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Synthesis and characterization of cyclomaltoheptaose (*Z*)-2-butenedioic monoesters

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ABSTRACT

The reactions of cyclomaltoheptaose (β-cyclodextrin) and maleic anhydride were investigated, and the resulting products characterized by i.r., proton, and carbon-13 n.m.r. spectroscopy. The ^{13}C-n.m.r. spectra show preferential esterification at HO-6 under various conditions of temperature and concentration of reactants and of catalyst. An increase of temperature, catalyst concentration, or initial molar ratio of maleic anhydride to cyclodextrin increases the rate of esterification.

INTRODUCTION

Cyclodextrins (CDs) and their chemically modified derivatives have been extensively investigated for separation and other purposes[1,2]. It is well known that CDs possess a remarkable ability to include selectively a wide variety of organic and inorganic molecules or ions into their hydrophobic cavities. The inclusion process is reversible, that is the included compound may be easily recovered if a suitable displacer is employed. Hence, CDs have, potential use as media in chromatography and other separation processes. However, CDs are slightly soluble in aqueous solution, and require modification for use as solid adsorbents for selective recovery of compounds from aqueous solution. This may be achieved through one of the following reaction routes to form water-insoluble, CD-containing resins: (*a*) attachment of CD onto water-insoluble polymers, (*b*) reaction with cross-linking agents, and (*c*) modification of the CD to introduce other functional groups, such as C–C double bonds, amino, or carboxyl groups, which may be further modified by methods (*a*), (*b*), or polymerization.

The chemical modification of CDs is, in general, limited to the hydroxyl groups on C-2,3, and 6 of the glucopyranose residue, so that the central cavity is not disrupted. Furthermore, the location of modification is important in determining accessibility for

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the guest molecule. It has been shown that modification at O-6 is more desirable than elsewhere because the guest molecule has ready access from the wider, open side of the cavity surrounded by secondary hydroxyl groups.[3]

This paper presents synthesis and characterization of a series of new modified CDs, prepared by treating cyclomaltoheptaose (*β*-CD, **1**) with maleic anhydride (**2**) in the presence of *N,N*-dimethylbenzylamine. The modified CD contains C–C double bond(s) and carboxyl group(s). Such dual functional groups give more flexibility in the formulation of water-insoluble, *β*-CD-containing polymers. For example, the C–C double bond can be made to react with some monomers to form copolymers, and the carboxyl group may bond covalently with other functional groups. Potentially, this compound could serve as a raw material to produce CD-based membranes and resins for chemical separation purposes.[4]

EXPERIMENTAL

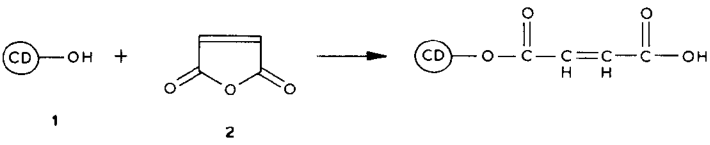
Materials. — Cyclomaltoheptaose (*β*-CD, **1**), obtained from the Anspec Company, was washed with cyclohexane several times to displace impurities included inside the CD cavity, and dried under vacuum for 24 h at 80° and then stored in a desiccator for routine use. Maleic anhydride (**2**), *N,N*-dimethylbenzylamine, and *N,N*-dimethylformamide (DMF) (Aldrich Chemical Company Fisher Scientific Company) were of reagent grade with purity $99^{+}\%$, and were used without further purification. A stock solution of 20mM NaOH was prepared using a mixed solution of tetrahydrofuran and water (4:1, v/v). The concentration of the solution was determined by back titration with potassium hydrogenphthalate.

Synthesis of cyclomaltoheptaose 6-[(Z)-2-butenedioic monoesters]. — In the general procedure, *β*-CD (**1**, 3.0 g) and maleic anhydride (**2**, 1.3 g) were added to a 250 mL three-necked round-bottom flask containing 100 g of DMF. The flask was heated in a water bath until the desired temperature was reached (25, 50, and 75°). The reaction was then initiated by addition of $PhCH_2NMe_2$ (0.15 g) and allowed to continue for 24 h. Aliquots were removed at given time intervals, diluted with 50 mL of distilled water, and then titrated with NaOH stock solution. A control reaction containing all reagents except **1** was also carried out. The final mixture was purified by evaporation with a rotary evaporator and then adding acetone to precipitate the products. The products were further washed 3 times with acetone and twice with cyclohexane, and then centrifuged at 6000 r.p.m. for 15 min and dried under vacuum overnight at 60°.

Identification. — I.r. spectra were recorded between 4000–600 cm^{-1} with a Beckman IR-33 spectrophotometer. ^{1}H- and ^{13}C-n.m.r. spectra were recorded on Nicolet NTC-470 and NTC-200 n.m.r. spectrometers operating at 470 and 50 MHz, respectively.

RESULTS AND DISCUSSION

Synthesis and kinetics. — The (*Z*)-2-butenedioic monoesters of *β*-CD (**1**) were formed by the reaction shown in the following scheme.



The proportion of carboxyl groups incorporated into **1** was determined by titration. The conversion is expressed as the total amount of ester linkages formed or the average number of ester linkages formed per CD molecule. The total amount of ester linkages formed is equal to the net change of the carboxyl groups in the hydrolyzed reaction-mixture before and after the reaction, because one carboxyl group is consumed in the formation of one ester linkage. The average number of ester linkages formed per CD molecule, $\bar{N}$, is determined by the following equation,

$$\bar{N} = \frac{[CO_2H]_{initial} - [CO_2H]_{final}}{[CD_0]_0} \qquad (1)$$

in which $[CD_0]_0$ is the initial concentration of **1**.

Fig. 1 shows time-course curves of the reaction with the initial molar ratio of **1** to **2** ($[2]_0/[CD_0]_0$) ranging from 2.5–7.5 at 75°. An increase in this ratio increases the number of ester linkages formed per CD molecule. Also, it may be seen that $\bar{N}$ levels off, even in the cases of high initial molar ratios of **2** to **1**, as the reaction proceeds. The final conversion of **2** drops from 80 to 55% when the initial molar ratio of **2** to **1** changes from 2.5 to 7.5. These observations suggest that increasing the number of (*Z*)-2-butenedioyl groups on the CD molecule decelerates the further esterification on the same molecule, possibly through steric hindrance.

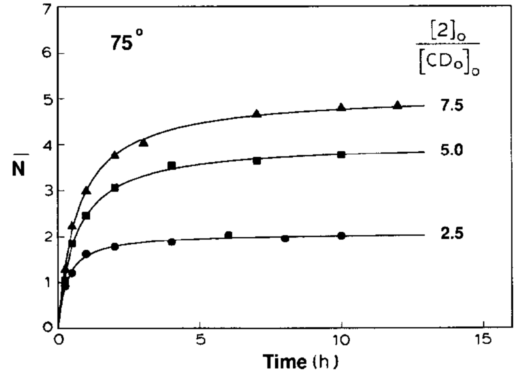


Fig. 1. Effect of the initial molar ratio of **2** to **1** on the average number of ester linkages ($\bar{N}$) formed in each CD molecule with $[CD_0]_0$ = 24mM and [*N*,*N*-dimethylbenzylamine] = 11mM.

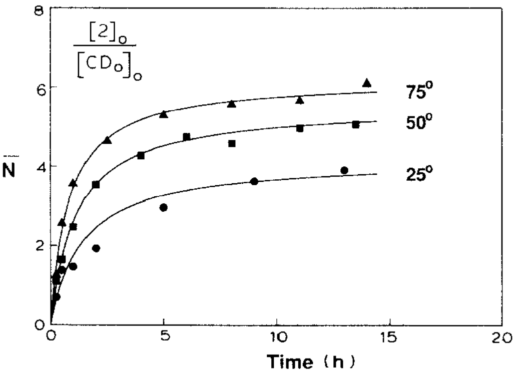


Fig. 2. Effect of temperature on the average number of ester linkages(N) formed in each CD molecule with $[CD_0]_0$ = 24mM and [N,N-dimethylbenzylamine] = 11mM.

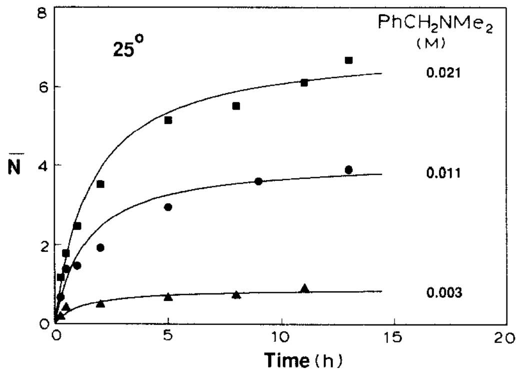


Fig. 3. Effect of catalyst on the average number of ester linkages ($\bar{N}$) formed in each CD molecule with $[CD_0]_0$ = 24 mM and **2** = 240mM.

Figures 2 and 3 give time-course curves of the reaction at various temperatures and catalyst concentrations, respectively. The use of $PhCH_2NMe_2$ has been shown[5] to be effective for catalyzing the reaction between hydroxyl groups and **2**, and this is also true in this reaction system. The increase of catalyst concentration increases the average number of ester linkages per CD molecule, indicating that $PhCH_2NMe_2$ is also effective in catalyzing multiple esterifications on the same CD molecule. An increase of temperature predictably increases the rate of reaction and the final conversion (Fig. 2).

Considering the reactions involved in the synthesis, we postulate that the process is consecutive, that is, the reacted CD serves as the precursor for the next modified CD. For example, **1** (CD_0) reacted with **2** to form a maleic monoester (CD_1) of the CD which could further react with **2** to form a bis(maleic monoester) (CD_2) of **1**, and so on. These reactions may be briefly expressed as:

$$CD_{i-1} + \mathbf{2} \rightarrow CD_i \tag{2}$$

in which the subscript "i" denotes the number of ester linkages formed in the same CD molecule. The actual average number of ester linkages formed through this reaction mechanism should be:

$$\bar{N}_a = \frac{\sum_{i=1}^{21} i[CD_i]}{[CD_o]_o} \qquad (3)$$

Theoretically, the product structures may be more complicated than those indicated, if the location of esterification on the cyclodextrin molecule is taken into consideration. The reactions involve a series of esterifications between **2** and 21 hydroxyl groups. The possible structural isomers increase greatly with increase in the number of ester linkages. However, the actual number of structural isomers in the products is lower because esterification occurs preferentially at O-6.

The use of $PhCH_2NMe_2$ favors the reactions already mentioned; however, some side reactions might occur when $\bar{N}_a$ is large and the reaction is prolonged. Possible side reactions are intermolecular and intramolecular esterifications between the second carboxyl group of the maleic acid and a hydroxyl group. In such cases, the value of $\bar{N}$ determined from Eq. *1* also takes into account the number of ester linkages resulting from these side reactions and, therefore, $\bar{N}_a$ should be smaller than $\bar{N}$.

Some kinetic information may be extracted from the time-course data, even though many reactions are involved in the synthesis. For example, the rate of formation of (*Z*)-2-butenedioic monoesters of **1** may be determined from the initial rate of reaction. Fig. 4 shows the effect of **2** on the rate of formation (r_1) of the monoesters. The dependence is of the Michaelis–Menten type, namely $r_1 \alpha [\mathbf{2}]/(K_m + [\mathbf{2}])$, instead of being linear. The dependence of catalyst concentration on the rate of formation of the monoesters may be obtained from Fig. 3, and they are linearly interrelated.

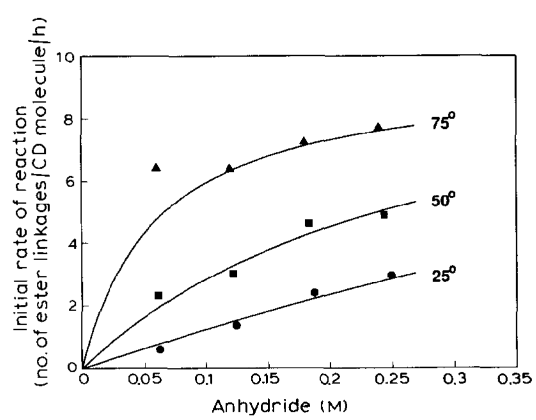


Fig. 4. The dependence of initial rate of reaction on maleic anhydride concentration and temperature with $[CD_0]_0$ = 24 mM and [*N*,*N*-dimethylbenzylamine] = 11 mM.

Structure characterization. — The i.r. spectra of the purified products clearly indicate the formation of (*Z*)-2-butenedioic monoesters of **1**. Absorption bands at 1700, 1645, and 1560 cm^{-1} correspond to the stretching vibrations of C–O of ester, C–O of carboxylic acid, and C–C, respectively. The unusually low frequencies of these absorption bands as compared with their normal absorption frequencies may be attributed to conjugation.[6]

Proton and ^{13}C-n.m.r. spectra of **1** and the monoesters (isolated after 4 h of reaction at 25°) are shown in Fig. 5 and 6, respectively. The vinylenic proton signals at 6.2 and 6.4 p.p.m. are assigned to protons adjacent to ester linkage and free acid,

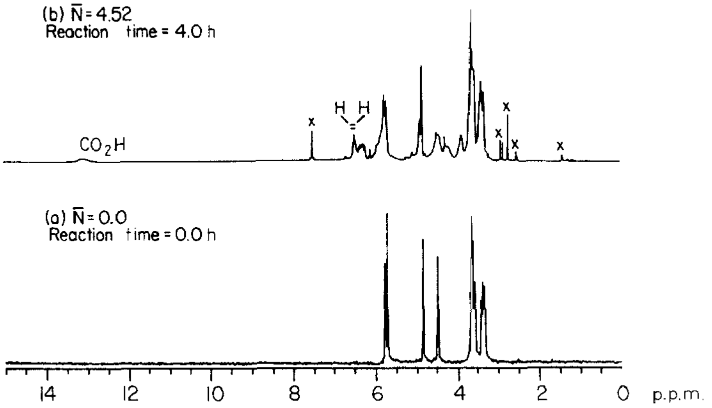


Fig. 5. Proton n.m.r. spectra of pure **1** and the (*Z*)-2-butenedioic monoester of **1** with [CD$_0$]$_0$ = 56 mM [**2**]$_0$ = 560mM, [*N*,*N*-dimethylbenzylamine] = 23.5 mM, and T = 25°. The signals marked x are due to residual solvents and reagents used during the synthesis and purification.

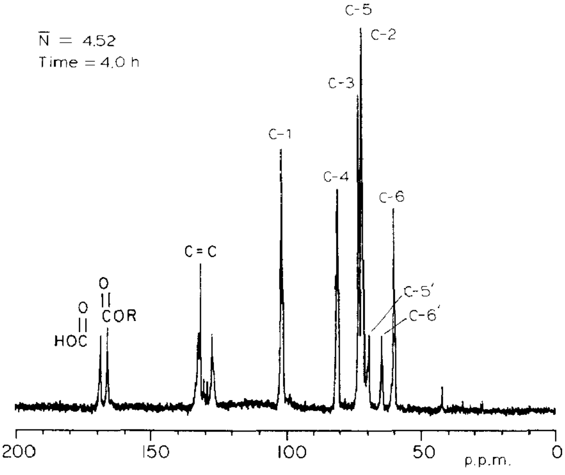


Fig. 6. The ^{13}C-n.m.r. spectrum of the (*Z*)-2-butenedioic monoester of **1** isolated after 4 h of reaction, with other reaction conditions as shown in Fig. 5.

respectively, and the carboxyl protons resonate as a broad signal at 13 p.p.m. The area of vinyl protons compared to the total protons of CD indicated 2.5 ester linkages per CD molecule.

Assignment of ^{13}C-n.m.r. spectra accords with literature data[7,8]. The resonance at 64.4 p.p.m. is assigned to C-6 carbon atoms bearing a substituted hydroxyl group by reference to the chemical shift of C-6 in **1**. Such a downfield shift of C-6 is consistent with the fact that the esterification of hydroxyl groups of cello-oligosaccharides and cellulose causes strong deshielding[9,10]. The relative areas of unsubstituted to substituted C-6 are 1.5:1.0, indicating a degree of substitution of 2.8 out of the 7 6-hydroxyl groups of **2**. This is in agreement with the number of ester linkages calculated from the ^{1}H-n.m.r. spectrum of the same sample.

It is also known that the esterification of a hydroxyl group of glucopyranosyl compounds causes an upfield shift of the resonance of adjacent carbon atoms[10]. No upfield signals correspond to C-1 and C-4, indicating that the esterification at O-2 and O-3 of the cyclodextrin is not as significant as that at O-6. However, a signal at 69.2 p.p.m. could be attributed to the signal of C-5 adjacent to esterified C-6 carbon atoms. The upfield shift of C-5 caused by esterification at C-6 is 2.6 p.p.m. The resonance at 168.7 p.p.m. is assigned to the C–O of carboxylic groups, and that at 166.1 p.p.m. to C–O of ester groups. The signal at 132 p.p.m. is assigned to a vinylenic carbon adjacent to the ester linkage. The vinylenic carbon adjacent to the free carboxylic group resonates at 127 p.p.m.

Esterification at O-2 and O-3 appears possible, but not favorable. ^{13}C-n.m.r. spectrum shows no significant esterification at O-2 and O-3 of **1** after 24 h of reaction at 25°. A similar observation has been made in the substitution of cellulose[9,11].

Preferential esterification at C-6 is desirable from the viewpoint of formation of inclusion complexes, because these modified CDs are then still able to access the guest molecule via the mouth of the cavity after they have been attached to water-insoluble resins or reacted with cross-linking agents.

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REFERENCES

1 W. L. Hinze, *Separation and Purification Methods*, 10 (1981) 159–237.
2 J. Szejtli, *Cyclodextrin Technology*, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1988.
3 D. W. Griffiths and M. L. Bender, *Adv. Catal.* 23 (1973) 209–261.
4 Z. Y. Shen, G. J. Tsai, Z. X. Chen, and G. T. Tsao, in N.N. Li (Ed.) *Proc. 2nd Intern. Conf. Separation Technol.*, (1987–1988), in press.
5 Z. Chen, X. Wei, T. Zhang, and Q. Zhang, *Chinese J. Appl. Chem.*, 1 (1984) 7–11.

6 R. M. Silverstein and G. C. Bassler, *Spectrometric Identification of Organic Compounds*, John Wiley & Sons, Inc., New York, 1974 pp. 73–119.
7 P. Colson, H. J. Jennings, and I. C. P. Smith, *J. Am. Chem. Soc.*, 96 (1974) 8081–8087.
8 A. Harada, M. Furue, and S. -I. Nozakura, *Macromolecules*, 9 (1976) 701–704.
9 T. Miyamoto, Y. Sato, Y. Shibata, and H. Inagaka, *J. Polym. Sci.*, 22 (1984) 2363–2370.
10 B. Capon, D. S. Rycroft, and J. W. Thomson, *Carbohydr. Res.*, 70 (1979) 145–149.
11 T. K. Wu, *Macromolecules*, 13 (1980) 74–79.