ISOLATION, PURIFICATION, AND CHARACTERIZATION OF CYCLOMALTODECAOSE (ε-CD)

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ABSTRACT

ε-Cyclodextrin (ε-CD) is a cyclic oligosaccharide, composed of ten α-1,4-linked D-glucoses reported by French *et al.* in 1965[1)], but has not been studied because of the difficulty in the preparation and purification of large-ring CDs composed of more than nine α-1,4-linked D-glucose units. This report describes the purification and characterization of ε-CD. Furthermore, the crystal and molecular structure of ε-CD hydrate (ε-CD $19H_2O$) was elucidated by X-ray analysis.

1. INTRODUCTION

We have already reported that one of the large-ring CDs, cyclomaltononaose(δ-CD), which is composed of nine α-1,4-linked D-glucose units, has a lower aqueous solubility than either α-CD or γ-CD[2)]. Large-ring CDs may have some unique characteristics in comparison with other conventional CDs. Furthermore, we have previously reported the isolation, purification, and characterization of η-CD (cyclomaltododecaose, composed of twelve α-1,4-linked D-glucose units)[3)], ζ-CD (cyclomaltoundecaose, composed of eleven α-1,4-linked D-glucose units) and θ-CD (cyclomaltotridecaose, composed of thirteen α-1,4-

linked D-glucose units)[4]. Here, we isolated and crystallized ε-CD (cyclomaltodecaose, composed of ten α-1,4-linked D-glucose units) as its hydrate form for the first time and elucidated its structure by X-ray analysis.

2. MATERIALS AND METHODS

2.1. Materials

CD powder(Dexypearl K-50) was purchased from Ensuiko Sugar Refining Co., Ltd. All other chemicals were commercial sources and used without further purification.

2.2. Purification method of ε-CD

The large-ring CDs mixture was prepared in the same way as described previously.[2,3] Purification of ε-CD from the large-ring CD mixture powder(ca. 240g) was mainly carried out by HPLC, and Fig.1 shows its flow chart. The fractions containing pure ε-CD were collected and concentrated by vacuum evaporation. A prismatic precipitate separated out quickly, and then it was recrystallized with acetonitrile-water (65:35) solution. The final product(ε-CD) was obtained in a yield of ca.160mg as a fine crystal powder.

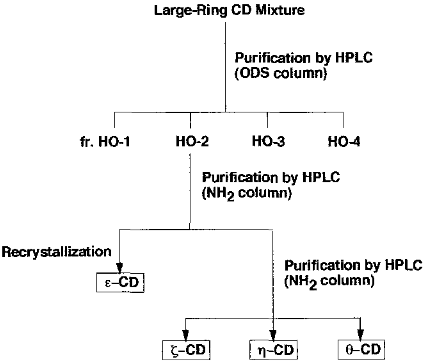


Figure 1. Purification Method of Large-Ring CDs.
ODS Column: Senshu Pak ODS-5251-SS,
Eluent: CH_3OH : H_20=6:100, Flow Rate: 6.0mL/min.
NH_2 Column: Asahipak NH2P-50,
Eluent: CH_3CN : H_2O=58:42, Flow Rate: 2.0mL/min.

2.3. X-ray analysis of ε-CD hydrate

Crystallization of ε-CD $19H_2O$ was performed by slow evaporation of acetonitrile-water (50:50) solution containing ε-CD at room temperature. A transparent colorless, prismatic crystal of 0.60 x 0.50 x 0.40 mm in size was used for the X-ray analysis. The crystal data are: $C_{60}H_{100}O_{50}$ $19H_2O$, F.W. 1962.3, Monoclinic, C2, Z=2, a=29.338(3), b=9.982(2), c=19.340(2)Å, β=121.025(6)°, V=4853(1)Å^3, D_{calc} 1.356 g/cm^3, μ=11.07 cm^{-1}. X-ray intensity data were measured on a Rigaku automatic four circle diffractometer (AFC-7R, Cu-K_α, λ=1.5418Å, ω-2θ scan with a 2θ<130.2°). In the structure determination by SIR88[5] and in the following refinements by full-matrix least-squares procedures 8746 independent reflections with $|F_0| > 3\sigma(|F_0|)$ were used. Hydrogen atoms were included but not refined. The final refinement was done by anisotropic temperature factors for oxygen and carbon atoms, and converged R to 0.108. All calculations were performed using a teXsan crystallographic software package of Molecular Structure Corporation.

3. RESULTS AND DISCUSSION

3.1. Identification of ε-CD

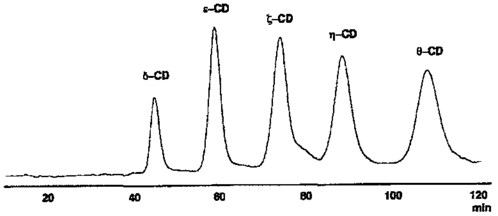


Figure 2. Elution Profile Large-Ring CDs on an Amino Column.
Column : Asahipak NH2P-50, Eluent : $CH_3CN:H_2O$=65:35
Flow rate : 0.7 mL/min

ε-CD was subjected to analytical HPLC columns. The purity of ε-CD was almost 100% by HPLC determinations. Fig.2 shows the HPLC chromatogram of large-ring CDs. The values of elution time increased with an increasing number of glucose units in the order of : δ-CD < ε-CD < ζ-CD < η-CD < θ-CD. In the mass spectra of ε-CD, high parent ion peaks appeared at 1621.7, corresponding to the molecular weight plus proton$(M+H)^+$. The molecular weight of ε-CD was determined to be 1620, and this value corresponded to that of ε-CD $\{(C_6H_{10}O_5)_{10}\}$. ^{1}H-NMR, ^{13}C-NMR ^{13}C -^{1}H COSY two dimensional NMR were observed with a JEOL GX-400 spectrometer(400 MHz). The peaks of H1, H3, H2 and H4 of ε-CD were recognized, but the peaks of H5 and H6 were not completely assigned by the ^{1}H -NMR spectrum. The ^{13}C -NMR spectrum of ε–CD had six clear single peaks and as Table 1 shows its chemical shifts originated from the acyclic structure of CD itself in a similar manner as the other CDs. The measurement of ^{13}C -^{1}H COSY two dimensional NMR also supported this finding; the same peaks attributed to a cyclic structure of conventional CD could be obtained in ε-CD(data not shown in this proceedings).

Table 1. ^{13}C-NMR Chemical Shifts of CDs.

Carbon	α–CD	β–CD	γ–CD	δ–CD	ε–CD
1	101.80	102.22	102.03	100.51	99.34
2	72.18	72.30	72.78	72.65	72.24
3	73.79	73.55	73.40	73.32	73.28
4	81.66	81.58	80.90	78.74	77.57
5	72.49	72.55	72.25	71.88	71.35
6	60.94	60.83	60.77	60.84	61.06

3.2. The crystal and molecular structure of ε-CD hydrate

Figs. 3 and 4 show the molecular structure of ε-CD. The overall shape of ε-CD is elliptic and its longer axis is parallel to a line from glucose unit G2 to G7 and shorter axis from G4 to G9 or from G3 to G8. The distance along the longer axis is about 12.58~14.02 Å(G1-O6•••O6-G6~G2-O2•••O2-G7), and that along the shorter axis is about 6.45~8.63 Å(G4-O6•••O6-G9~G3-O2•••O2-G8). Moreover, glucose units,G3,G4,G5,G8, G9 and G10 fall inside the ε-CD cavity. From the side view of ε-CD, the molecule takes a boat form or U character, that is, the glucosidic oxygen atoms(O4) connecting neighboring glucose unit by α-1,4-linkage,are not in a plane. All glucose units took a chair form. Bond lengths and angles of ε-CD are normal. In the ε-CD $19H_2O$, 19 water molecules are distributed over 20 positions, five in the cavity(6 water molecules), and fifteen in the interstices(13 water molecules).

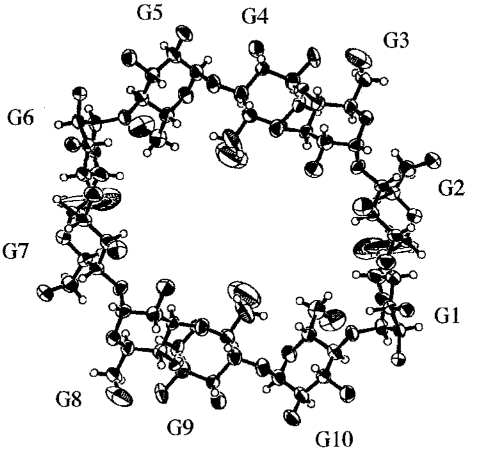
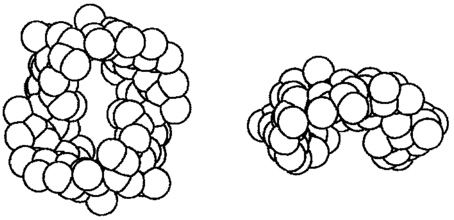


Figure 3. Molecular structure and numbering of ε-CD.



(a) (b)

Figure 4. Spacefilling drawing of ε-CD.
(a) view from O6 side
(b) side view

REFERENCE

[1] French, D., Pulley, A.O., Effenberger, J.A., Rougvie, M.A., Abdullah, M., *Arch. Biochem. Biophys.*, **111**, 153-160(1965).
[2] Miyazawa, I., Ueda, H., Nagase, H., Endo, T., Kobayashi, S., Nagai, T., *Eur. J. Pharm. Sci.*, **3**, 153-162(1995).
[3] Endo, T., Ueda, H., Kobayashi, S., Nagai, T., *Carbohydr. Res.*, **269**, 369-373(1995).
[4] Ueda, H., 6th Int.Seminar on Inclusion Compounds, Istanbul, TURKEY, August 1995, p 45.
[5] SIR88: Burla,M.C., Camail,M., Cascarano,G., Giacovazzo,C., Polidori,G., Viterbo,D., *J. Appl. Cryst.*, **22**, 389-303(1989).