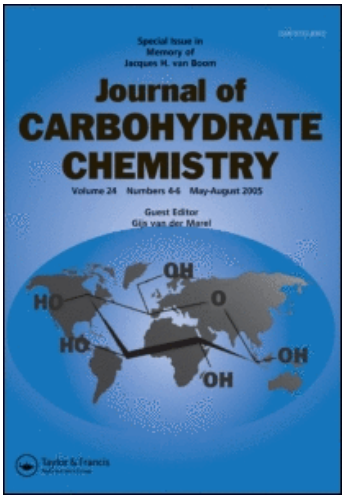

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ANALYSES OF A MIXTURE OF GLUCOSYL-CYCLOMALTOHEPTAOSES PREPARED ON AN INDUSTRIAL SCALE[1]

Kyoko Koizumi,* Yasuyo Okada, Noriko Nakanishi, Toshiko Tanimoto, Yosuke Takagi,* Masaki Ishikawa,* Hiroshi Ishigami,* Kozo Hara,* and Hitoshi Hashimoto*

Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 11-68 Koshien Kyuban-cho, Nishinomiya 663, Japan and
*Carbohydrate Research Laboratory, Ensuiko Sugar Refining Co., Ltd. 13-46 Daikoku-cho, Tsurumi-ku, Yokohama 230, Japan

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ABSTRACT

A mixture of glucosyl-cyclomaltoheptaoses (β-cyclodextrins, βCDs) was prepared by glucoamylolysis of a mixture of maltosyl-βCDs which was produced on an industrial scale from maltose and βCD through the reverse action of *Klebsiella pneumoniae* pullulanase. Glucosyl-βCDs in the mixture were separated by HPLC on a reversed phase column and their molecular weights were measured by FAB-MS. In addition, the number of side-chains in each molecule was confirmed by methylation analysis and it was proved that the mixture comprised mainly of a monoglucosyl-βCD [G-βCD] and diglucosyl-βCDs [$(G)_2$-βCDs], and as a minor component triglucosyl-βCDs [$(G)_3$-βCDs], and that G-, $(G)_2$-, and $(G)_3$-βCDs were produced in the ratios of 50%:45%:5%. The structures of three positional isomers of $(G)_2$-βCD were established by HPLC analysis of partial hydrolyzates, ^{13}C NMR spectroscopy, and chemical synthesis. Four regioisomeric $(G)_3$-βCDs which could be isolated were characterized by ^{13}C NMR spectroscopy.

INTRODUCTION

Glucosyl-cyclomaltoheptaoses [G- and $(G)_2$-βCDs] have much higher solubility in water and aqueous methanol solution,[2] weaker hemolytic activity on human erythrocytes,[2,3] and significantly lower local tissue irritancy[3] than their parent βCD. Therefore, glucosyl-βCDs have attracted much interest and are expected to be used in various fields.

We produced a mixture of glucosyl-βCDs on an industrial scale and separated individual glucosyl-βCDs by HPLC, and characterized them by FAB-MS, methylation analysis, fragmentation analysis, and ^{13}C NMR spectroscopy. Furthermore, the structures of three positional isomers of $(G)_2$-βCD were confirmed by chemical syntheses.

RESULTS AND DISCUSSION

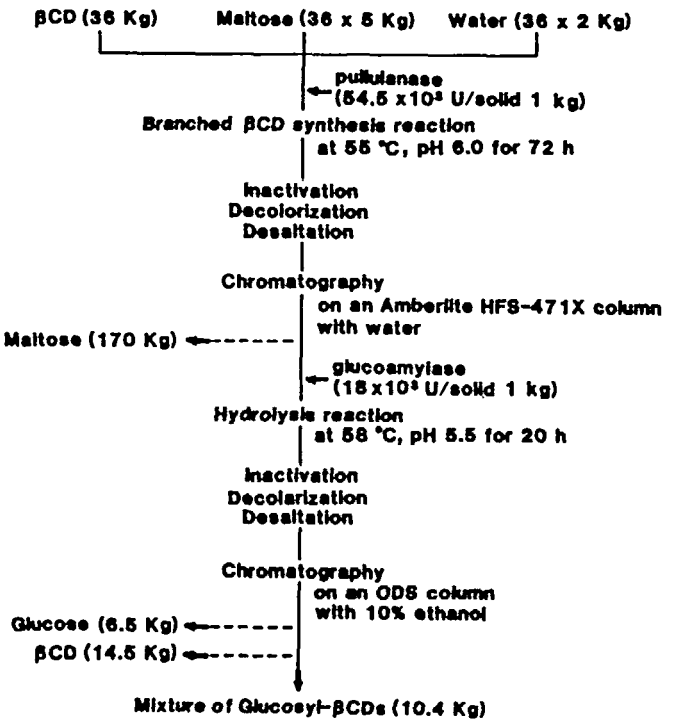
Production. It was reported that branched CDs having side-chains of malto-oligomers could be produced by incubating parent CDs and malto-oligosaccharides having desired degree of polymerizations (DPs) with debranching enzyme such as isoamylase (glycogen 6-glucanohydrolase, EC 3.2.1.68)[4-6] or pullulanase (pullulan 6-glucanohydrolase, EC 3.2.1.41).[7-9] Although debranching enzyme normally hydrolyzes α-(1→6) glucosidic linkages in α-glucans such as amylopectin, pullulan, and glycogen, the reverse condensation reaction of the enzyme can combine malto-oligosaccharides to CDs under specific conditions. However, glucosyl-CDs are prepared by shortening the side-chains of maltosyl- or maltotriosyl-CDs with glucan 1,4-α-glucosidase (glucoamylase, EC 3.2.1.3).

We produced a mixture of glucosyl-βCDs on an industrial scale using the method as shown in SCHEME 1. Though branched CDs having side-chains longer than maltotriosyl are possible, to be effectively produced using isoamylase, we selected maltose and pullulanase as the initial substrate and the enzyme for reasons of the costs of substrate and enzyme, in relation to application value of the products on an industrial use.

Isolation. A mixture of glucosyl-βCDs obtained was analyzed by HPLC on a reversed phase column and six peaks [(a)—(f)] were observed (FIG. 1). The components (A—F) corresponding to each peak were separated by semi-preparative HPLC using a larger size column with 7—10% methanol as the eluent. The peak (b) fraction was subdivided into three fractions by repeating the chromatography and three components, B1, B2, and B3 were isolated.

Characterization. The molecular weights confirmed by FAB-MS are 1620 for A, B1, B2, and B3, 1458 for C, D, and E, and 1296 for F. These results and methylation analysis data, summarized in TABLE 1, suggested that A, B1, B2, and B3 were triglucosyl-βCDs [$(G)_3$-βCDs]; C, D, and E were diglucosyl-βCDs [$(G)_2$-βCDs]; and F was a monoglucosyl-βCD [G-βCD]. The ratios of G-, $(G)_2$-, and $(G)_3$-βCDs, as measured from the chromatogram of FIG. 1 were

SCHEME 1. Production and purification of a mixture of glucosyl-βCDs



50%:45%:5%.

TABLE 1. Data of Methylation Analysis for Glucosyl-βCDs A—F.

Product	Retention Time[a] (min)	Molar Ratio A, B1, B2, B3	C, D, E	F
1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol	6.4	3	2	1
1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol	18.0	4	5	6
1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-glucitol	38.4	3	2	1

a. Chromatographic conditions: column, 0.3% OV-275—0.4% GEXF-1150 on Shimalite W (AW-DMCS), 80-100 mesh (2 m × 3 mm i.d.); column temperature, 160 °C; carrier gas and flow rate, N_2 and 30 $mL \cdot min^{-1}$.

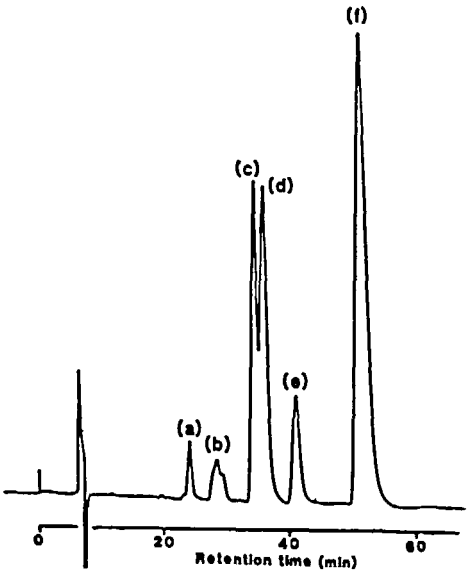


FIG. 1. Elution profiles of glucosyl-βCDs on YMC-Pack AQ-323 ODS column (250 × 10 mm i.d.) with 7% methanol. Detector: Shodex RI SE-61, flow rate: 2 mL·min^{-1}, temperature: 30 °C.

Compound F was identified as 6-*O*-α-D-glucopyranosyl-βCD[10] by chromatographic behavior and by ^{13}C NMR spectroscopy.

Compounds C and D were identified as 6^1,6^3-di-*O*-(α-D-glucopyranosyl)-βCD and 6^1,6^4-di-*O*-(α-D-glucopyranosyl)-βCD, respectively. These two regioisomeric diglucosyl-βCDs had been obtained from the mother liquors of a large-scale preparation of βCD with *Bacillus ohbensis* cyclomaltodextrin glucanotransferase (EC 2.4.1.19) and had been characterized by HPLC analysis of partial hydrolyzates and by ^{13}C NMR spectroscopy.[11] Another diglucosyl-βCD E had been predicted to be 6^1,6^2-di-*O*-(α-D-glucopyranosyl)-βCD on the basis of comparison of its ^{13}C NMR spectrum with those of C and D.[12] To confirm the structure of E, detailed study on HPLC analysis of partial hydrolyzates of E together with reinvestigation of partial hydrolyzates of C and D was attempted.

Three series of linear saccharides were found in each chromatogram of partial hydrolyzates of (G)$_2$-βCDs, as obtained by HPLC on an amino-bonded silica column. Each of the first and the second series consisted of homogeneous malto-oligosaccharides (DP 2—7) and malto-oligosaccharides containing one 1→6 linkage (DP 2—8), respectively, while each of the third series contained two 1→6 linkages (DP 4, 5 or 6—9). The smallest member of the third series found in each chromatogram of partial hydrolyzates of three

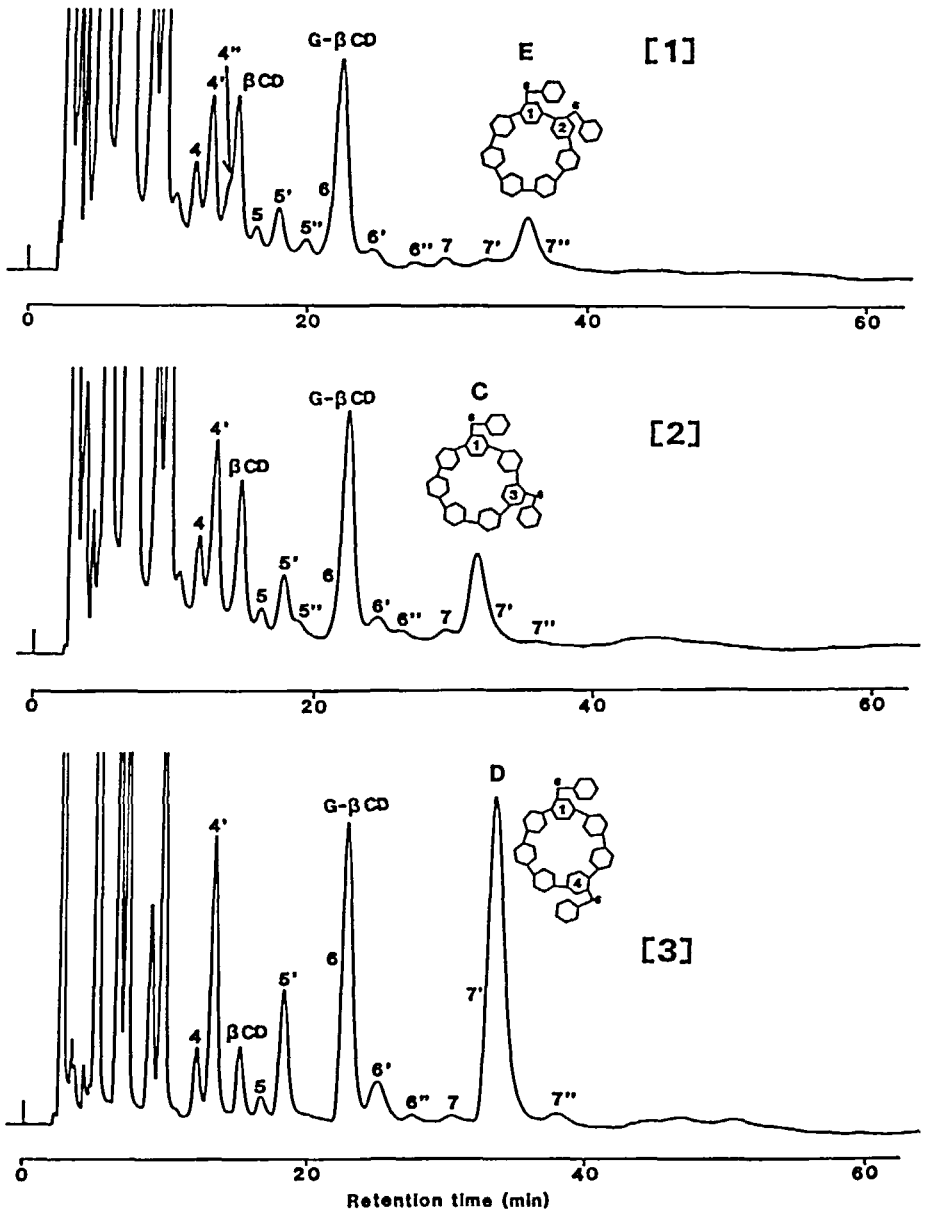


FIG. 2. Chromatograms of partial hydrolyzates of $(G)_2$-βCD E [1], C [2], and D [3]. The number on each peak shows DPs of malto-oligosaccharides, the primed number shows DPs of malto-oligosaccharides each containing one 1→6 linkage, and the double primed number shows DPs of malto-oligosaccharides each containing two 1→6 linkages. Chromatographic conditions: column, Hibar LiChrosorb NH_2 (250 × 4 mm i.d.); eluent, 66% acetonitrile; flow rate, 1 $mL \cdot min^{-1}$; the other conditions as in FIG. 1.

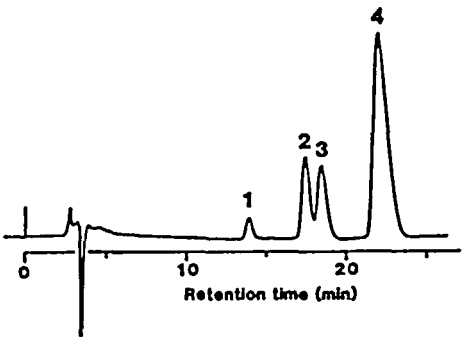


FIG. 3. Chromatogram of products from IV, $6^1,6^4$-di-*O*-(D-glucopyranosyl)-βCDs. 1 = β,β substituted product, 2, 3 = α,β or β,α substituted products, 4 = α,α substituted product. Chromatographic conditions: column, YMC-Pack A-312 ODS (150 × 6 mm i.d.); eluent, 4% methanol; flow rate, 1 mL·min^{-1}.

regioisomeric $(G)_2$-βCDs C, D, and E can be expected to suggest the positions of two branches in the molecule. To make sure the DP of the smallest member of the third series, the conditions of hydrolysis and chromatography were modified, and then chromatograms as shown in FIGs. 2[1], [2], [3] were obtained. In these chromatograms, peak 4'' can be observed only in [1], while peak 5'' can be seen in [1] and [2]. The smallest member of the third series in [3] has a DP 6. These facts indicate that E is $6^1,6^2$-di-*O*-(α-D-glucopyranosyl)-βCD, and C and D are $6^1,6^3$- and $6^1,6^4$-disubstituted isomers, respectively.

These structures of C, D, and E were confirmed by direct comparison with authentic samples synthesized chemically via regiospecifically $6^1,6^n$-di-*O*-*tert*-butyldimethylsilylated βCDs (n = 2, 3, and 4). Three regioisomeric $6^1,6^n$-di-*O*-(*tert*-butyldimethylsilyl)-βCDs (I, II, and III) were prepared by reaction of dry βCD with 2.5 molar equivalents of *tert*-butyldimethylsilyl chloride in pyridine, isolated by HPLC, and characterized by ^{13}C NMR spectroscopy. Furthermore, their unambiguous structures were evidenced by conversion to the known compounds $6^1,6^n$-di-*O*-(toluenesulfonyl)-βCDs. Compounds I, II, and III, in the order of elution from a C_{18}-bonded silica (ODS) column, were $6^1,6^4$-, $6^1,6^3$-, and $6^1,6^2$-disubstituted βCDs, respectively. The ratios of I, II, and III in the silylation products were almost 1:1:1. Isolated compounds I, II, and III were acetylated and desilylated with 47% boron trifluoride etherate to obtain bis(2,3-di-*O*-acetyl)pentakis(2,3,6-tri-*O*-acetyl)-βCDs (IV, V, and VI) as the required glucosyl acceptors. According

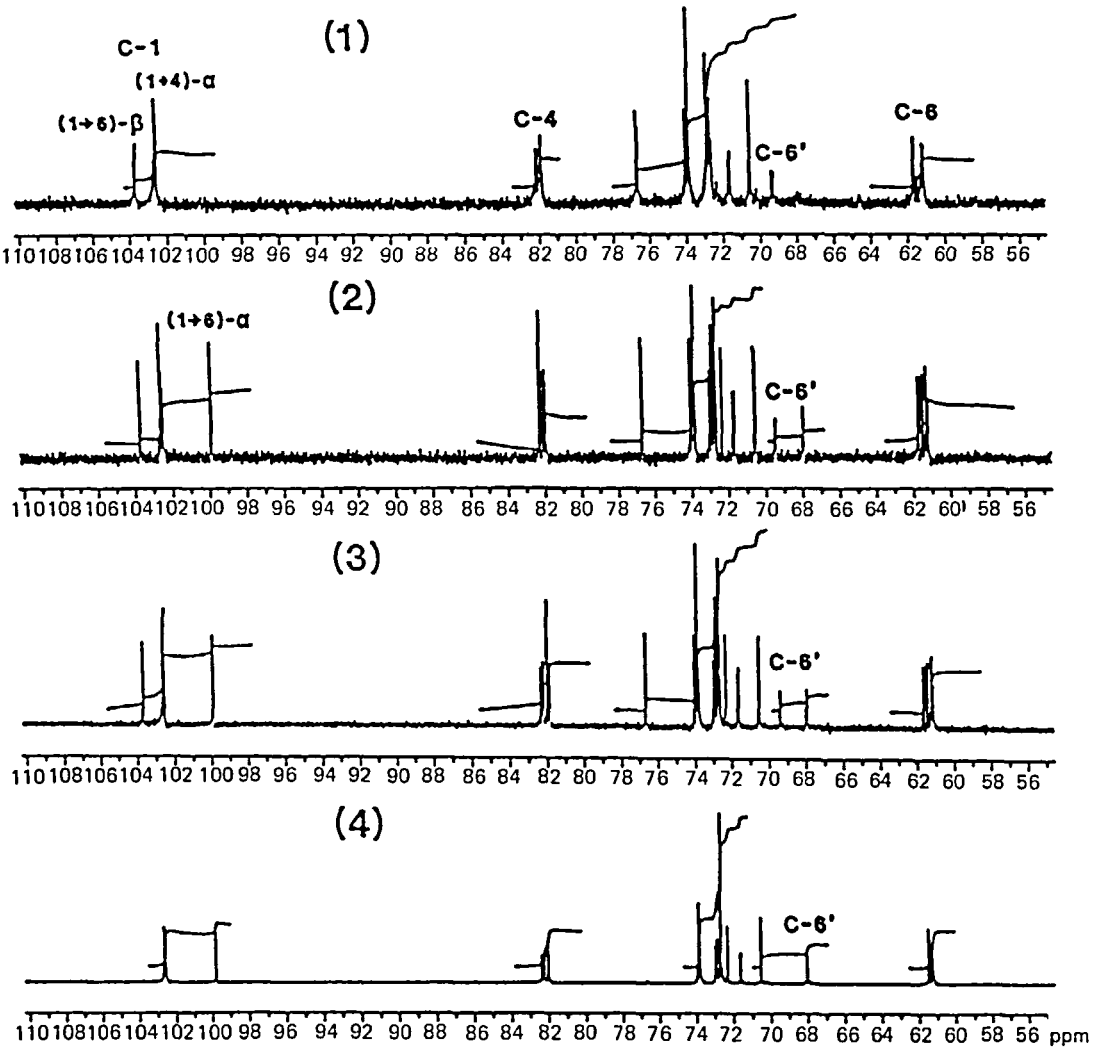


FIG. 4. ^{13}C NMR spectra of $6^1,6^4$-di-*O*-(D-glucopyranosyl)-β CDs measured in D_2O at 125.65 MHz. C-6': the carbon atom of the branch point.

to the procedure of Fügedi *et al.*[14] glucosylation of IV, V, and VI was satisfactorily achieved by reaction with 2,3,4,6-tetra-*O*-benzyl-1-*O*-trichloroacetimidoyl-α-D-glucopyranose (VII)[15,16] in dichloromethane at -20 ℃, using trifluoromethanesulfonic acid as catalyst. Each product from IV, V, and VI was subjected to catalytic debenzylation (Pd/C) and deacetylation. As shown in FIG. 3, HPLC of the products from IV, 6^1, 6^4-diglucosyl-β CDs revealed the presence of four components, which were isolated, and characterized by ^{13}C

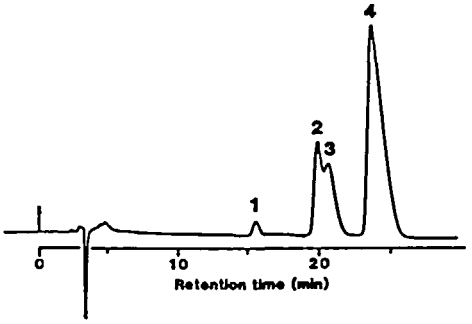


FIG. 5. Chromatogram of products from V, $6^1,6^3$-di-*O*-(D-glucopyranosyl)-β CDs. Others as in FIG. 4.

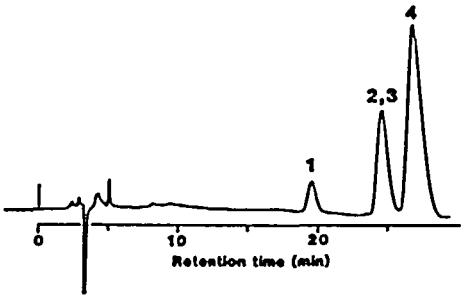


FIG. 6. Chromatogram of products from VI, $6^1,6^2$-di-*O*-(D-glucopyranosyl)-β CDs. Eluent, 5% methanol, others as in FIG. 4.

NMR spectroscopy. The C-1 resonances of the glucose residues of the CD ring, side-chain residues α(1→6)-linked and β(1→6)-linked to the CD ring appeared at δ ~102.7, ~99.9, and ~103.7 ppm, respectively. In the ^{13}C NMR spectrum of the main product, corresponding to the peak 4 in the chromatogram, two α(1→6)-linked C-1 signals were observed and hence the main product was the required compound. The compounds corresponding to the peaks 2 and 3 showed two kinds of (1→6)-linked C-1 signals at δ ~99.9 and ~103.7 ppm, and the minor component corresponding to the smallest peak 1, showed two β(1→6)-linked C-1 signals at δ ~103.7 ppm. These facts indicate that both compounds corresponding to the peaks 2 and 3 are configurational isomers each one having α and β(1→6)-linkage in a molecule and the minor component is $6^1,6^4$-di-*O*-(β-D-glucopyranosyl)-β CD. The spectral data relating to C-6 signals were consistent with the structures of these configurational isomers. The assignments of C-6 signals were confirmed by the INEPT method[17] using $\Delta=3/4J$. The large downfield shift of two C-6 signals indicates that the side-chain D-glucose residues are attached to oxygens on these carbon atoms. The signal of C-6 involved in α(1→6)-linkage appears at δ 68.0 ppm and that involved in β(1→6)-linkage is observed at δ69.4 ppm. The ratios in the signal intensities of CD ring C-6 (at δ ~61.2), side-chain C-6 (at δ ~61.5 and/or ~61.7) and branch-point C-6 were 5:2:2. Chromatograms of $6^1,6^3$- and $6^1,6^2$-diglucosyl-β CDs shown in FIGs. 5 and 6, respectively, are similar to that of $6^1,6^4$-di-glucosyl-β CDs (FIG. 4), and the component corresponding to the last peak in each chromatogram is the desired α,α-diglucosyl derivative. The

TABLE 2. Ratios of Configurational Isomers in the Glucosylation Products.

Products	α,α	α,β and β,α	β,β
$6^1,6^4$-disubstituted	65.3	31.4	3.3
$6^1,6^3$-disubstituted	65.4	31.5	3.1
$6^1,6^2$-disubstituted	65.7	27.9	6.4

molar ratios of configurational isomers in the glucosylation products are summarized in TABLE 2.

Figure 7 shows ^{13}C NMR spectra of tri-*O*-(α-D-glucosyl)-βCDs A, B1, B2, and B3. By comparison of these four spectra and those of three di-*O*-(α-D-glucosyl)-βCDs C, D and E, it was assumed that A might be $6^1,6^3,6^5$-tri-*O*-(α-D-glucopyranosyl)-βCD and three B might be $6^1,6^2,6^4$-, $6^1,6^2,6^5$-, and $6^1,6^2,6^6$-tri-*O*-(α-D-glucopyranosyl)-βCDs. These structures will be confirmed by chemical syntheses in the near future.

By the way, when the activity of glucoamylase used for shortening side-chains of maltosyl-βCDs was weaker, another peak was observed between peaks (e) and (f) in a chromatogram as shown in FIG. 1. The molecular weight of this compound was 1620, measured by FAB-MS, and methylation analysis and ^{13}C NMR spectroscopy (FIG. 8) revealed that the compound had one glucose and one maltose side-chain. Rehydrolysis of the compound with glucoamylase gave $6^1,6^2$-di-*O*-(α-D-glucopyranosyl)-βCD and hence, the compound was 6^1 or 6^2-*O*-α-D-glucosyl-6^2 or 6^1-*O*-α-maltosyl-βCD.

EXPERIMENTAL

General Procedures. Melting points were measured with Yanagimoto micro melting-point apparatus and are uncorrected. Optical rotations were determined with a JASCO digital polarimeter, model DIP 360. TLC was performed on Silica gel 60 TLC plates (Merck) with appropriate developing solvents by spraying with sulfuric acid. A Harrison Centrifugal Thin Layer Chromatotron, model 7924 was used for centrifugal chromatography (Cen. C.). HPLC was performed with a JASCO 880-PU pump, a Waters U6K universal injector, and a Showa Denko SE-61 refractive index monitor. The columns used were a YMC-Pack AQ-323 ODS (250 × 10 mm i.d.), YMC-Pack SH-343-5 AQ (250 × 20 mm i.d.), and Hibar LiChrosorb NH_2 (250 × 4 mm i.d.). HPLC

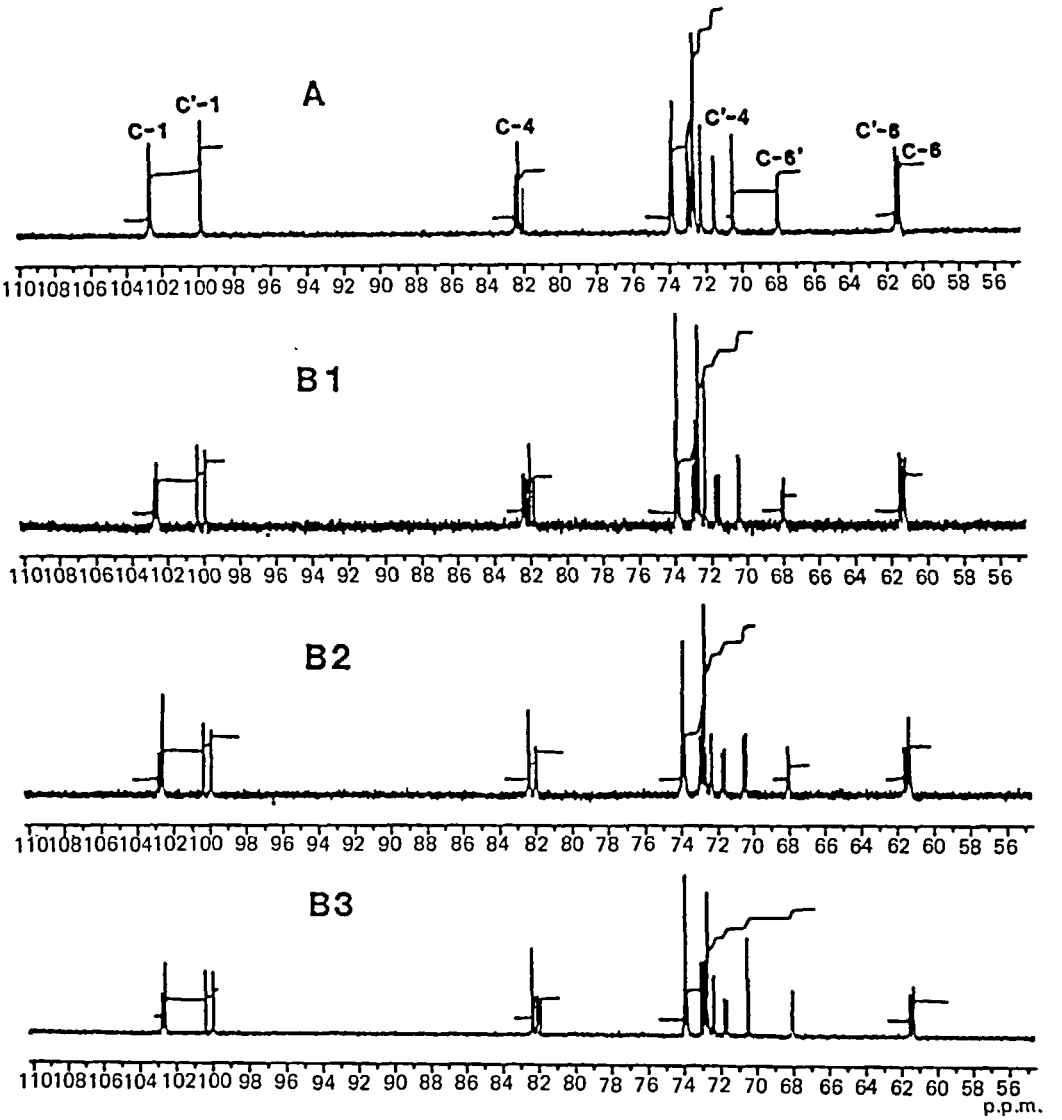


FIG. 7. ^{13}C NMR spectra of tri-*O*-(α-D-glucopyranosyl)-βCDs measured in D_2O at 125.65 MHz. C: the carbon atom of the ring D-glucose unit. C': the carbon atom of the branched unit. C-6': the carbon atom of the branch point.

analyses at constant temperature were conducted by the use of a column oven SSC 3510C (Senshu Scientific Co.). A Shimadzu Chromatopac C-R3A digital integrator was used for quantitative analyses. FAB-MS was performed with a JEOL JMS-DX 303 mass spectrometer. ^{13}C NMR spectra (125.65 MHz) were recorded at ambient temperature on 2-3% solutions in D_2O with JEOL GSX-500 spectrometer.

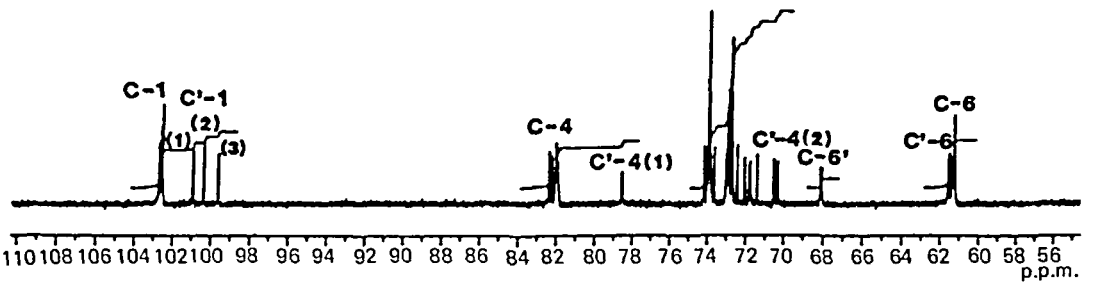


FIG. 8. ^{13}C NMR spectrum of 6^1 or 6^2-*O*-α-D-glucosyl-6^2 or 6^1-*O*-α-maltosyl-βCD measured in D_2O at 125.65 MHz. C: the carbon atom of the ring D-glucose unit. C': the carbon atom of the branched unit. C-6': branch point. C'-1(1): (1→4)-linked, (2) and (3): (1→6)-linked. C'-4(1): linked, (2): free.

Materials. βCD and maltose were commercial products of Ensuiko Sugar Refining Co., Ltd. and Sanwa Denpun Kogyo Co., Ltd., respectively. Amberlite HFS-471X resin (Na^+ form) and C_{18}-bonded silica (ODS) were purchased from Japan Organo Co., Ltd. Pullulanase from *Klebsiella pneumoniae* "Pullulanase AMANO 3" (3000 U/mL) and glucoamylase from *Rhizopus niveus* "GURUKUZAI-MU AF" (6000 U/mL) were both commercial products of Amano Pharmaceutical Co., Ltd. One unit of pullulanase activity is defined as the amount of enzyme that causes an increase of reduction corresponding to one μmol of glucose from pullulan per minute, and one unit of glucoamylase activity is the amount of enzyme that forms 10 mg of glucose from soluble starch in 30 min at 40 ℃, pH 4.5. All reagents were of analytical grade. Reagent-grade organic solvents used for chromatography were dried and freshly distilled before use. Water used in solvent preparations was distilled, deionized, and redistilled.

Methylation Analysis. Methylation of branched βCDs was performed by the method of Prehm[18] with 2,6-di-(*tert*-butyl)pyridine and methyl trifluoromethanesulfonate in trimethyl phosphate. The products were hydrolyzed, converted to their alditol acetates, and then analyzed with a Hitachi gas chromatograph Model 063 fitted with a flame-ionization detector.

Fragmentation Analysis. Samples (7 mg each) were partially hydrolyzed in 2 mL of 0.3 M or 0.5 M trifluoroacetic acid for 90—150 min at 100 ℃. The solution containing the hydrolyzates was concentrated to dryness *in vacuo*. The residue was dissolved in 200 μL of water and aliquots (2 μL) were analyzed by HPLC.

Syntheses of $6^1,6^n$-Di-*O*-(α-D-glucopyranosyl)-cyclomaltoheptaoses. $6^1,6^n$-Di-*O*-(*tert*-butyldimethylsilyl)-βCDs (I, II, III). According to the procedure described previously,[13] compounds I (n = 4), II (n = 3), and III (n = 2) were synthesized and characterized: I, mp 287 ℃ (dec.), $[\alpha]_D^{25}$ +118.8° (*c* = 1.2, CH_3OH); II, mp 282 ℃ (dec.), $[\alpha]_D^{25}$ +125.6° (*c* = 0.9, CH_3OH); III, mp 289 ℃ (dec.), $[\alpha]_D^{25}$ +130.4° (*c* = 1.0, CH_3OH).

Bis(2,3-di-*O*-acetyl)pentakis(2,3,6-tri-*O*-acetyl)-βCDs (IV, V, and VI). Compounds I (284 mg), II (512 mg), and III (625 mg) were individually dissolved in dry pyridine (20-50 mL) and acetic anhydride (10-30 mL) was added. The solution was stirred overnight at 100 ℃ and then concentrated. The residue was extracted with chloroform and the extract was washed with aqueous sodium hydrogencarbonate and water, dried, and concentrated. The residue was dissolved in dichloromethane (20-40 mL) and 47% boron trifluoride etherate in ether (0.44-1.10 mL) was added. The mixture was stirred for 1 h at room temperature, diluted with dichloromethane, and poured into ice-water. The dichloromethane layer was separated, rinsed successively with water, aqueous sodium hydrogencarbonate, and again water, then dried, and concentrated. The product was purified by Cen. C. with hexane-acetone (3:2) to give IV (328 mg, 81.4%), V (605 mg, 83.3%), VI (689 mg, 77.8%). The $[\alpha]_D^{26}$ values in $CHCl_3$ were +119.0° (*c* = 1.2) for IV, +111.7° (*c* = 1.2) for V, +112.5° (*c* = 0.9) for VI.

2,3,4,6-Tetra-*O*-benzyl-1-*O*-trichloroacetimidoyl-α-D-glucopyranose (VII). To a solution of 2,3,4,6-tetra-*O*-benzyl-D-glucose (1.0 g) in 10 mL of absolute dichloromethane, sodiumhydride (40 mg) and trichloroacetonitrile (1.5 mL) were added, and the mixture was vigorously stirred. After 1 h, 90 mg of sodium hydride was added under cooling, and stirring was continued for 1 h. The reaction mixture was filtered through Celite and concentrated. The crude product was purified by Cen. C. with petroleum ether:ether (2:1) and the desired product (VII) was obtained 82.6% in yield.

Glucosylation of IV. A mixture of IV (534 mg) and powdered 4 Å molecular sieves (1.582 g) in 15 mL of dry dichloromethane was stirred under nitrogen at -20 ℃. A solution of VII (547 mg) in dichloromethane (5 mL) was added and after 30 min, a solution of trifluoromethanesulfonic acid (80 μL) in dichloromethane (3 mL) was added dropwise. After 30 min, as an examination by TLC (hexane-acetone, 1:1) suggested that the reaction had not been completed, another 547 mg of VII and 35 μL of trifluoromethanesulfonic acid in 1 mL of dichloromethane were added. After completion of glucosylation, triethylamine (2 mL) was added, and the mixture was filtered through Celite,

rinsed with 1 M sulfuric acid, saturated aqueous sodium hydrogencarbonate, and water, then dried, and concentrated. Cen. C. with hexane–acetone (1:1) of the residue gave chromatographicaly pure $6^1,6^4$-di-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-βCD peracetate (VIII, 692 mg, 84.1%).

Glucosylation of V and VI. In the same manner as described above, $6^1,6^3$-di-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-βCD peracetate (IX, 699 mg, 75.0%) and $6^1,6^2$-di-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-βCD peracetate (X, 749 mg, 71.7%) were obtained from V (605 mg) and VI (678 mg), respectively.

$6^1,6^4$-Di-*O*-(α-D-glucopyranosyl)-βCD. Compound VIII (692 mg) was dissolved in methanol containing 10% formic acid (20 mL). This solution was added to a stirred suspension of 10% Pd/C (2.5 g) in the same solvent mixture (80 mL) and stirring was continued in a nitrogen atmosphere. The catalyst was filtered off, and washed with methanol and water, and solvent was evaporated. The residue was dissolved in methanolic 0.05 N sodium methoxide (50 mL) and stirred at room temperature for 1 h. The deposited solid was dissolved by the addition of water and the solution was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated. The residue was comprised of a mixture of configurational isomers (329 mg, 97.1%) and separation of the residue on a column of YMC-Pack AQ-323 ODS with 5% methanol gave the desired compound, $6^1,6^4$-di-*O*-(α-D-glucopyranosyl)-βCD, $[\alpha]_D^{30}$ +165.5°(*c* = 1.0, H_2O).

$6^1,6^3$-, and $6^1,6^2$-Di-*O*-(α-D-glucopyranosyl)-βCDs were synthesized from compound IX (700 mg) and X (749 mg) in the same manner as described above. The yields were 312 mg (91.0%) and 341 mg (92.9%), respectively: $[\alpha]_D^{30}$ both +165.8°(*c* = 1.0, H_2O).

Specific Rotation $[\alpha]_D^{30}$ of Triglucosyl-βCDs (A, B1, B2, B3) and Glucosyl maltosyl-βCD (G) in H_2O. A: +165.5°, B1: +162.8°, B2: +162.6°, B3: +163.8°, G: +165.1°.

REFERENCES AND FOOTNOTES

1. This work was presented at the *XVth International Carbohydrate Symposium*, Yokohama, Japan, August 12–17, 1990.

2. Y. Okada, Y. Kubota, K. Koizumi, S. Hizukuri, T. Ohfuji, and K. Ogata, *Chem. Pharm. Bull.*, 36, 2176 (1988).

3. M. Yamamoto, A. Yoshida, F. Hirayama, and K. Uekama, *Int. J. Pharm.*, 49, 163 (1989).

4. J. Abe, N. Mizowaki, S. Hizukuri, K. Koizumi, and T. Utamura, *Carbohydr. Res.*, 154, 81 (1986).

5. J. Abe, S. Hizukuri, K. Koizumi, Y. Kubota, and T. Utamura, *Carbohydr. Res.*, 176, 87 (1988).

6. S. Hizukuri, J. Abe, K. Koizumi, Y. Okada, Y. Kubota, S. Sakai, and T. Mandai, *Carbohydr. Res.*, 185, 191 (1989).

7. M. Abdulah and D. French, *Arch. Biochem. Biophys.*, 137, 483 (1970).

8. Y. Sakano, M. Sano, and T. Kobayashi, *Agric. Biol. Chem.*, 49, 3391 (1985).

9. S. Hizukuri, S. Kawano, J. Abe, K. Koizumi, and T. Tanimoto, *Biotechnol. Appl. Biochem.*, 11, 60 (1989).

10. K. Koizumi, T. Utamura, M. Sato, and Y. Yagi, *Carbohydr. Res.*, 153, 55 (1986).

11. K. Koizumi, T. Tanimoto, Y. Okada, and M. Matsuo, *Carbohydr. Res.*, 201, 125 (1990). In this paper, 6-*O*-α-isomaltosyl-βCD was erroneously assigned to $6^1,6^2$-di-*O*-(α-D-glucopyranosyl)-βCD. (see Reference 12.)

12. K. Koizumi, T. Tanimoto, Y. Okada, N. Nakanishi, N. Kato, Y. Takagi, and H. Hashimoto, *Carbohydr. Res.*, accepted.

13. N. Nakanishi, J. Tsujikawa, N. Hara, T. Tanimoto, and K. Koizumi, *YAKUGAKU ZASSHI*, 110, 477 (1990).

14. P. Fügedi, P. Nánási, and J. Szejtli, *Carbohydr. Res.*, 175, 173 (1988).

15. R. R. Schmidt and J. Michel, *Angew. Chem.*, 92, 763 (1980).

16. R. R. Schmidt and M. Stumpp, *liebigs Ann. Chem.*, 1249 (1983).

17. A. Morris and R. Freeman, *J. Am. Chem. Soc.*, 101, 760 (1979).

18. P. Prehm, *Carbohydr. Res.*, 78, 372 (1980).