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Short communication

Retention behavior of positional isomers of disubstituted cyclomalto-oligosaccharide (cyclodextrin) derivatives on an ODS column

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Abstract

The correlation between hydrophobic effects and structures of three and four positional isomers of $6^1,6^n$-di-*O*-triphenylmethyl (trityl)- or $6^1,6^n$-di-*O*-*tert*.- butyldimethylsilyl (*tert*.-$BuMe_2Si$)-cyclomaltohexaoses (cG_6s, α-cyclodextrin) ($n=2–4$), -cyclomaltoheptaoses (cG_7s, β-cyclodextrin) ($n=2–4$), and -cyclomaltooctaoses (cG_8s, γ-cyclodextrin) ($n=2–5$) on an ODS column are discussed. Cyclodextrins with two hydrophobic-substituted groups bonded to hydroxyl groups tended to show low retention of positional isomers in which the binding positions of the two substituted groups on the cyclodextrin ring were far apart from each other.

Keywords: Positional isomers; Cyclodextrins; Oligosaccharides

1. Introduction

Cyclomalto-oligosaccharides [cG_ns, cyclodextrins (CDs)] have unique structures, properties, and the ability to form inclusion compounds with various kinds of inorganic and organic compounds. These compounds and their derivatives have come to be used in many fields [1–4]. In particular, modified CDs [5–7] have been widely studied to equip them with special characteristics in order to allow their use as chiral selectors in capillary zone electrophoresis [8,9], as enzyme models [10], and as intermediates for chemical syntheses of branched CDs [11–14]. Other promising applications are anticipated in various fields.

Many useful derivatives can be synthesized as these compounds have many hydroxyl groups which can be modified. However, it is difficult to conduct selective reactions on each kind of alcoholic function and problems arise with the formation of many complex isomers and difficulties in isolating and determining the desired compounds. Thus, careful selection of the synthetic methods and conditions are required and effective purification by high-performance liquid chromatography (HPLC) becomes very important.

This makes it necessary to devise suitable HPLC procedures for the separation and determination of CD derivatives. Furthermore, if these procedures can also be used to identify the isomers and substituted positions, this should facilitate the development of many useful CD derivatives. Here we describe our

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study on the retention behavior of structurally established positional isomers of disubstituted CD derivatives, 6^1,6^n-di-*O*-trityl- or 6^1,6^n-di-*O*-*tert*.-$BuMe_2Si$-αCDs (n=2–4), -βCDs (n=2–4), and -γCDs (n=2–5) (see Scheme 1 for structures) on an ODS column using HPLC.

2. Experimental

2.1. Apparatus and column

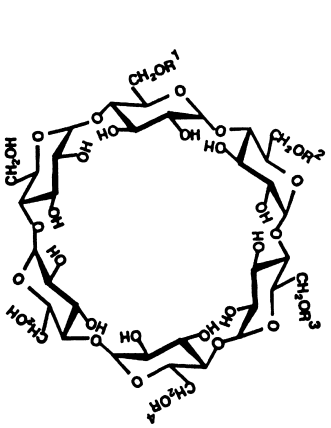
A HPLC system was assembled with a Tri Rotar SR-1 pump (Jasco), U6K universal injector (Waters), SE-71 refractive index (RI) detector (Showa Denko), CO-1093C column oven (Uniflows), and C-R5A data processor (Shimadzu). The column used was a YMC-Pack A-312 ODS, S-5 μm, 120 Å, 150×6 mm I.D.

2.2. Materials

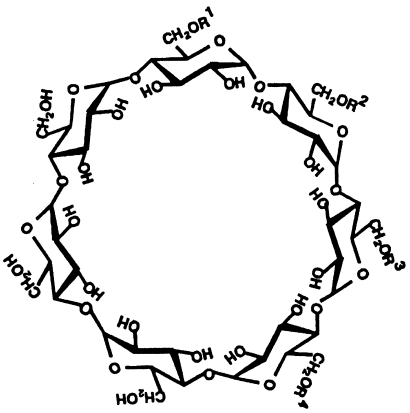
Trityl or *tert*.-$BuMe_2Si$-αCDs, -βCDs, and -γCDs derivatives used for the analyses were prepared and characterized for their positional isomers as reported previously [11–14]. Standard materials, alkyl alcohols and hydrocarbons, were obtained from Nacalai Tesque (Kyoto, Japan). Most samples were made up with ca. 20% methanol and injection volumes were about 1–5 μl. The mobile phases were made up by volume from LC grade solvents (Nacalai Tesque) and distilled, deionized water.

3. Results and discussion

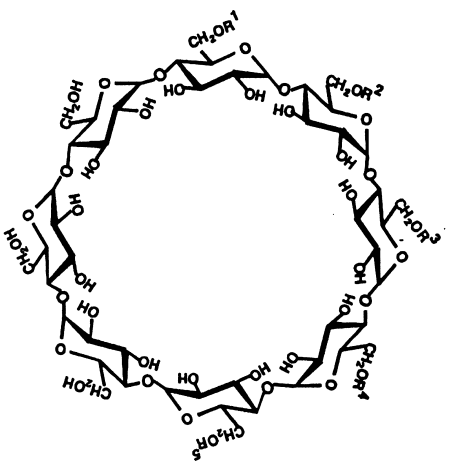
Fig. 1 shows chromatograms of three and four positional isomers of dityrityl-αCDs, -βCDs and -γCDs obtained under the same conditions on an ODS column with methanol–water; the baseline



	R^1	R^2	R^3	R^4
1	H	H	H	H
2	Tr	Tr	H	H
3	Tr	H	Tr	H
4	Tr	H	H	Tr
5	Si	Si	H	H
6	Si	H	Si	H
7	Si	H	H	Si



	R^1	R^2	R^3	R^4
8	H	H	H	H
9	Tr	Tr	H	H
10	Tr	H	Tr	H
11	Tr	H	H	Tr
12	Si	Si	H	H
13	Si	H	Si	H
14	Si	H	H	Si



	R^1	R^2	R^3	R^4	R^5
15	H	H	H	H	H
16	Tr	Tr	H	H	H
17	Tr	H	Tr	H	H
18	Tr	H	H	Tr	H
19	Tr	H	H	H	Tr
20	Si	Si	H	H	H
21	Si	H	Si	H	H
22	Si	H	H	Si	H
23	Si	H	H	H	Si

Tr, trityl; Si, *tert*-$BuMe_2Si$

Scheme 1.

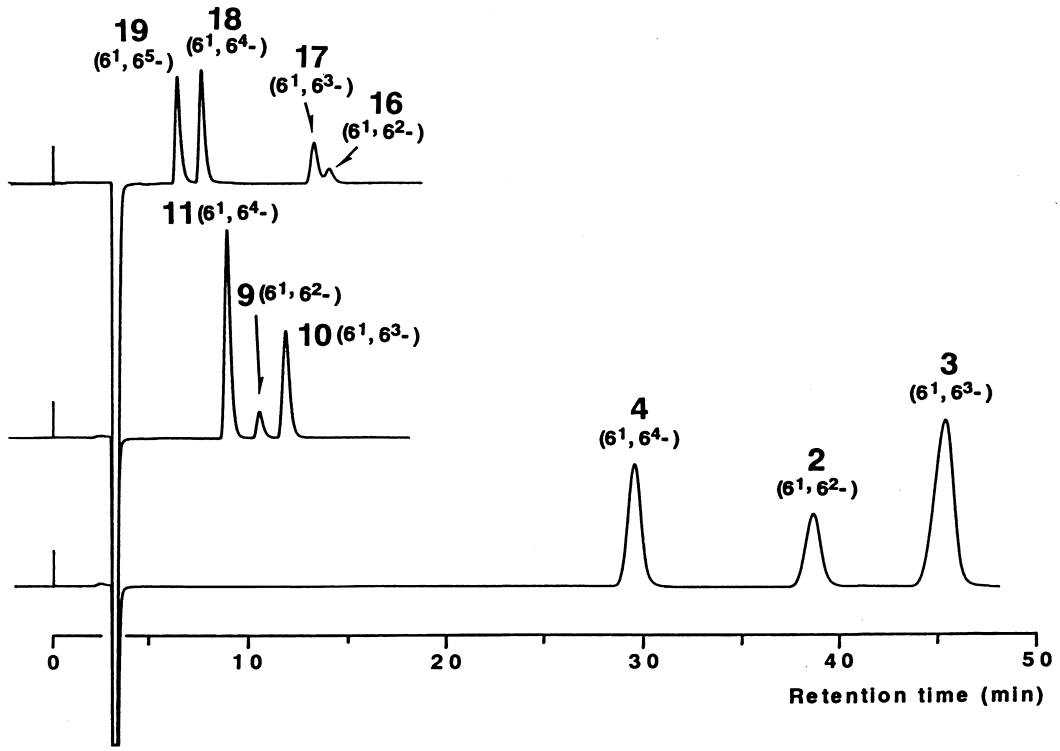


Fig. 1. Elution profiles of three and four positional isomers of $6^1,6^n$-di-*O*-triphenylmethyl (trityl)-cyclomaltohexaoses (cG_6s, αCD) (n=2–4, **2**–**4**), -cyclomaltoheptaoses (cG_7s, βCD) (n=2–4, **9**–**11**), and -cyclomaltooctaoses (cG_8s, γCD) (n=2–5, **16**–**19**). Chromatographic conditions: column, YMC-Pack A-312 ODS (150×6 mm I.D.); eluent, methanol–water (75:25); flow-rate, 1.0 ml/min; detector, Shodex RI-71 at $32\cdot10^{-6}$ RI units full scale; temperature, 30°C.

separation of all positional isomers was confirmed. The extents of retention of βCD and γCD trityl derivatives were similar, while much greater retention of the αCD trityl derivatives was observed. Table 1 lists the capacity factors k' which are directly calculable from the chromatogram in Fig. 1. The αCD trityl derivatives have higher k' values. The amount of glucose constituting CD is smaller, and the effect of hydrophobic-substituted groups contributes greatly to retention.

Table 1
Retention of $6^1,6^n$-di-*O*-trityl-CDs

Compound	Capacity factor, k'			
	$6^1,6^5$-	$6^1,6^4$-	$6^1,6^3$-	$6^1,6^2$-
Tr_2-αCD		8.77 (**4**)	14.00 (**3**)	11.79 (**2**)
Tr_2-βCD		1.66 (**11**)	2.85 (**10**)	2.42 (**9**)
Tr_2-γCD	1.00 (**19**)	1.42 (**18**)	3.32 (**17**)	3.58 (**16**)

The elution order of the three positional isomers of di-*O*-trityl-αCDs and -βCDs was $6^1,6^4$- (**4**, **11**), $6^1,6^2$- (**2**, **9**), and $6^1,6^3$- (**3**, **10**) derivatives, and that of di-*O*-trityl-γCDs having four positional isomers was $6^1,6^5$- (**19**), $6^1,6^4$- (**18**), $6^1,6^3$- (**17**) and $6^1,6^2$- (**16**) derivatives (Fig. 1). In general, the farther apart the binding positions of the two trityl groups were on the CD, the less the retention tended to be.

In reversed-phase chromatography, the retention decreases for isomers with hydrophilic substituent group bonds close to the hydrophobic functional group [15–24]. For example, with the alkyl alcohol, the retention was so small as to link the hydroxyl group in the vicinity of the molecular center (Table 2). The hydroxyl group lowered the surrounding hydrophobicity, that is, the hydrophilic effect of the hydroxyl group was larger when there was linking with the molecular center rather than the molecular edge. Similarly, for CD derivatives with binding

Table 2
Retention of alkyl alcohols on silica C_{18} in 80% methanol

Alkyl alcohol	Capacity factor, k'
CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-OH	12.0
CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH(OH)-CH_3	10.6
CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH(OH)-CH_2-CH_3	9.9
CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH(OH)-CH_2-CH_2-CH_3	9.3
CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH(OH)-CH_2-CH_2-CH_2-CH_3	9.0
CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH(OH)-CH_2-CH_2-CH_2-CH_2-CH_3	8.8
CH_3-CH_2-CH_2-CH_2-CH_2-CH(OH)-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3	8.8

Table 3
Retention of the isomer of hydrocarbons on silica C_{18} in 70% methanol

Hydrocarbon	Capacity factor, k'
CH_3-CH_2-CH_2-CH_2-CH_2-CH_3	14.1
CH_3-CH(CH_3)-CH_2-CH_2-CH_3	12.7
CH_3-CH_2-CH(CH_3)-CH_2-CH_3	12.3
CH_3-CH(CH_3)-CH(CH_3)-CH_3	11.1
CH_3-C(CH_3)(CH_3)-CH_2-CH_3	10.6

positions of two trityl groups on the CD ring farther apart from each other, the trityl groups were hydrated by the hydrophilicity of the hydroxyl groups on glucose units around the trityl groups, leading to less hydrophobicity of the molecule as a whole and thus less retention.

Isomers with two trityl groups on the CD ring in adjacent binding positions do not display a more hydrophilic effect due to the hydroxyl group, and the retention increases as the binding positions of the two trityl groups become farther apart. However if two hydrophobic-substituted groups are brought very close together, they overlap with each other, and the effective hydrophobic surface area displaying hydrophobic interaction with the C_{18} decreases, while the retention decreases. This behavior has been widely observed in reversed-phase chromatography [15–24]. For example, for hydrocarbon isomers with the same molecular mass as shown in Table 3, a decrease in surface area due to their round shape accompanied by an increase in the number of branches, can decrease retention.

We found that dityrityl-αCD and -βCD derivatives, 6^1,6^4- isomers (**4** and **11**) having two trityl groups on the CD ring at binding positions far apart were eluted first, followed by 6^1,6^2- isomers (**2** and **9**) for which the hydrophobic surface area was decreased by overlapping of two substituted groups. As a result, the retention times of 6^1,6^3- isomers (**3** and **10**) were long when there was overlapping of the substituted groups and the contribution from hydroxyl groups was smallest. As for dityrityl-γCDs, though the retention mechanism was basically almost the same as for αCD and βCD trityl derivatives, the retention times of the 6^1,6^2- isomer (**16**) and the 6^1,6^3- isomer (**17**) were reversed. Thus, slight differences in distances between two trityl groups accompanied by

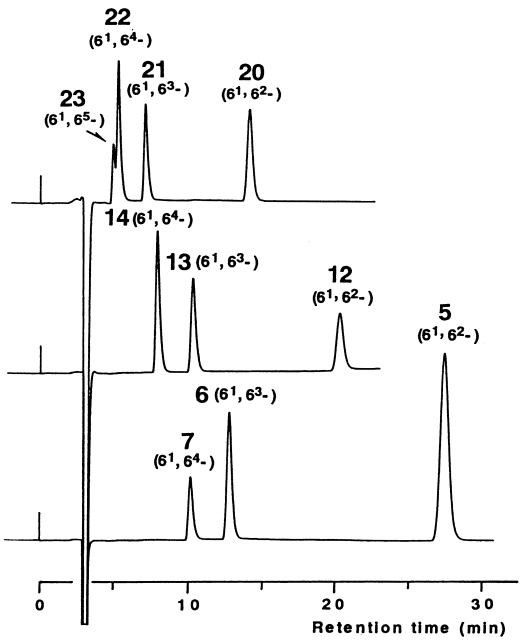


Fig. 2. Elution profiles of three and four positional isomers of 6^1,6^n-di-(*tert.*-butyldimethylsilyl)-cyclomaltohexaoses (cG_6s, αCD) (n=2–4, **5**–**7**), -cyclomaltoheptaoses (cG_7s, βCD) (n=2–4, **12**–**14**), and -cyclomaltooctaoses (cG_8s, γCD) (n=2–5, **20**–**23**). Chromatographic conditions as in Fig. 1.

Table 4
Retention of $6^1,6^n$-di-*O*-*tert*.-$BuMe_2Si$-CDs

Compound	Capacity factor, k'			
	$6^1,6^5$-	$6^1,6^4$-	$6^1,6^3$-	$6^1,6^2$-
(*tert*.-$BuMe_2Si)_2$-αCD		2.30 (**7**)	3.13 (**6**)	7.90 (**5**)
(*tert*.-$BuMe_2Si)_2$-βCD		1.52 (**14**)	2.29 (**13**)	5.55 (**12**)
(*tert*.-$BuMe_2Si)_2$-γCD	0.58 (**23**)	0.68 (**22**)	1.28 (**21**)	3.58 (**20**)

a difference in the number of glucose units constituting the CD seem to contribute to reversing the retention times.

The elution profiles of three and four positional isomers of $6^1,6^n$- di-*O*-*tert*.-$BuMe_2Si$-αCDs, -βCDs and -γCDs under the same conditions on an ODS column with methanol–water are shown in Fig. 2. In all these cases, the retention order of $6^1,6^5$-, $6^1,6^4$-, $6^1,6^3$- and $6^1,6^2$- derivatives and the elution patterns were almost the same (Table 4). The *tert*.-butyldimethylsilyl group was found to be a sterically more compact substituted group compared with the trityl group, and its elution profile was easy to understand and was as expected. In the $6^1,6^2$- isomer, there was no decrease of the hydrophobic surface area by overlapping of two substituted groups.

4. Conclusions

CDs with two hydrophobic-substituted groups bonded to hydroxyl groups tend to show low retention of positional isomers in which the binding positions of the two substituted groups on the CD ring are far apart from each other.

Studies on the correlation between the retention on an ODS column and the structures of evident positional isomers of CD derivatives should throw light on new possibilities for CD use. It would be useful to be able to predict the unknown structures of CD derivatives from their retention behavior, establish the optimum separation conditions for isolating new CD derivatives from a mixture, and design useful new CD derivatives based on their retention behavior.

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