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Preparation of $3^A,6^A$-Anhydro-β-cyclodextrin and Its Taka Amylolysis

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$3^A,6^A$-Anhydro-β-cyclodextrin was prepared by the reaction of 6-O-(p-tosyl)-β-cyclodextrin with aqueous alkali. This anhydrocyclodextrin was enzymatically hydrolyzed by Taka amylase to give 3",6"-anhydromaltotetraose exclusively.

Cyclodextrins are enzymatically hydrolyzed by Taka-amylase.[1] Melton and Slessor reported that 6-O-substituted α-cyclodextrins were hydrolyzed by Taka-amylase to give selectively 6'-O-substituted maltoses.[2] We also found that 6-O-substituted α- or β-cyclodextrins,[3] 2-O-substituted α- or β-cyclodextrins,[4] or 3-O-substituted α- or β-cyclodextrins[4] gave a specifically substituted maltooligosaccharide. Although providing information about interactions between substrates and the subsites of Taka-amylase and developing one-step synthetic method for substituted oligosaccharides, these studies are limited to the systems where all glucose units in cyclodextrins possess $^4C_1$ conformations. If a novel cyclodextrin derivative which contains a building block different from the normal $^4C_1$ glucose unit as a component of the macrocyclic structure is used as a substrate for the enzymatic hydrolysis, it will provide new information about the enzyme and a preparation method for a new type of oligosaccharide. We describe here synthesis of a novel cyclic oligosaccharide, $3^A,6^A$-anhydro-β-cyclodextrin which contains a 3,6-anhydroglucose

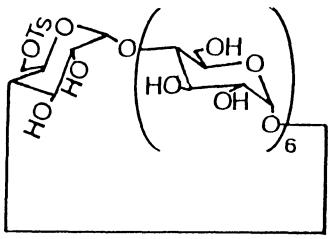
unit with $^1C_4$ conformation[5] as one of the structure components of the macrocycle and describe also its Taka amylolysis to give 3",6"-anhydromaltotetraose.

A solution of 6-O-(p-tosyl)-β-cyclodextrin (**1**) (800 mg) in 1 mol dm^{-3} NaOH or saturated aqueous $Ba(OH)_2$ (10 mL) was kept at 40 °C. After 11 h, the starting material disappeared completely and a new spot was observed on silica gel TLC around the R_f value of β-cyclodextrin. The mixture was neutralized by addition of dilute HCl or H_2SO_4, filtered and applied on a reverse-phase column (Lobar Column LiChroprep RP8, size B, Merck). After eluting with H_2O (500 mL), 1% aqueous MeOH (300 mL), and 3% aqueous MeOH (300 mL), 7% aqueous MeOH (1100 mL) and then 20% aqueous MeOH (300 mL) were applied. The elution of the 20% aqueous MeOH gave β-cyclodextrin (57.8 mg, 8.2%). The elution of 7% MeOH gave a novel product (609 mg, 87.9%) whose R_f value on TLC (0.09) was slightly smaller than that of β-cyclodextrin (0.11). This compound was assigned to $3^A,6^A$-anhydro-β-cyclodextrin (**2**) on the basis of the following spectral data. Its FABMS spectrum showed a correct molecular ion ($M + H^+$) at m/z 1117. The ^{13}CNMR spectrum of **2** and the INEPT ^{13}C NMR spectrum demonstrated the presence of a unique methylene carbon at δ 68.2 other than normal methylene carbons (-CH_2OH, δ 59.8). The chemical shift of the unique carbon was very close to that of a methylene carbon (C_6) in methyl 3,6-anhydro-α-D-glucoside **3**[6] (δ68.1), demonstratingthat the unique carbon was the carbon of the 3,6-anhydro-bridge. The ^1H NMR spectrum (400 MHz) of **2** gave a decisive evidence of its 3,6-anhydroglucoside structure (Fig. 1A). This was similar to that of methyl 3,6-anhydro-α-D-glucoside **3** (Fig. 1B). The absorptions of **2** and **3** were easily assigned by the decoupling experiments and by comparing their coupling constants with reported ones of methyl 3,6-anhydro-β-D-glucoside[7] and methyl 2,4-di-O-acetyl-3,6-anhydro-α-D-glucoside[8] (Table 1).[9]

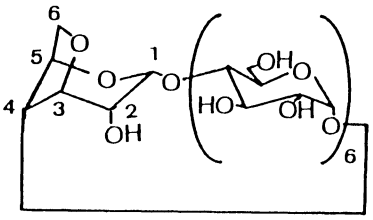
The anhydrocyclodextrin **2** was enzymatically hydrolyzed by Taka amylase as follows. A solution of **2** (50 mg) and Taka amylase (50 mg, amylase Type IV, Sigma) in 5 mL of acetate buffer (pH 5.5, 0.2 mol dm^{-3}) containing $CaCl_2$ (0.01 mol dm^{-3}) was kept at 40 °C for 72 h. After usual workup procedure,[4] the mixture was chromatographed with a reverse-phase column to give 3",6"-anhydromaltotetraose (25 mg, 85.4%), whose FAB mass spectrum showed a correct molecular ion. The structure determination of the oligosaccharide was carried out

as follows. The oligosaccharide was reduced with $NaBH_4$ to give the glucitol derivative, which was completely acetylated and analyzed by EI mass spectroscopy. The fragmentation pattern of the mass spectrum showed clearly that the 3,6-anhydroglucose unit was located at the second from the nonreducing end of the oligosaccharide.

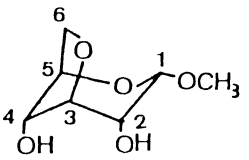
Since 3-O-substituted cyclodextrins and 6-O-substituted cyclodextrins gave



1



2



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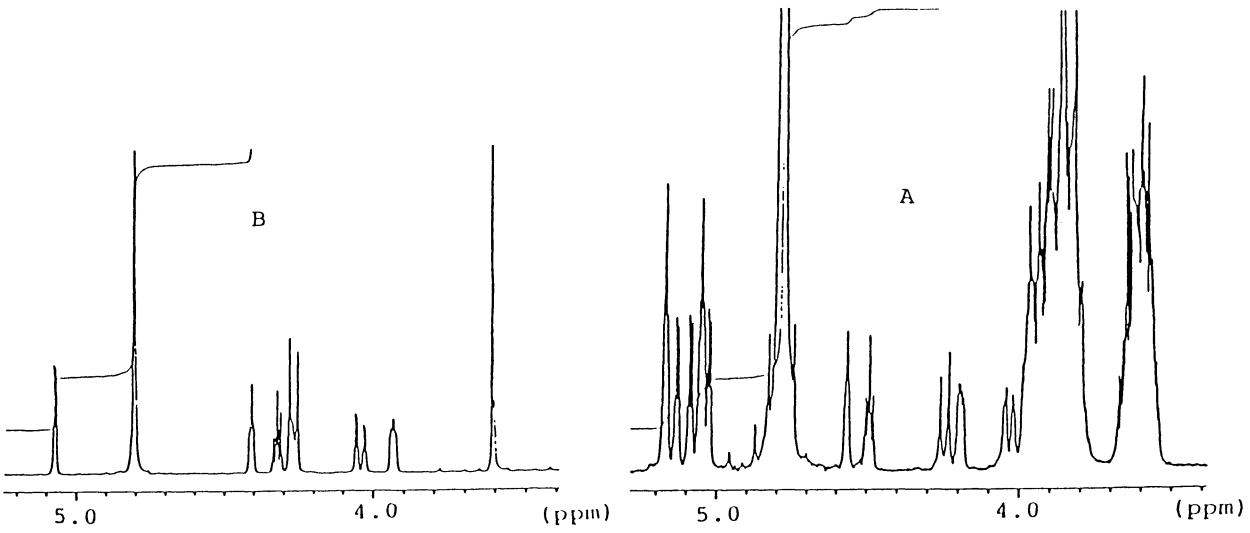

Fig. 1. ^1H NMR spectra (400 MHz) of $3^A,6^A$-anhydro-β-cyclodextrin **2** (A) and methyl 3,6-anhydro-α-D-glucoside **3** (B) in D_2O.

Table 1. 400 MHz ^1H NMR Chemical Shifts and Coupling Constants of $3^A,6^A$-Anhydro-β-cyclodextrin (**2**) and Methyl 3,6-Anhydro-α-D-glucoside (**3**) in D_2O

	Chemical shift (δ, ppm)							Coupling constant/Hz						
	H_1	H_2	H_3	H_4	H_5	H_6	$H_{6'}$	J_{12}	J_{23}	J_{34}	J_{45}	J_{56}	$J_{56'}$	$J_{66'}$
2	5.18	≈3.9	4.49	4.19	4.57	4.04	4.25	≈2.6	4.8	4.8	2.0	2.4	0	11.2
3	5.07	3.94	4.32	4.27	4.41	4.04	4.27	2.6	4.8	4.8	2.8	2.8	0	10.8

3'-substituted maltotrioses and 6'-substituted maltose, respectively,[2-4] in the enzymatic hydrolysis, 3,6-O-substituted β-cyclodextrin such as **2** is expected to afford enzymatically 3',6'-O-substituted maltotriose. However, the exclusive formation of 3",6"-anhydromaltotetraose was observed, demonstrating that the $^1C_4$ conformation of the 3,6-anhydroglucose unit was important factor controlling the cleavage pattern of the oligosaccharide by Taka amylase. Also, the substitution of a $^1C_4$ anhydroglucose unit for a $^4C_1$ normal glucose unit of cyclodextrins will develop a new type of sugar host having unique cavity shape without C_n symmetry axis. The present results that $3^A,6^A$-anhydro-β-cyclodextrin can be easily prepared and that Taka amylolysis of the anhydrocyclodextrin gave exclusively 3",6"-anhydromaltotetraose will be applicable to determination of regiochemistry of $6^A,6^X$-O-di(sulfonyl)-γ-cyclodextrins and 6^A-O-sulfonyl-6^X-substituted-β-cyclodextrins. These results will be reported in the near future.

References

1) K. Suetsugu, S. Koyama, K. Takeo, and T. Kuge, J. Biochem., 76, 57 (1974).

2) L. D. Melton and K. N. Slessor, Can. J. Chem., 51, 327 (1973).

3) K. Fujita, A. Matsunaga, and T. Imoto, Tetrahedron Lett., 1984, 5533; I. Tabushi, T. Nabeshima, K. Fujita, A. Matsunaga, and T. Imoto, J. Org. Chem., 50, 2638; K. Fujita, A. Matsunaga, Y. Ikeda, and T. Imoto, Tetrahedron Lett., 1985, 6439; K. Fujita, A. Matsunaga, and T. Imoto, J. Am. Chem. Soc., 106, 5740 (1984).

4) K. Fujita, T. Tahara, S. Nagamura, T. Imoto, and T. Koga, J. Org. Chem. 52, 636 (1987).

5) A X-ray crystallographic study on the structure of methyl 3,6-anhydroglucoside; B. Lindberg, B. Lindberg, and S. Svensson, Acta Chem. Scand., 27, 373 (1982).

6) W. H. Haskins, R. M. Hann, and C. S. Hudson, J. Am. Chem. Soc., 68, 628 (1980).

7) S. Angyal and Y. Kondo, Carbohydr. Res., 81, 35 (1980).

8) J. Defaye and D. Horton, Carbohydr. Res., 65, 283 (1978).

9) Although the H_2 signal was overlapped on the signals of the other glucose units, its presence was evidenced by the decouplings with H_1 and H_3.

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