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Selective Preparation of Substituted Maltooligosaccharides through Enzymatic Hydrolysis of Substituted β-Cyclodextrins by Bacterial α-Amylase (Saccharifying Type). A Novel Method for Determining Regiochemical Structure of Disubstituted β-Cyclodextrin

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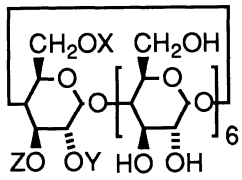
Specifically substituted maltooligosaccharides were prepared through the enzymatic hydrolysis of β-cyclodextrin derivatives by bacterial α-amylase (saccharifying type).

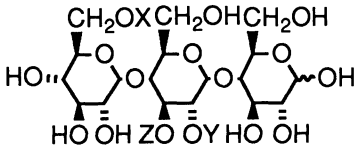
A few amylases such as Taka amylase A (TAA)[1] and bacterial α-amylase (saccharifying type) of *Bacillus subtilis* (BSA)[2] can hydrolyze β-cyclodextrin. We have reported selective preparation of substituted maltooligosaccharides through enzymatic hydrolysis of substituted β-cyclodextrin by TAA.[3] BSA and TAA can hydrolyze starch, but give different products from each other.[2] Therefore, the combination of BSA and substituted β-cyclodextrins is reasonably expected to provide new substituted maltooligosaccharides which cannot be obtained by hydrolysis with TAA. In this report, we describe BSA-hydrolysis of some β-cyclodextrin derivatives which were prepared by selective chemical modification of β-cyclodextrin.[3]

A solution of BSA (1 mg) in ice-cooled water (1.9 mL) was added to a solution of 6-*O*-(*p*-tosyl)-β-cyclodextrin **1** (21.9 mg) in a mixture of DMSO (1.2 mL) and water (1.9 mL) under ice-cooling. The solution was allowed to stand at room temperature. An additional BSA (1 mg) was added to the solution every 4-5 days. The starting material **1** disappeared after 21 days by using total 4 mg of BSA. The course of reaction was monitored by silica gel TLC (elution solvent; n-C_3H_7OH/AcOH/H_2O (7/7/5)). The solution was kept in boiling water for

10 min, precipitates were filtered, and the filtrate was analyzed by reversed-phase HPLC (Fig. 1A) and chromatographed on a reversed-phase column with gradient elution from water to 40% aqueous CH_3CN to give 6"-*O*-(*p*-tosyl)maltotriose **6** (5.6 mg, 50%) (Fig. 2). Under the similar conditions using water as the solvent, other β-cyclodextrin derivatives **2a**, **2b**, **3**, **4**, and **5** gave specifically modified oligosaccharides **7a**, **7b**, **8**, **9**, and **10**, respectively (for example; Fig. 1B and 1C). The reaction conditions and the results are summarized in Table 1.

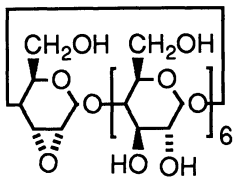


1 ; X = Ts, Y = Z = H
2a; Y = 1-Ns, X = Z = H
2b; Y = CH_3, X = Z = H
3; Z = 2-Ns, X = Y = H



6 ; X = Ts, Y = Z = H
7a; Y = 1-Ns, X = Z = H
7b; Y = CH_3, X = Z = H
8; Z = 2-Ns, X = Y = H

Ns = naphthalenesulfonyl, Ts = *p*-toluenesulfonyl



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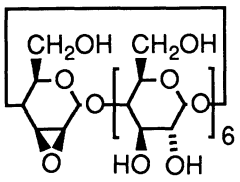

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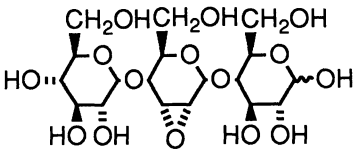

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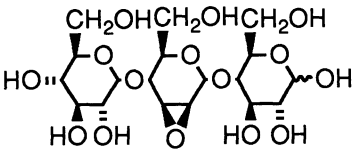

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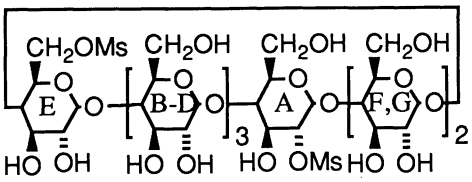

11, $2^A,6^E$-bis(*O*-mesitylenesulfonyl)-β-cyclodextrin
Ms = mesitylenesulfonyl

Table 1. Hydrolysis of Modified β-Cyclodextrins with Bacterial α-Amylase (Saccharifying Type)

CD derivative (mg)	BSA (mg)	Solvent (mL)	Reaction period/d	Product
1 (21.9)	4.0	H_2O (1.9) + DMSO (1.2)	21	**6** (5.6 mg, 50.0%)
2a (211.0)	32.3	H_2O (40)	25	**7a** (61.2 mg, 55.5%)
2b (30.1)	3.0	H_2O (5)	3.8	**7b** (8.7 mg, 64.1%)
3 (20.9)	1.5	H_2O (4)	7	**8** (7.1 mg, 63.9%)
4 (20.4)	1.5	H_2O (4)	10	**9** (6.9 mg, 78.9%)
5 (20.3)	1.3	H_2O (4)	10	**10** (7.3 mg, 83.4%)

The structure of each product was determined as follows. The fast-atom-bombardment (FAB) mass spectrum of each compound contained the molecular ion. The compounds **6**, **7a**, **8**, **9**, and **10** were identified by comparing their HPLC retention times and FAB mass spectra with those of the authentic compounds.[3] Compound **7b** was converted to the completely acetylated maltotriose derivative by conventional method with

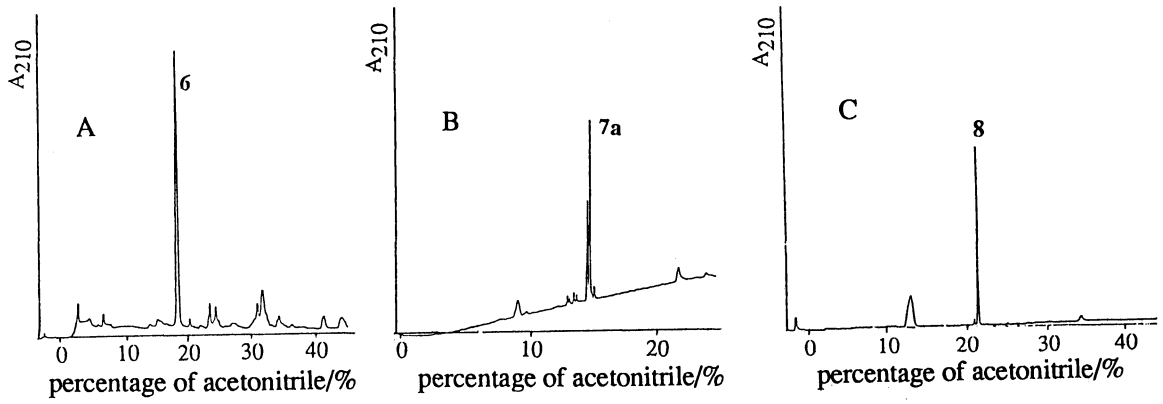

Fig. 1. Reversed-phase HPLC (TSKgel ODS-80T$_M$, 0.56x150 mm, Tosoh) of the mixture obtained by the reaction of **1** (A), **2a** (B), or **3** (C) with bacterial α-amylase (saccharifying type) of *Bacillus subtilis*. Linear gradient elution of acetonitrile was used.

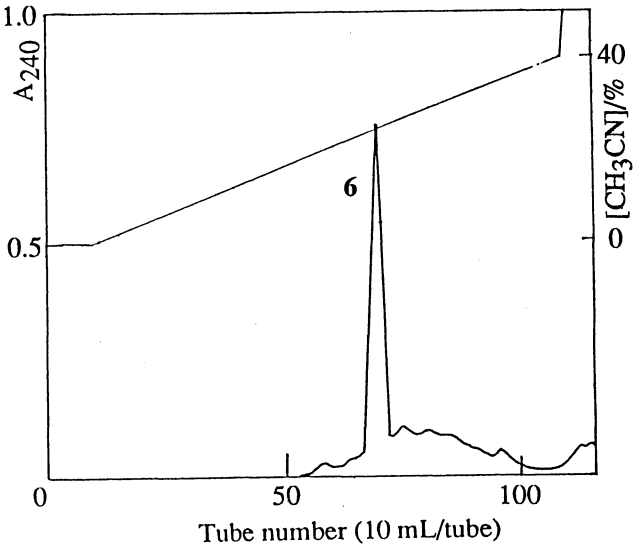

Fig. 2. Reversed-phase column chromatography (Lobar Column Lichroprep RP18, size B, Merck) of the mixture obtained by the reaction of **1** with bacterial α-amylase (saccharifying type) of *Bacillus subtilis*. Linear gradient elution of acetonitrile was used.

acetic anhydride-pyridine in 80% yield. Analysis of the fragmentation pattern in EI mass spectrum demonstrated that the 2-*O*-methylglucose unit was located at the center of the maltotriose structure.

The formation of **7a,b** from **2a,b**, respectively, demonstrated that the position of the modified glucoside unit in the oligosaccharide and the chain length of the oligosaccharide depend on the position of the modified hydroxyl group, but not the bulkiness of the modified group.

Although **8** is produced also by the TAA-catalyzed hydrolysis of **3**,[3] the others (**6**, **7a**, **9**, and **10**) are particular products which are obtained by BSA-catalysis of the β-cyclodextrin derivatives. Furthermore, these compounds are troublesome to be prepared by purely chemical synthesis, but can be prepared in one-step by BSA-catalysis. The present results are valuable for determining the regiochemical structure of various modified β-cyclodextrins which cannot be determined by TAA hydrolysis method. For example, TAA hydrolysis cannot discriminate among $2^{A},6^{C}$-, $2^{A},6^{D}$-, and $2^{A},6^{E}$-bis(*O*-mesitylenesulfonyl)-β-cyclodextrin since TAA converts them to an equimolar mixture of 2''-*O*-(mesitylenesulfonyl)maltotriose and 6'-*O*-(mesitylenesulfonyl)maltose. However, since only the $2^{A},6^{E}$-isomer (**11**) can produce 2'-*O*-(mesitylenesulfonyl)maltotriose and 6'-*O*-(mesitylenesulfonyl)maltotriose by BSA-catalyzed hydrolysis, this particular disubstituted isomer can be easily discriminated from the other isomers.[5]

Very similar results to those described in this report have been obtained by the reaction of γ-cyclodextrin derivatives with porcine pancreatic amylase (PPA)[4] which cannot hydrolyze the β-cyclodextrin analogs, suggesting that BSA and PPA are very similar in the number of the subsites and in reaction mechanism to each other, but are different in the steric arrangement of the substrate binding site.

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