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Carbohydrate Research 269 (1995) 369–373

CARBOHYDRATE RESEARCH

Note

Isolation, purification, and characterization of cyclomaltododecaose (η-cyclodextrin)

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Received 11 July 1994; accepted 24 October 1994

Keywords: Cyclomaltododecaose; η-Cyclodextrin (η-CD); Isolation; Purification

Because of difficulties in the isolation and purification of large-ring cyclomaltoses (cyclodextrins), little is known, except for the paper by French and co-workers in 1965 [1], about those that are composed of more than nine α-(1 → 4)-linked D-glucose units. This is in sharp contrast to α-CD, β-CD, γ-CD, and their derivatives for which much has been documented [2,3]. Frömming and Szejtli have noted that preparation of large-ring CDs would seem to be an unrewarding challenge on account of their expected high solubility in water and predicted weak complex-forming abilities [4]. However, 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 that either α-CD or γ-CD [5]. Large-ring CDs may have some unique characteristics in comparison with those of other conventional CDs. Even if large-ring CDs are not applicable for industrial useage, elucidation of their structures and physicochemical properties may provide useful information and serve as otherwise interesting developments in basic science. This report describes the isolation, purification, and characterization of η-CD, which is composed of twelve α-(1 → 4)-linked D-glucose units.

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SSDI 0008-6215(94)00359-9

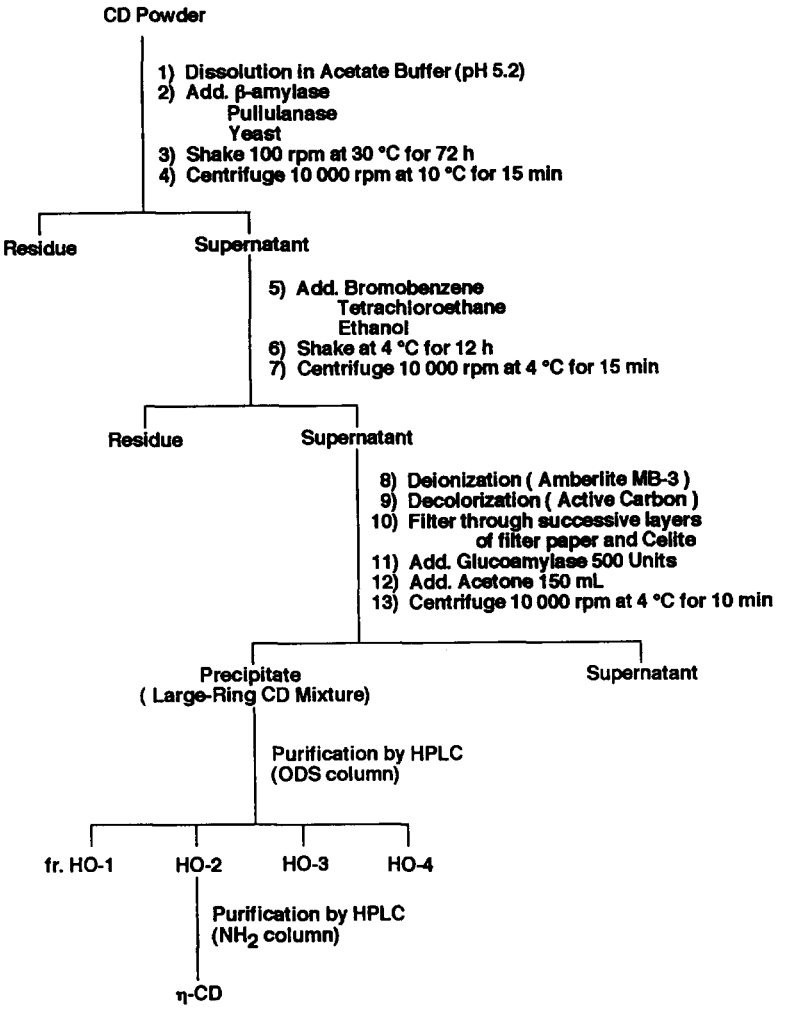

Fig. 1. Preparation and purification method for η-CD.

Large-ring CDs were prepared by a method developed by Kobayashi et al. [6]. The process is outlined in Fig. 1. The process was carried out as follows. Commercially available CD powder (Dexypearl K-50) was dissolved in acetate buffer (pH 5.2) and incubated at 30°C for 3 days with a mixture of β-amylase, pullulanase, and yeast. Through this process, dextrins and branched CDs in the Dexypearl K-50 underwent breakdown to D-glucose and CDs by an enzyme-catalyzed reaction with β-amylase and pullulanase, and the free glucose was digested by the yeast. Following centrifugation, the α-, β-, and γ-CDs were removed from the supernatant as insoluble complexes of α-CD with tetrachloroethane and β- and γ-CD with bromobenzene. Uncomplexed dextrins were precipitated by ethanol and removed by centrifugation, giving a supernatant that appeared to contain many kinds of large-ring CDs. The supernatant was

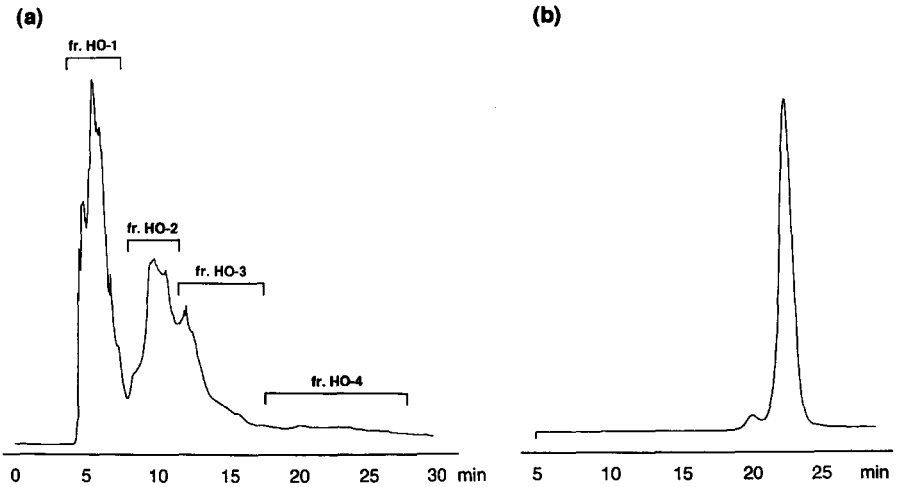

Fig. 2. HPLC chromatogram of (a) large-ring CDs and (b) η-CD. Conditions: (a) Column, Senshu Pak ODS-5251-SS; eluent, 6:100 CH_3OH–H_2O; flow rate, 6.0 mL/min. (b) Column, Asahipak NH2P-50; eluent, 58:42 CH_3CN–H_2O; flow rate, 0.7 mL/min.

subjected to deionization, decolorization, and hydrolysis using glucoamylase, and then the large-ring CD mixture was precipitated by acetone. To obtain pure η-CD, the precipitate consisting of large-ring CDs, was redissolved in water and was first roughly fractionated by high-performance liquid chromatography (HPLC) on an ODS (C_{18}) column (see the chromatogram in Fig. 2). Then fraction HO-2 was further purified by HPLC on an amino-bonded (NH_2) column. The fraction so obtained yielded about 10 mg ($\sim 0.01\%$) of η-CD that analyzed on the NH_2 column as $> 98\%$ pure [Fig. 2(b)]. Fast-atom bombardment mass spectrometry (FABMS) gave m/z 1945.6 $[M + H]^+$, which is in agreement with the calculated molecular weight of η-CD (MW = 1944).

Finally, the two-dimensional ^1H–^{13}C correlated (H,C COSY) NMR spectrum was measured (Fig. 3). Although most assignments could be made from the data, the free $-CH_2OH$ function could not be discerned. However, the ^{13}C NMR spectrum of η-CD showed six clear, distinct, single peaks at δ 63.25, 73.54, 74.27, 75.41, 80.59, and 101.94. The same peaks that have been attributed to the cyclic structures of the conventional CDs were obtained with η-CD (Table 1).

In this work we have established a purification method for η-CD from commercially available CD powder and have isolated η-CD as a freeze-dried powder. The existence of η-CD was confirmed by up-to-date analytical techniques, including HPLC, and one- and two-dimensional NMR spectroscopy. We suspect that large-ring CDs other than δ-CD and η-CD exist, and we intend to isolate and characterize them.

1. Experimental

Materials.—CD powder (Dexypearl K-50) was purchased from Ensuiko Sugar Refining Company, Ltd. (Yokohama, Japan). β-Amylase [α-(1 → 4)-glucan maltohy-

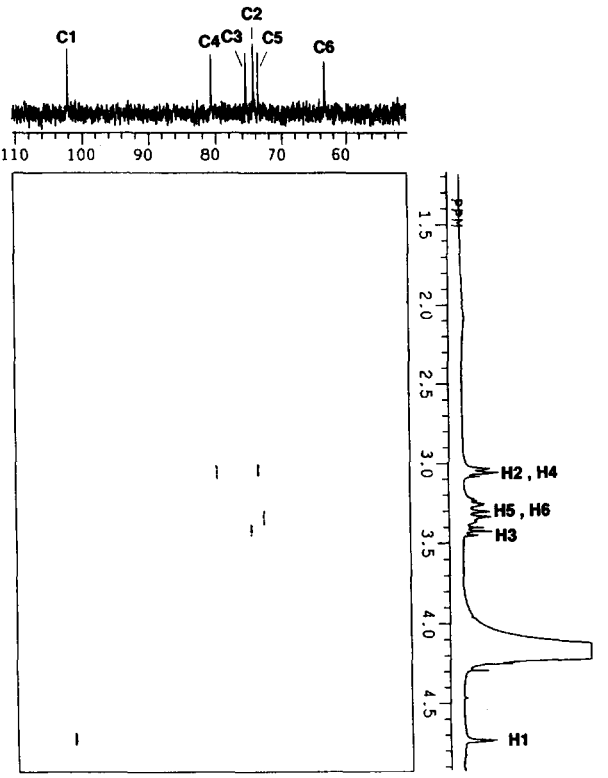

Fig. 3. Two-dimensional $^{1}H-^{13}C$ correlated (H,C COSY) NMR spectrum of η-CD.

drolase] was purchased from Tokyo Kasei Company, Ltd. (Tokyo, Japan), and glucoamylase [α-(1 → 4)-glucan glucohydrolase] was purchased from Seikagaku Kogyo Company, Ltd. (Tokyo, Japan). Pullulanase [α-(1 → 6)-glucosidase, Promozym 200L™) was kindly donated by Novo Nordisk Bioindustry Company, Ltd. (Chiba, Japan). All other chemicals were from reliable commercial sources and were used without further

Table 1
^{13}C NMR chemical shifts of CDs [a]

Carbon	α-CD [b]	β-CD [b]	γ-CD [b]	δ-CD [c]	η-CD
1	102.5	102.87	102.68	102.72	101.94
2	72.8	72.10	73.35	74.86	74.27
3	74.4	74.11	73.96	75.53	75.41
4	82.3	82.16	81.50	80.98	80.59
5	73.1	72.87	72.84	74.10	73.54
6	61.5	61.35	61.30	63.05	63.25

[a] Reported as ppm downfield from external Me_4Si at 30°C in D_2O.
[b] From [7].
[c] From [5b].

purification. Milli-Q water (Millipore Corporation, Milford, MA, USA) was used as the purified water in all preparations and purifications.

General methods.—HPLC was performed (*a*) on an ODS (C_{18}) reversed-phase column (Shenshu Pak ODS-5251-SS, 20 × 250 mm), an SSC Flow System 3100F pump, a DEGASYS DG-1200 degasser, and an ERC-7530 refractive index (RI) monitor, and (*b*) on an amino-bonded (NH_2) column (Asahipak NH2P-50, 4.5 × 250 mm), an SSC Flow System 3100J pump, a Shodex DEGAS KT-16 degasser, and an ERC-7512 RI monitor. An SIC Chromatocorder 12 was used for integration of peak areas. FABMS was performed with a Jeol SX-102A mass spectrometer using glycerin as the matrix. The acceleration voltage was 10 kV. ^{13}C and two-dimensional $^{1}H-^{13}C$ correlation (H,C COSY) NMR analyses were performed with a Jeol GX-400 spectrometer (400 MHz ^{1}H). The sample was dissolved in 99.8% D_2O, and chemical shifts are reported in δ-units (ppm) downfield from the signal of external Me_4Si.

Acknowledgements

We are grateful to Dr. J. Shigihara for the FABMS measurement and to Mrs. Y. Sawano for the NMR measurements. We gratefully ackcnowledge the generous supply of Promozym 200L™ from Novo Nordisk Company, Ltd. Thanks are due also to Messrs. Y. Suzuki, T. Nakao, and N. Futatsugi for their assistance.

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