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ALKYLATION OF CYCLOMALTO-OLIGOSACCHARIDES (CYCLODEXTRINS) WITH DIALKYL SULFATE–BARIUM HYDROXIDE: HETEROGENEITY OF PRODUCTS AND THE MARKED EFFECT OF THE SIZE OF THE MACROCYCLE

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ABSTRACT

The alkylation of cyclomalto-oligosaccharides (cyclodextrins, CDs) with dialkyl sulfate–barium hydroxide has been claimed to yield 2,6-di-*O*-alkyl derivatives. Re-investigation by plasma desorption-m.s. of the products of laboratory methylation of αCD, βCD, or γCD and ethylation of βCD and several commercial preparations revealed them to be mixtures with broad and roughly symmetrical distributions of the degree of substitution. Recrystallization separated the components only partially. Analysis of the product of methylation of a mixture of CDs established the order of reactivity $\gamma \gg \alpha \geq \beta$. The reactivity of γCD thus resembles that of amylose.

INTRODUCTION

Partial substitution reactions on cyclomalto-oligosaccharides (cyclodextrins, CDs) usually yield mixtures of products, but the Kuhn–Trischmann method[1] was claimed[2,3] to yield 2,6-di-*O*-alkylated products. The technique was then used extensively[4–8] to prepare similar compounds. Eventually, these alkyl derivatives gained acceptance in biomedical applications[9,10] and one of them, heptakis(2,6-di-*O*-methyl)cyclomaltoheptaose, is now available commercially. The technical importance of alkylated CDs and the difference between the results of Kuhn–Trischmann alkylation and other reactions prompted a re-investigation. During this

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work*, a product of the Kuhn–Trischmann methylation of cyclomaltoheptaose (βCD) was also investigated by chromatography and found to contain two major and four minor components, three of which were fully characterized[11]. N.m.r. spectroscopy of this product revealed only 65% of the expected compound and chromatography of the benzoylated derivative was required for its isolation[12].

EXPERIMENTAL

Preparations. — The Kuhn–Trischmann procedure[1] involves dissolution of the carbohydrate in methyl sulfoxide, the addition of 1 vol. of *N,N*-dimethylformamide, followed by barium oxide and barium hydroxide hexahydrate. Dialkyl sulfate is added and reaction is allowed to proceed at room temperature for 2 days. After decomposition of the mixture with aqueous ammonia, the products are extracted into chloroform and precipitated by the addition of light petroleum. The yields of crude products are in the range 60–90%. There have been various modifications of reaction conditions[4–8] and, in this work, the conditions described in ref. 3 were used with the molar ratios 1:20:3.9 for glucose residue, barium bases, and dialkyl sulfate. Samples of the commercial preparations examined were obtained prior to the summer of 1987.

Mass spectrometry. — A ^{252}Cf plasma desorption mass spectrometer was used, which was constructed by Dr. R. D. Macfarlane (Texas A and M University, College Station, TX) for N.H.L.B.I. No derivatization was needed. Solutions in methanol or other suitable solvent were electrosprayed or otherwise deposited as a thin film on a window and introduced into the vacuum chamber. Ions are formed by attachment of adventitious alkali (usually sodium) cations to the carbohydrate and subsequent desorption through the effects of fission fragments of ^{252}Cf. In the few instances where peaks belonging to the potassium species were also observed, the sums of sodium and potassium peaks were used for quantification.

In this mode of mass spectrometry, every effective radioactive disintegration of ^{252}Cf leads to ionization in the sample and simultaneously starts the clock of the detector; the masses of the ions formed are measured on the basis of their time-of-flight to the detector. Radioactive disintegration occurs at about every ms and the spectra presented were accumulated for 120–540 min, which resulted in ~2000 counts/channel in the area of $(M + Na)^+$ while the noise level was ~10 counts/channel. The resulting signal-to-noise ratio of ~200 indicates the signal to be significant. Furthermore, closely related substances are expected to provide about the same intensities of the $(M + Na)^+$ ions. The method determines only the content of the surface layer of molecules. A problem in the m.s. of *O*-alkylated carbohydrates is the estimation of the extent to which fragmentation of $(M + Na)^+$ ions distorts the interpretation of the results. The difference between the $(M + Na)^+$

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TABLE I

COMPOSITION OF METHYLATED DERIVATIVES OF βCD

No.	*Preparation and purification procedure*	*Average d.s.*	*Number of methyl groups per βCD*											
			8	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*
			(% of content)											
1	2,6-Di-*O*-methyl-βCD (Kuhn–Trischmann method, recrystallized twice from water)	14.2				4	7	11	39	22	14	3		
2	2,6-Di-*O*-methyl-βCD (Aldrich Chemical Co., Inc.)	14.4	1	2	3	4	5	7	22	30	16	7	3	
3	2,6-Di-*O*-methyl-βCD (Toshin Chemical Co., Ltd.)	14.5						8	41	44	7			
4	2,6-Di-*O*-methyl-βCD (Chinoin Chem. Pharm. Works)	14.3		2	3	4	5	8	30	31	10	3	2	2
5	Partially methylated βCD "half 'n half": 2-*O*-64%, 3-*O*-43%, 6-*O*-99% (Sanraku Inc.)	14.3			3	3	6	6	37	26	12	5	2	

TABLE II

COMPOSITION OF ETHYL DERIVATIVES OF βCD

No.	*Preparation and purification procedure*	*Average d.s.*	*Number of ethyl groups of βCD*																
			4	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*
			(% of content)																	
1	2,6-Di-*O*-ethyl-βCD (Kuhn–Trischmann method, crude product)	12.8	1	2	2	3	3	4	5	8	11	16	24	7	3	3	2	2	3	1
2	2,6-Di-*O*-ethyl-βCD (Kuhn–Trischmann method, triturated with water)	13.5							4	8	8	18	44	14	4					

ion of the compound with one unsubstituted hydroxyl group (M − 14) and that from the compound methylated to the extent expected, and which lost a methyl group through fragmentation (M − 15), is just 1 mass unit: that is, <0.1% of the mass of the compounds in question. Furthermore, the considerable half-width (≤5 mass units) of bands in the area of interest may result in overlap or lead to composite bands with maxima between those of its components[13]. In order to evaluate these effects, mass spectra of two highly purified compounds, kindly provided by Dr. K. Koizumi[14,15], were investigated. Thus, each spectrum contained only four ions in the area of interest: heptakis(2,6-di-*O*-methyl)cyclomaltoheptaose, *m/z* 1376.6 (0.1), 1354.1 (1), 1339.1 (0.2), 1323.3 (0.2), baseline (0.02); hexakis(2,6-di-*O*-methyl)cyclomaltohexaose, *m/z* 1179.2 (0.2), 1163.1 (1), 1147.6 (0.3), 1131.9

(0.2), baseline (0.05). Corrections for baseline and overlap[13] yielded results suggesting that the fragmentation of the $(M + Na)^+$ species into a species interfering with analysis (*e.g.*, loss of methyl group) was $\geq 15\%$ of its intensity. Since all the differences of interest in the data in Tables I and II are significantly larger, the interpretation of data is not invalidated. Thus, for example, for the recrystallized preparation (Table I, No. 1), the signal at m/z of the species containing 13 methyl groups is more than three times higher than any signal near that position in the standard preparation. The data in Tables I and II were corrected only for baseline.

RESULTS

When the Kuhn–Trischmann alkylation procedure[3] was applied to cyclomalto-hexaose (αCD), -heptaose (βCD), and -octaose (γCD), the inhomogeneity of the crude products was shown by the mass spectra in Fig. 1, in which only species differing by molecular weight are recorded. The distributions of products seem to be symmetrical, suggesting that the reactivities of the hydroxyl groups do not differ greatly. Comparison of the distributions of products obtained from the three CDs

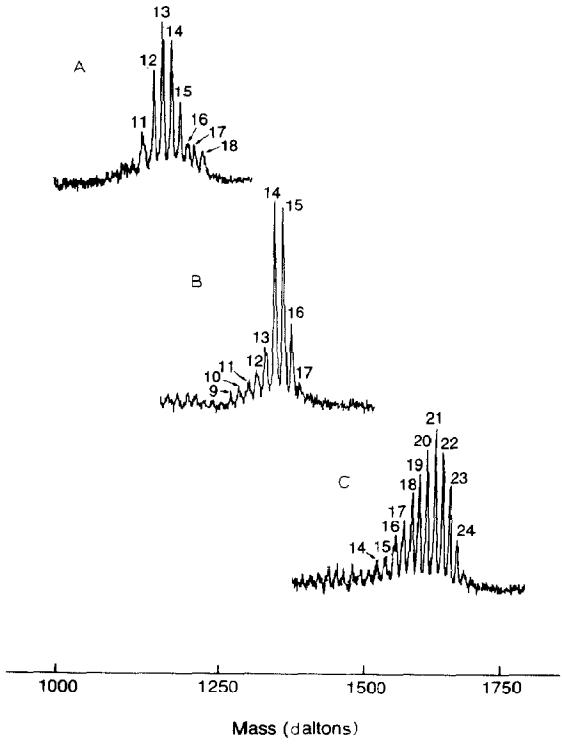

Fig. 1. Mass spectra of products of Kuhn–Trischmann methylation of A, αCD; B, βCD; C, γCD. The numbers above the peaks denote the number of methyl groups per CD. Crude products, obtained by precipitation of chloroform extracts with light petroleum, were used.

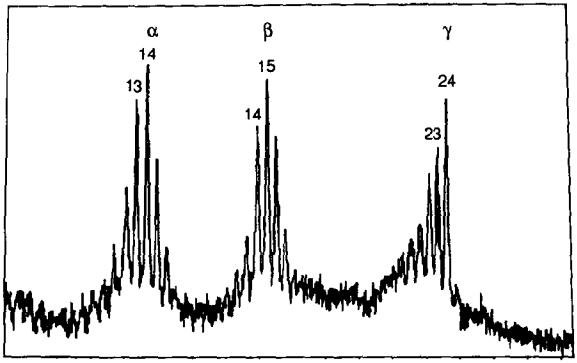

Fig. 2. Mass spectrum of the unfractionated product of Kuhn–Trischmann methylation of an equimolar mixture of αCD, βCD, and γCD. The numbers above the peaks denote the number of methyl groups per CD unit.

(Fig. 1) indicates some differential reactivity. Consequently, an equimolar mixture of the three CDs was methylated. The mass spectrum of the mixture of products (Fig. 2) showed that the average degree of substitution (d.s.) of αCD was 13.6 (*i.e.*, ~2.3 substituents per glucose residue) with a principal species with d.s. 14. For βCD, the corresponding values were 15.0 (*i.e.*, ~2.1 substituents per glucose residue), and 15, respectively. Both distributions were nearly symmetrical. In contrast, the principal species from γCD had d.s. 24, *i.e.*, the fully methylated derivative, and consequently the distribution was skewed toward this peak with an average d.s. of 22.4 (*i.e.*, ~2.8 substituents per glucose residue). Thus, the reactivity of γCD was higher than those of αCD and βCD, which differed only slightly.

Several related preparations of heptakis(2,6-di-*O*-methyl)cyclomaltoheptaose were examined and, with the exception of a preparation that had been purified by chromatography and served as a standard (see Experimental), all the other were heterogeneous (Table I). Preparation **1** was made by repeated recrystallization from water[9], but comparison of the results in Table I and Fig. 1 indicate that co-crystallization occurs. Recrystallization from methanol enriched the crystals in heptakis(2,6-di-*O*-methyl)cyclomaltoheptaose and in its under-methylated products, and the mother liquors were enriched in the over-methylated products (results not shown). Preparations **2–4** in Table I were commercial materials none of which was homogeneous. Preparation **5** of Sanraku, Inc., is referred to as "half 'n half"; its synthesis involved an alkali rather than alkali earth hydroxide, a process which resulted in full methylation of position 6, whereas positions 2 and 3 were only half-methylated. The solubility of **5** in water does not decrease with increase in temperature as does that of heptakis(2,6-dimethyl)cyclomaltoheptaose. The distribution found for preparation **5** is similar to those of the products obtained by Kuhn–Trischmann methylation.

The lack of individuality is not exclusive for methyl derivatives of CDs and was observed for products of ethylation of βCD (Table II).

DISCUSSION

The Kuhn–Trischmann alkylation procedure was originally developed for full methylation of starch[1] and when applied[2,3] to βCD was found to stop at the di-*O*-methyl stage. With αCD, a slightly higher d.s. (2.1–2.3 substituents per glucose residue) was achieved[3]. The present findings of symmetrical and broad distributions of substituents in each of the preparations examined suggest that the end point of this reaction is not as distinct as was supposed but is similar to that of other reactions, *e.g.*, the reaction of CDs with epoxides[16].

CDs differ in the reactivities of their hydroxyl groups. As expected, the hydrolysis of CDs, both chemical and enzymic, is affected by the size of the macrocycle[17]. However, it is surprising that the reactivities of the hydroxyl groups are affected. The difference in the reactivities of CDs in the described alkylation reaction ($\gamma \gg \alpha \geq \beta$) can hardly be due to differences between their pK_a values (12.3, 12.2, and 12.1, for αCD, βCD, and γCD, respectively[18,19]); rather, the recognition of the size of macrocycle by reactants may be the cause.

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