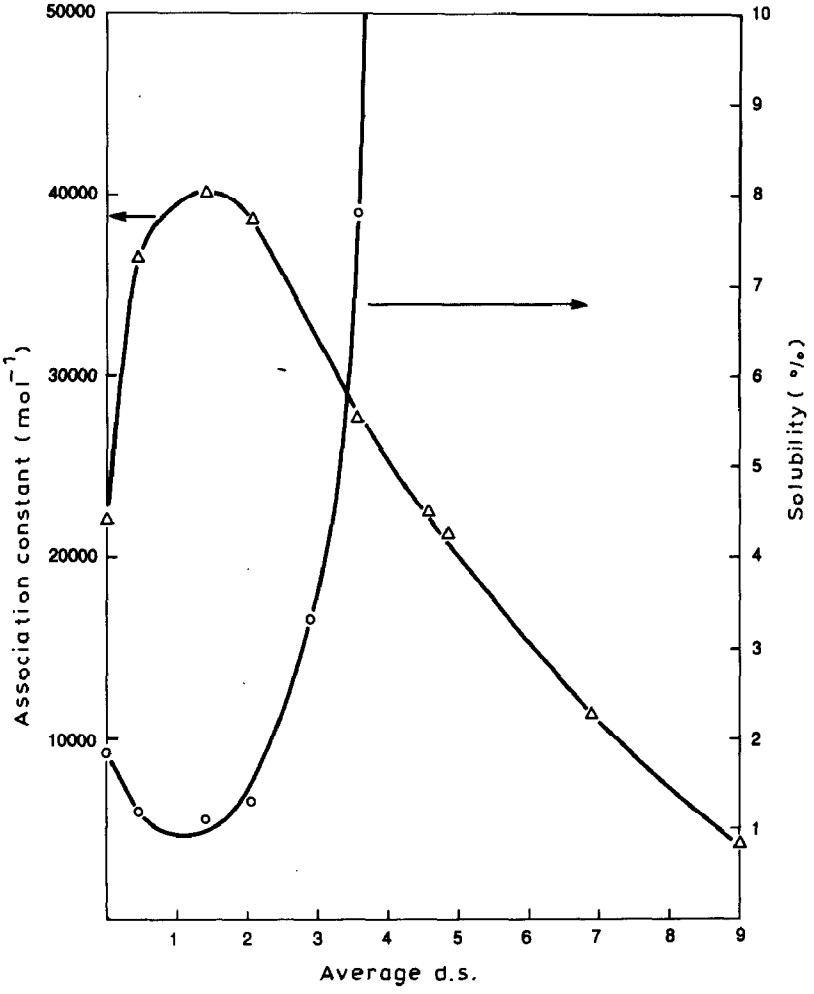

Fig. 3. Solubilities and association constants *versus* d.s.

cyclomaltoheptaose, indicating a high content of 2-*O*-[(*S*)-2-hydroxypropyl]cyclomaltoheptaose (compare Fig. 1), which is less soluble than cyclomaltoheptaose[10]. At higher d.s. values, when the number of individual species increases rapidly, a steep increase in solubility was observed.

The ability of the preparations to form complexes with phenolphthalein[11] was also investigated, and was found first to increase and then to decrease with increasing d.s. (Table I and Fig. 3). The decrease was expected, because high substitution will make it more difficult for the guest molecule to enter the cavity of the host. However, the increase at low d.s. was not expected, and may be due to increased lipophilic binding of the guest molecule by the hydroxypropyl groups. As the pharmaceutical usefulness of the hydroxypropylated cyclomaltoheptaoses depends both upon their solubility and their ability to form complexes, it is essential to find a compromise between these parameters.

The effect of five different bases, namely, lithium hydroxide, sodium hydroxide, potassium hydroxide, tetramethylammonium hydroxide, and barium hydroxide as promoters of the reaction between cyclomaltoheptaose and (*S*)-propylene oxide was investigated. The concentration of hydroxyl anions was approximately the same in these experiments, and the d.s. values were comparable. As seen from Table II, no significant differences in the substitution pattern could be detected when different bases were used.

Hydroxypropylation of cyclomalto-hexaose, -heptaose, and -octaose in 1.2M aqueous sodium hydroxide, to approximately the same d.s., gave the results listed in Table III. There are some differences in the substitution pattern for the three cyclodextrins; thus, cyclomaltohexaose shows considerably lower reactivity at O-3 and higher at O-6 than the other two homologues. However, more systematic experiments are needed in order to explain these observations.

EXPERIMENTAL

General methods. — Plasma-desorption m.s. (^{252}Cf, positive mode) was performed on a spectrometer constructed by Dr. R. D. Macfarlane for N.H.L.B.I. F.a.b.-m.s. (positive and negative modes) was performed on a VG 70 70 E HF instrument (University of Minnesota, Mass Spectrometry Service Laboratory), using a thioglycerol matrix. Absorbances were measured with a Beckman DU-50 spectrometer. G.l.c. was performed on a Hewlett–Packard 5830 A instrument fitted with a flame-ionisation detector, with hydrogen as the carrier gas. G.l.c.–m.s. was performed on a Hewlett–Packard 5890-5970 system with helium as the carrier gas. A Hewlett–Packard Ultra 2 fused silica, capillary column was used. Solutions were concentrated at 40° under reduced pressure.

Hydroxypropylation of cyclomaltoheptaose. — Cyclomaltoheptaose (13.1 g, corresponding to 11.35 g of the anhydrous compound; 10 mmol) was dissolved in 0.4M sodium hydroxide (50 mL). The solution was cooled in an ice bath, (*S*)-propylene oxide (8.3 g, 143 mmol) was added during 1 h, and the mixture was then brought to room

TABLE II

Substitution pattern and relative reactivities of the 2-, 3-, and 6-positions of cyclomaltoheptaose on hydroxypropylation, promoted by different bases

Base	*D.s.*	*Ether[b] (mole %)*								*Rel. reactivity[c]*		
		S_0	S_2	S_3	S_6	S_{23}	S_{26}	S_{36}	S_{236}	k_2	k_3	k_6
0.40M LiOH	2.5	67.9	19.8	5.9	2.2	3.3	0.8	–	–	1	0.30	0.11
0.38M NaOH	2.0	74.4	14.6	4.8	2.6	2.2	0.9	0.5	–	1	0.32	0.21
0.40M KOH	2.5	69.8	17.7	5.7	1.8	3.4	0.8	0.8	–	1	0.32	0.15
0.4M Me_4NOH	3.6	57.7	23.1	7.4	2.7	6.0	1.5	1.0	0.5	1	0.32	0.16
0.20M $Ba(OH)_2$	1.3	83.3	9.7	3.6	1.2	1.0	0.2	0.5	0.3	1	0.35	0.18

[a] Determined from methylation analysis. [b] S_2 = mole % of D-glucopyranosyl residues substituted at O-2, *etc.* [c] k_2 = relative reactivity at O-2, *etc.*

TABLE III

Relative reactivities of the 2-, 3-, and 6-positions of cyclomalto-hexaose, -heptaose, and -octaose on hydroxypropylation, promoted by 1.3M sodium hydroxide

Substance	*D.s.*[a]	*Ether[b] (mole %)*								*Rel. reactivity*			
		S_0	S_2	S_3	S_6	S_{23}	S_{26}	S_{36}	S_{236}	k_2	k_3	k'_3	k_6
CM-hex	5.5	36.5	26.4	1.9	10.4	6.2	12.9	1.8	3.7	1	0.08	0.56	0.50
CM-hep	5.5	43.0	24.2	6.6	7.3	9.6	5.2	1.6	2.4	1	0.26	0.97	0.34
CM-oct	6.9	33.7	25.2	5.7	8.1	11.3	8.7	2.5	4.7	1	0.24	0.84	0.40

[a] Determined from methylation analysis. [b] S_2 = mole % of D-glucopyranosyl residues substituted at O-2, *etc.* [c] k_2 = relative reactivity at O-2, *etc.*

temperature (22–23°). Aliquots (4 mL) were withdrawn after 1, 2, 3, 4.5, 6.5, 8.5, 10.5, 12.5, 21.5, and 84 h. Each aliquot was neutralised with M hydrochloric acid, then dialysed against distilled water for 4 h, and the retained solution was concentrated to dryness. Samples 1–10 were obtained in this manner.

Determination of solubilities. — Measured quantities of water were successively added to a weighed quantity of each sample (~25 mg), followed by sonification, until a clear solution was obtained.

Determination of association constants[11]. — Stock solutions of an average molar concentration of $\sim 6 \times 10^{-4}$ (as calculated from the d.s. values) were prepared from each sample. The solution (5, 7, 10, 12, and 14 mL) was added to a solution of phenolphthalein (2 mL), prepared by diluting 3.75mM phenolphthalein in aqueous 95% ethanol (10 mL) to 100 mL with water. Sodium carbonate (0.04M, 2.5 mL) was added and the volume brought to 25 mL by addition of water. The absorbances at 550 nm were measured and the association constants determined using the Benesi–Hildebrand method. In this method, the total molarity of phenolphthalein is divided by the absorbance at 550 nm, and these values, for each dilution, are plotted against the reciprocal of the total molarity of the hydroxypropylcyclomaltoheptaose. The slope at the least-squares line between these points, divided by the value of the intercept, gives the reciprocal of K_{ass}.

Hydroxypropylation of cyclomaltoheptaose using different bases. — A solution of cyclomaltoheptaose (6.44 g, corresponding to 5.60 g of the anhydrous compound, 4.94 mmol) in the base [25 mL of 0.4M LiOH, NaOH, KOH, or NMe_4OH, or 0.2M $Ba(OH)_2$] was cooled in an ice bath, (*S*)-propylene oxide (4.06 g, 70 mmol) was added during 1 h, and each reaction mixture was stirred for 12 h with cooling and then for 4 h at room temperature. The mixture was then processed as described above, and the product (6.2–6.9 g) was dried *in vacuo*.

Hydroxypropylation of cyclomaltohexaose. — To a solution of cyclomaltohexaose (5.41 g, corresponding to 4.87 g of the anhydrous compound, 5 mmol) in 1.32M aqueous sodium hydroxide (13.5 mL), cooled in an ice bath, was added (*S*)-propylene oxide (3.5 g, 60 mmol). A propylene oxide–cyclomaltohexaose complex (1:2 from its n.m.r. spectrum) was temporarily formed and disappeared slowly during the reaction, which was allowed to proceed for 6 h under cooling and then for 12 h at room temperature. The reaction mixture was processed as described above, to give the product (5.1 g).

Hydroxypropylation of cyclomalto-octaose. — A solution of cyclomalto-octaose (15.05 g, corresponding to 12.97 g of the anhydrous product, 10 mmol) and (*S*)-propylene oxide (9.3 g, 160 mmol) in 1.32M sodium hydroxide (27 mL) was kept for 6 h under cooling with ice and then for 39 h at room temperature. The reaction mixture was worked-up as above. The syrup obtained was dissolved in ethanol (100 mL), the solution was concentrated, and this procedure repeated. The resulting foam was stirred with ethyl acetate (75 mL) for 30 min, filtered, and dried, to give the product (18.5 g).

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