Synthesis and Isolation of Regiospecific Mono Iodo-Cyclodextrin as a Fragile Intermediate to Amino-Cyclodextrin

Keiko TAKAHASHI* and Kenjiro HATTORI**

Iodo-CD(II) was prepared from toluenesulfonyl-CD (I) and purified by preparative HPLC. Iodo-CD (II) was easily converted to the amino-CD (III). Examination of the structure of the hydrolyzate from amino-CD (III) showed that iodination and amination proceeded with inversion of configuration in both reactions.

Introduction

Cyclodextrins exhibit unique and significant characteristics for providing hydrophobic binding site in aqueous solution, which are often conveniently and successfully utilized as enzyme models[1]. To prepare more refined and sophisticated enzyme models, various types of modification of cyclodextrin (CD) had been carried out. Most of these involved modification of the primary hydroxyl group site of CD, and so it is difficult to prepare regiospecific monosubstituted cyclodextrin. We have already reported an effective modification; the regiospecific monotosylated α-, and β-CD[2]. In the procedures, the tosyl group of CD was introduced onto the secondary hydroxyl site of CD accompanying 1 : 1 complex formation between tolylsulfonyl chloride and CD in aqueous solution. Several effective enzyme models were derived from this regiospecific monotosylated CD; for example, histamine-α-CD[2a], histidine-β-CD and nicotinamide-β-CD[4,5].

In this paper, we wish to report the synthesis and isolation of regiospecific iodo-CD (II) that is very significant in preparing regiospecific modified enzyme models *via* mono aminocyclodextrin (III).

Experimental

Materials: NaI and DMF were obtained from Wako Pure Chemical Industries Ltd. The latter was purified by distillation over calcium hydride. The Tos-CD(I) used in this work was prepared by the treatment with *p*-tolylsulfonyl chloride and β-CD in aqueous solution, as reported previously[2b].

Preparation of iodo-β-cyclodextrin (II): A solution of I(5.4 g 4 mmol) and sodium iodide (6.0 g 40 mmol) in DMF (100 ml) was treated for 15 hr at 133±1°C. The reaction temperature was controlled in the silicone oil bath with a Taiyo Thermominder H-80. The reaction mixture was poured into acetone with stirring to precipitate the product. The precipitate was filtered off, dissolved in water, and freeze-dried. Crude iodo-CD was obtained in 4.5 g

* Part-time Research Assistant
** Associate Professor
Department of Industrial Chemistry
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(89%). The HPLC data, which were obtained using an amino-silicagel column (Toyo Soda LS-450, ϕ 0.5×30 cm) eluted with 70% acetonitrile in water and detected by refractive index, indicated that there were two species in this crude product. One was identified as β-CD and the other as II.

Preparative HPLC: II was purified by preparative HPLC, using Toyo Soda HLC-827 with an amino-silicagel column (Toyo Soda LS-450 ϕ 2.5×30 cm) eluted with 70% acetonitrile in water at 40°C and detected by refractive index. The sampling column size was 8 ml, and the concentration of crude product injected was about 3% weight per volume.

Volhard titration: The purity of II was determined by Volhard titration by use of 0.1 M silver nitrate solution and 0.02 M potassium thiocyanate solution in the usual way[6].

Preparation of aminocyclodextrin (III): 10 g of crude iodo-CD was added to excess ammonia solution (28% in water, 100 ml), and the mixture was maintained at 160°C for 48 hr. The product was precipitated in acetone. The crude III was treated by cation exchange chromatography with a CM-Sephadex column, eluted with water and then by 1 M-ammonia solution. The latter eluent was evaporated to dryness. The yield of product was 3.0 g: paper chromatography indicated that the product was pure. The R_f value 0.24 (solvent: 1-butanol-dimethylformamide-water, 2 : 1 : 1; detecting reagent: ninhydrin). Elemental analysis, Found : N, 1.24%; C/N, 35.53. Calcd for $C_{42}H_{57}O_{34}N$: N, 1.24%; C/N, 35.87.

Partial Hydrolysis of III: III was partially hydrolyzed in a 1 M-HCl solution at 80°C for 6 hr. The reaction mixture was evaporated to dryness and dissolved in water, followed by cation exchange chromatography; the column was washed with water, then eluted with 0.33 M-HCl and 4 M-HCl. The fractions colored by the ninhydrin test were collected and evaporated, extracted with methanol, and then recrystallized in acetone. Chromatographic separation was carried out with a Sephadex G-15, column giving a product with an R_f value (0.04) in the paper chromatography: the product was confirmed as hexose by NMR characterization; δ 3.6, 4.1, 4.7(ppm) due to nonanomeric C-H. $[\alpha]_D^{25}=45.0°$.

Results and Discussion

Preparation of II: The reaction was followed by taking HPLC data at intervals with an amino-silicagel column under the same conditions described above. Fig. 1 shows the process

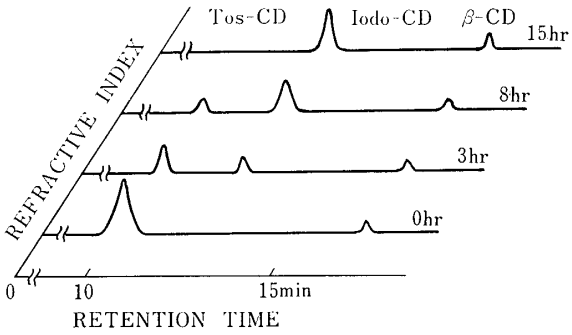


Fig. 1 The Process of Iodination Reaction Followed by HPLC
The reaction was carried out under the conditions shown at the preparation of II in the Experimental.

of the iodination reaction at 133°C in DMF. In the initial stage of the reaction, there were two peaks in the chromatography, one agreed with I and the other with β-CD. A peak which agreed with II then appeared with the passage of time. In contrast, the peak of I decreased and disappeared after 15 hr. β-CD's peak of I increased slightly with the passage

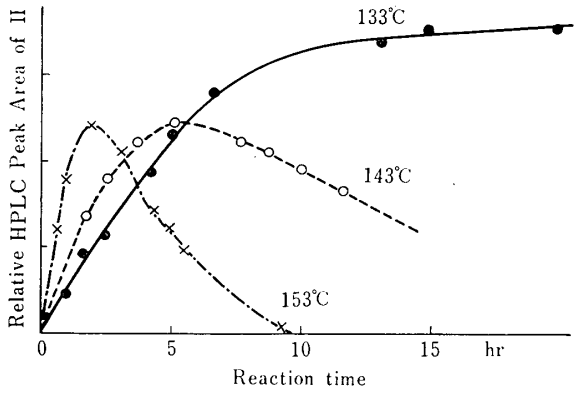


Fig. 2 Relative Amount of II Plotting with Time in the Iodination at Various Temperature
The reaction conditions were the same as in the Figure 1 except for the temperature.

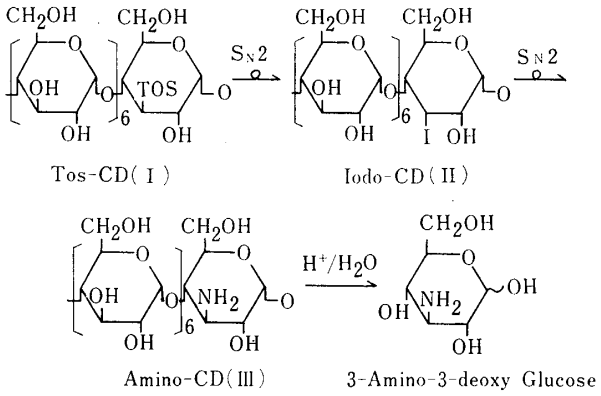
of time. At higher temperature this increase became remarkable, which was probably due to the decomposition of II. As is shown in Fig. 2, the reacton at 133°C for 15 hrs was the optimum condition. A short reaction time gave a low substitution of tosylate by iodine atoms, and prolonged reaction (over 25 hrs) caused II to undergo breakdown to β-CD and iodine. The optimum reaction temperature in the range up to 153°C was about 133°C. At low temperature, iodination proceeded slowly, and below 100°C. II was rarely produced. The reaction was more rapid with increasing temperature; at the same time the decomposition of the product occurred, so that the product was obtained in a low yield. II was so unstable as to be decomposed by heat, light irradiation and oxygen. Reprecipitation with acetone also decomposed II to β-CD and iodine.

Purification of II: The instability of II described above made the purification of II difficult. Some different methods were tried in an attempt to purify II: by using Sephadex G-10 and highly porous polystyrene gel (Diaion, HP-20). This was the first successful to attempt to isolate II by preparative HPLC with an aminosilicagel column. The purity was 99.5% by iodine estimation in a Volhard titration.

Assumed structure of II in relation to the reaction mechanism: In these procedures, II was prepared from I as a starting material. It has been reported that tosylation of β-CD in aqueous solution provided the regiospecific monotosylated β-CD[2]. Because the glucose unit of β-CD is in the C 1 conformation (chair form)[8], the secondary hydroxyl groups are equatorial, and so the tosyl moiety attached to β-CD should be equatorial. Futhermore, partial hydrolysis of tosyl-CD gave the same R_f value of paper chromatography of authentic 3-tosyl glucose and not that of authentic 6-tosylglucose that was obtained by the hydrolysis of Tos-CD prepared in pyridine solution without any complex formation[9]. The above result shows the evidence that the tosyl moiety attached at the C-3 position of the glucose of the CD molecule. S_N2 nucleophilic displacement was applicable to iodination by I; the tosyl group was replaced by an iodine atom with inversion of the configuration at the tosylated carbon atom, and thus the introduced iodine should be axial. S_N2 nucleophilic displacement, with hydrazine, ammonia and azides of secondary sulfonyloxy groups in sugar is known[10]. Displacement with iodide of the secondary methylsulfonyloxy group can be accomplished in same cases[11]. This II was easily converted to the amino derivatives by treatment with aqueous ammonia in methanol at 160°C for 48 hrs, then this was partially hydrolyzed to an amino hexose. This amino hexose was identified by optical rotation and paper chromatography and agreed with 3-aminoglucose[7]. This finding suggests that the amino group is

attached to β-CD at an equatorial site on the C-3 positionof glucose.

The following scheme can be suggested to account for the above result:



Scheme 1. Suggested Reaction Mechanism for the Synthesis of Regiospecific Amino -CD (Ⅲ) from Tos-CD (Ⅰ) via Iodo -CD (Ⅱ) Intermediate

As mentioned previously, the sulfonyloxy group undergoes attack under alkaline conditions by an unsubstituted hydroxyl group to give an anhydride. If the hydroxyl group is adjacent and configurationally trans, an epoxide ring is formed[11]. But in this reaction from II to III, the configurational geometry between adjacent iodine and hydroxyl groups was the cis form, so that an epoxide ring is not easily formed. Thus the amino group replaced the iodine attached to β-CD, with inversion of the configuration: S_N2 nucleophilic displacement.

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