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Carbohydrate Research 266 (1995) 75–80

CARBOHYDRATE RESEARCH

Regioselectivity of alkylation of cyclomaltoheptaose (β-cyclodextrin) and synthesis of its mono-2-*O*-methyl, -ethyl, -allyl, and -propyl derivatives

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Received 7 February 1994; accepted 10 June 1994

Abstract

Mono-2-*O*-methyl-, -2-*O*-ethyl-, and -2-*O*-allyl-cyclomaltoheptaose were prepared by alkylations of cyclomaltoheptaose in dilute aqueous alkali, and mono-2-*O*-propylcyclomaltoheptaose was obtained by hydrogenation of the allyl derivative. All the 2-*O*-alkyl derivatives were less soluble in water than was cyclomaltoheptaose. All formed inclusion complexes with toluene in aqueous solution, but only the methyl ether was less soluble in the water–toluene system than in water. The solubilities of the other ethers in water were enhanced by the addition of toluene. Partial methylation of cyclomaltoheptaose with ^{13}C-enriched dimethyl sulfate in dilute aqueous alkali yielded mixtures of products. The substitution patterns were analyzed by GLC–MS of the alditol acetates, prepared by hydrolysis, reduction, and acetylation, and by ^{13}C NMR after complete permethylation with nonenriched reagent. The results showed that methylation at O-2 is a predominant but not an exclusive reaction; as expected, the regioselectivity decreases with increasing degree of methylation.

Keywords: Cyclomaltoheptaose (β-cyclodextrin), derivatives of; Cyclodextrin, β- (cyclomaltoheptaose); Synthesis

1. Introduction

Previously we demonstrated that the distribution of substituents on partial alkylation of cyclomaltoheptaose (β-CD) with oxiranes depends upon the alkali concentration in the

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SSDI 0008-6215(94)00251-7

aqueous reaction medium [1,2]. High and low alkali concentrations favor 6-*O* and 2-*O* alkylation respectively, as might be expected on the basis of the relative steric accessibility and acidity of the two hydroxyls [1,2]. When only a limited amount of base was present, β-CD was also found to be alkylated mainly in the O-2 position in dimethylformamide [3], but when an excess of base in dimethyl sulfoxide was used, methylation first occurred on O-6 [4]. Similarly, pyridine as a solvent favored reaction of β-CD with 1-(1-naphthyl)ethyl isocyanate on O-6, while in dimethylformamide O-2 reacted preferentially [5]. Several 2-(2-hydroxyalkyl) and 2-(2,3-dihydroxyalkyl) ethers of β-CD have lower solubilities than β-CD in water, but their inclusion complexes with toluene are more soluble in water than the complex of β-CD; this facilitated the isolation of these ethers from complex mixtures [6,7]. It was also demonstrated by X-ray crystallography that in the crystals of 2-*O*-[(*S*)-2-hydroxypropyl]-β-cyclodextrin each substituent is inserted into the adjacent molecule in the crystal lattice, which is the same lattice as that of β-CD [8]. This explains the low solubility of the 2-*O*-alkyl derivatives in water, as well as the higher water solubility of their inclusion complexes with toluene. We now report on the synthesis of the 2-methyl, 2-ethyl, 2-propyl, and 2-allyl monoethers of β-CD, and on the solubilities of these compounds. We also investigated the distribution of the substituents in partially methylated β-CD.

2. Results and discussion

In the preparation of the mono-2-*O*-alkyl derivatives, β-CD in 0.37 M aqueous sodium hydroxide was treated with dimethyl sulfate, diethyl sulfate, or allyl bromide. We isolated the products by fractional recrystallization from water–toluene, followed by chromatography on silica gel, and finally by recrystallization from water. In the preparation of the 2-methyl ether, which was the most difficult to purify, we also had to use chromatography of the per-*O*-acetylated derivative. Direct chromatography of mixtures without recrystallization produced only mixtures, probably of mono-*O*-alkyl regioisomers. The position of the substituents in the above compounds was ultimately confirmed by X-ray crystallography, partial results of which have already been published [9]. The 2-propyl ether was prepared by hydrogenation of the allyl ether.

We determined the solubilities of the 2-alkyl monoethers in water and in water–toluene as for the corresponding 2-(2-hydroxyalkyl) ethers [6]; these are summarized in Table 1. All the higher ethers have lower solubilities in water than β-CD, but considerably higher solubilities in water–toluene. Only β-CD and its 2-methyl ether produced stable crystalline complexes with toluene, and it is only for these two substances that the solubility in water is higher than in water–toluene. This probably reflects the less efficient inclusion of the small methyl group in the crystal lattice of this compound.

To determine the distribution of substituents among the 2-, 3-, and 6-positions, we did methylation experiments involving isotopic enrichment by ^{13}C. In each of these experiments we used the same amount of ^{13}C-enriched dimethyl sulfate, either as furnished or after dilution with nonenriched reagent to methylate β-CD; the molar ratios of dimethyl sulfate to carbohydrate in these experiments were 0.5, 1, 3.5, and 7, respectively. The products contained from mono- to hexa-methylated cyclomaltoheptaose, as shown by a thin-layer

Table 1
Solubilities of mono-2-*O*-alkylcyclomaltoheptaoses in water and in water saturated with toluene

Alkyl group	Solubility (%)	
	Water	Water–toluene
None	1.80	0.2
Methyl	1.70	1.25
Ethyl	0.53	0.93
Allyl	1.10	1.94
Propyl	0.67	1.29
(*S*)-2-Hydroxypropyl [a]	0.32	1.29

[a] Ref. [7]

chromatogram, which only separates by different degrees of substitution; we checked the result by mass spectrometry [10].

Determination of the distribution of substituents in the above four mixtures was made by hydrolysis, borohydride reduction, acetylation, and GLC–MS of the derived alditol acetates. The results are presented in Table 2. They show that the optimal regioselectivity in favor of O-2 occurred when the smallest molar ratio of dimethyl sulfate to cyclomaltoheptaose (0.5) was used and when the monosubstituted product predominated; nevertheless even then, 3-*O*- and 6-*O*-methyl derivatives were present. As the molar amount of alkylating agent increased, the regioselectivity declined, as one might expect on basis of the decrease in the number of available sites. The easy preparation of mono-2-*O*-alkyl derivatives, even though all three isomeric monosubstituted cyclomaltoheptaoses are always formed, is due to the combination of regioselectivity and the tendency of those derivatives to crystallize.

In the next experiment the partially methylated cyclomaltoheptaoses were subjected to exhaustive methylation with nonenriched methyl iodide. The ^{13}C NMR spectra of the permethylated samples were simple and mutually identical except for the different intensities of the methyl signals. These intensities reflected the manner in which ^{13}C-methyl was incorporated; the results (not shown here) were in agreement with those obtained by the GLC–MS method.

Table 2
Distribution of substituents in ^{13}C-methylation experiments

Methylated residue	Mole fractions at the indicated $(MeO)_2SO_2$–β-CD ratios			
	0.5	1	3.5	7
Glc	93.5	87.1	71.9	63.4
2-*O*-Me-Glc	5.2	9.5	13.7	15.1
3-*O*-Me-Glc	1.0	2.3	7.0	8.9
6-*O*-Me-Glc	0.4	1.2	4.8	6.8
2,3-Di-*O*-Me-Glc			1.7	2.9
2,6-Di-*O*-Me-Glc			1.0	1.9
3,6-Di-*O*-Me-Glc				1.1

3. Experimental

General methods.—We performed TLC on Kieselgel 60 F_{254} (E. Merck), using 6:1:3:1 1-propanol–EtOAc–H_2O–concd aq NH_3 as eluent, and developed the spots by briefly immersing the plate in Vaugh's reagent [1 g $Ce(SO_4)_2$, 24 g $(NH_4)_2MoO_4$, 50 mL concd H_2SO_4, 450 mL H_2O], then heating it moderately. Preparative column chromatography was performed using the system 6:1:3:1 1-propanol–toluene–H_2O–concd aq NH_3 for the separation of partially alkylated cyclodextrins, and $CHCl_3$–MeOH systems for separation of per-*O*-acetates. Solubilities were determined by the evaporation of a defined weight of a saturated solution (24°C) and weighing the dried residue. In a water–toluene system, 10% v/v of toluene was used. (^{13}C)Dimethyl sulfate (MSD Isotopes, Montreal, Canada) had 99.4% enrichment. ^{13}C NMR spectra were recorded at 90 MHz and ambient temperature in $CDCl_3$, using tetramethylsilane as an internal standard. Mass spectra were recorded with a ^{252}Cf mass spectrometer, constructed for the National Heart, Blood, and Lung Institute (NHBLI) of the National Institutes of Health by Dr. R.D. Macfarlane.

Analyses of partially alkylated β-CD.—The alkylated product was hydrolyzed, the resulting sugars were transformed into alditol acetates, and the mixtures were analyzed by GLC (flame ionization detector) and GLC–MS, as described previously for alkylated, fully methylated β-CD [1]. Relative molar response factors [11] were used to calculate the proportions of components.

Synthesis of mono-2-O-methyl-, mono-2-O-ethyl-, and mono-2-O-allyl-β-CD.—Dimethyl sulfate (2.7 mL, 29 mmol), diethyl sulfate (1.9 mL, 14 mmol), or allyl bromide (1.56 mL, 18 mmol) was added to a solution of β-CD hydrate (47 g, 36 mmol) in aq 0.37 M NaOH (180 mL, 66 mmol), and the mixture was stirred for 10 h at 0°C, then for 10 h at 24°C. The reactions were followed by TLC. The R_f values of the monomethyl, monoethyl, and monoallyl derivatives were 0.24, 0.26, and 0.36, respectively. The mixture was neutralized with H_2SO_4, toluene (15 mL) was added, the mixture was stirred at 0°C for 4 h, and the precipitate was collected.

The 2-alkyl ethers were enriched by fractional crystallizations from water or water–toluene. TLC was used to monitor the phase in which the desired substance was enriched; only that phase was then used in the next purification step.

In four recrystallizations of the methylated product from water–toluene (150 mL), the 2-*O*-methyl derivative was enriched in the crystalline phase. Then we performed the following recrystallizations: from water–toluene (500 mL), mother liquor collected; twice from water (20 and 16 mL), crystals collected; from water–toluene (150 mL), mother liquor collected; and from water (4 mL), crystals collected. The product (1.15 g), still containing traces of β-CD was converted to its per-*O*-acetate by treatment with pyridine (10 mL) and acetic anhydride (5 mL). The mixture was heated for 24 h at 80°C, then evaporated to dryness, and the residue was subjected to three additions and evaporations of toluene (50 mL). The resulting solid was chromatographed on a silica gel column (200 g) using 100:1 $CHCl_3$–MeOH. Deacetylation of the obtained per-*O*-acetate by the Zemplén [12] method and recrystallization from water yielded pure mono-2-*O*-methyl-β-CD (450 mg); MS: *m/z* 1172 $(M+Na)^+$. Anal. Calcd for $C_{43}H_{72}O_{35}\cdot 9H_2O$ (1311.2): C, 39.3; H 6.92. Found: C, 39.4; H, 6.95. The structure was confirmed by X-ray analysis [9]; the

hydration obtained from elemental analysis and that determined by crystallography were in approximate agreement.

The crude mixture after ethylation was recrystallized once from toluene–water (150 mL), with collection of the mother liquor, then twice from water (6 mL), with collection of the crystals. The product (1.37 g), in addition to the 2-*O*-ethyl derivative, still contained traces of β-CD and of disubstituted β-CD. Part of this material (1.00 g) was fractionated on a silica gel column (80 g) and recrystallized from water to yield pure mono-2-*O*-ethyl-β-CD (580 mg); MS: *m/z* 1186 $(M+Na)^+$. Anal. Calcd for $C_{44}H_{74}O_{35}\cdot 9H_2O$ (1325.2): C, 39.9; H, 7.00. Found: C, 40.0; H, 7.06. The structure was confirmed by X-ray analysis [9].

The precipitate after the allylation reaction was recrystallized from water–toluene (800 mL), with collection of the mother liquor. Evaporation of the mother liquor yielded a crystalline solid (13.3 g). Part of that solid (4 g) was chromatographed on a silica gel column (200 g) and the main fraction from the column was recrystallized from water (6 mL) to give pure mono-2-*O*-allyl-β-CD (1.1 g, corresponding to 9.5% overall yield); MS: *m/z* 1198 $(M+Na)^+$. Anal. Calcd for $C_{45}H_{74}O_{35}\cdot 8H_2O$ (1319.2): C, 41.0; H, 6.88. Found: C, 41.0; H, 6.90. The structure was confirmed by X-ray analysis [9].

Small-scale isolations of monoalkyl ethers from mixtures evaporated to dryness (800 mg of the obtained solid used) were performed by chromatography on a silica gel column (80 g). Isolated yields of mixtures of mono-*O*-alkyl-β-CDs (calculated on the basis of the alkylation reagent used) were *O*-methyl, 15%; *O*-ethyl, 16%; and *O*-allyl, 17%.

Mono-2-O-propyl-β-CD.—2-*O*-Allyl-β-CD (200 mg) in water (20 mL) was hydrogenated at 150 kPa over 5% Pd–C (50 mg) for 6 h at 24°C. The product, containing some β-CD because of partial hydrogenolysis of the *O*-allyl group, was fractionated on a silica gel column to yield pure mono-2-*O*-propyl-β-CD (110 mg); MS: *m/z* 1200 $(M+Na)^+$. Anal. Calcd for $C_{45}H_{76}O_{35}\cdot 8H_2O$ (1177.1): C, 40.91; H, 7.02. Found: C, 40.74; H, 7.02.

^{13}C-Methylation experiments.—β-CD hydrate (4.0 g, 3.2 mmol) was dissolved in aq NaOH (0.4 M, 15, 20, 70, and 140 mL, respectively) and the solutions were cooled to 0°C. Under stirring, mixtures of ^{13}C-enriched dimethyl sulfate (0.15 mL in all cases) and nonenriched dimethyl sulfate (0, 0.3, 0.9, and 1.95 mL, respectively) were added. The mixtures were then stirred for 10 h at 0°C and 12 h at 24°C. The solutions were neutralized with H_2SO_4 (2 M), concentrated to a volume of 40 mL, and freeze-dried. One fourth of the solid from each experiment was per-*O*-methylated by the following procedure [13]: to the vigorously stirred suspension of partially methylated sample and powdered NaOH (4 g) in dimethyl sulfoxide (50 mL), methyl iodide (20 mL) was slowly added. After a day of stirring water (100 mL) and chloroform (100 mL) were added. The mixture was stirred for an additional hour, and the chloroform layer was separated, washed three times with water (800 mL), and dried over sodium sulfate, filtered, and concentrated to dryness. The obtained solid was submitted to the same permethylation procedure for a second time. The ^{13}C NMR spectra of these permethylated samples were then recorded. The peaks of the three methyl carbons at positions 2, 3, and 6 are readily distinguished in the ^{13}C NMR spectra (58.51, 61.48, and 58.97 ppm, respectively); they were unambiguously attributed previously [14,15].

Acknowledgements

We thank Dr. Henry M. Fales (NHBLI) for measuring the mass spectra and Dr. David R. Miller (Spectral Data Services Inc., Champaign, IL) for the NMR measurements. Pre-

liminary experiments on preparing pure mono-2-*O*-alkyl derivative were performed by Dr. C.T. Rao; we thank him for his efforts.

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