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Regioisomeric $6^A,6^X,6^Y$-Tri-O-sulfonylated β-Cyclodextrin

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All of five regioisomeric $6^A,6^X,6^Y$-tri-O-(p-toluenesulfonyl)-β-cyclodextrins were prepared, isolated, and their structures were determined through a combination of the extended Körner method, 3,6-anhydration, and the Taka amylase A-catalyzed hydrolysis.

Regiospecifically $6^A,6^X$-di-O-sulfonylated cyclodextrins have attracted much attention as materials for preparing refined and sophisticated models of enzymes and receptors. The methods for their preparation were developed by Tabushi et al.[1] and Fujita et al.,[2] where the additional transannular disulfonylation of the transannularly disulfonylated cyclodextrins,[1] the extended Körner method,[2a,3] and the hydrolysis by Taka amylase A[2c] were used in determination of their regiochemical structures. However, for determining the structures of the trisulfonylated cyclodextrins which are more interesting materials, application of the first method is impossible and the others are not perfect, i.e., the extended Körner method cannot differentiate the $6^A,6^B,6^D$-isomer from the $6^A,6^B,6^F$-isomer (see Scheme 1), and the hydrolysis by Taka amylase A is applicable only for $6^A,6^B,6^C$-isomer. In special case, the $6^A,6^C,6^E$-isomer was assigned on the basis of its symmetry which was shown in the ^{13}C NMR spectrum.[3]

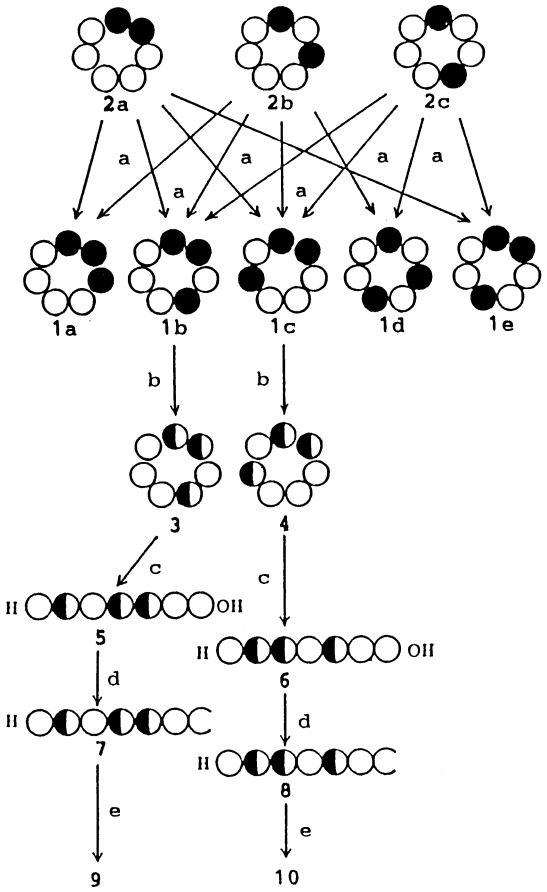
We describe here an effective method for determining the regiochemical structures of $6^A,6^X,6^Y$-tri-O-sulfonylated cyclodextrins.

A mixture of β-cyclodextrin and p-toluenesulfonyl chloride in pyridine was stirred at room temperature. The progress of reaction was monitored by silica gel TLC (H_2O/n-C_3H_7OH/AcOEt 5/7/7 (v/v/v)). After addition of water, the mixture was concentrated in vacuo, dissolved in 20% aqueous CH_3CN, and chromatographed on a reverse-phase column (ERC ODS 2922 column, 20 mm x 250 mm, 10 μm, Erma) with gradient elution from 20% aqueous CH_3CN (1 L) to 25% aqueous CH_3CN (1 L) followed by gradient elution from 25% aqueous CH_3CN (2 L) to 33% aqueous CH_3CN (2 L) to give **1**a-e. A regioisomeric mixture of the $6^A,6^X,6^Y$-tri-O-(p-toluenesulfonyl)-β-cyclodextrins was also prepared from the reaction of $6^A,6^X$-di-O-(p-toluenesulfonyl)-cyclodextrins **2**b,c. The numbers **1**a-**1**e of the tri-sulfonates are given in order of decreasing retention time in reverse-phase column chromatography. These reaction conditions and results are summarized in Table 1. R_f value on TLC; **1**a, 0.51; **1**b-e, 0.54. The fast-atom-bombardment mass spectra (hereafter abbreviated as FABMS) of each trisulfonate showed the correct molecular ion.

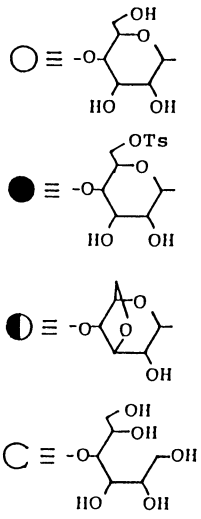
Table 1. Preparation of $6^A,6^X,6^Y$-tri-O-(p-toluenesulfonyl)-β-cyclodextrins[a)]

CD or CD derivative[b)]/mg	p-TsCl[c)] /mg	Py[d)] /mL	Reaction time/h	Trisulfonate/mg 1a	1b	1c	1d	1e
β-CD[f)] 500	800	5	3	10.8 (1.5%)	4.9[e)] (0.7%)	5.2[e)] (0.7%)	18.5 (2.6%)	7.8 (1.1%)
2b[g)] 220	120	2.2	2	6.7 (2.8%)	⌊ 15.1 ⌋ (6.2%)		23.4 (9.6%)	-
2c[h)] 300	150	3	2	15.8 (4.8%)	⌊ 16.9 ⌋ (5.1%)		-	11.0 (3.3%)

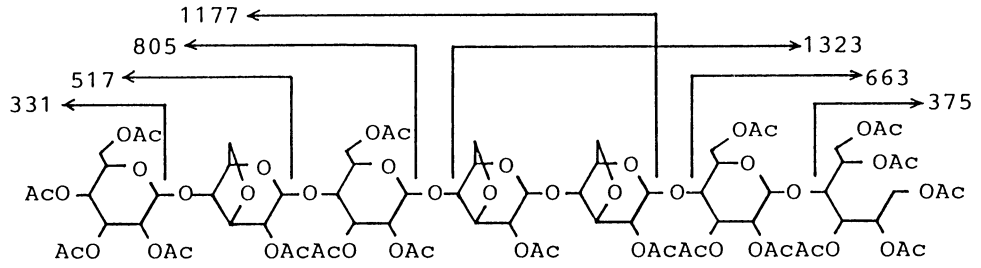
a) At room temperature. b) CD represents cyclodextrin. c) p-Toluenesulfonyl chloride. d) Pyridine. e) **1b** and **1c** were not completely separated by the reverse-phase column. The amount of pure trisulfonate which was obtained by collecting only desirable fractions is shown here. A mixture of **1b** and **1c** (13.3 mg, 1.9%) was also obtained by collecting other fractions. f) 6-Monosulfonate (135.7 mg, 23.97%), $6^A,6^B$-disulfonate (63.2 mg, 9.9%), $6^A,6^C$-disulfonate (73 mg, 11.5%), and $6^A,6^D$-disulfonate (71.7 mg, 11.3%) were also obtained. g) The starting material (87.3 mg, 39.7%) was recovered. h) The starting material (132.9 mg, 44.3%) was recovered.



(a) p-TsCl/Py
Ts = toluenesulfonyl
Py = pyridine
(b) 0.1 N $Ba(OH)_2$
(c) Taka amylase A
(d) $NaBH_4$
(e) $(CH_3CO)_2O$/Py



Scheme 1. Structure determination of $6^A,6^X,6^Y$-trisulfonates **1a-e**.



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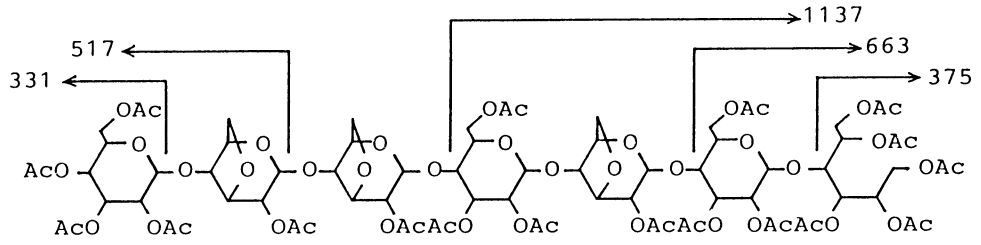

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Fig. 1. Fast-atom-bombardment mass spectral fragmentation patterns of **9** and **10**.

The ^{13}C NMR and ^{1}H NMR spectra of **1**a-e demonstrated that each compound possessed three p-toluenesulfonyl groups per cyclodextrin moiety. Although the spectra of the regioisomers are different from one another, we cannot assign each regiochemical structures from these differences.

The structures were determined through the extended Körner method where additional toluenesulfonylation of the known disulfonates **2**a-c[2c] was carried out. Theoretically, the $6^A,6^B$-disulfonate (**2**a), the $6^A,6^C$-disulfonate (**2**b), or the $6^A,6^D$-disulfonate (**2**c) should give a mixture of $6^A,6^B,6^C$-, $6^A,6^B,6^D$-, $6^A,6^B,6^F$-, and $6^A,6^B,6^E$-trisulfonates, a mixture of $6^A,6^B,6^C$-, $6^A,6^B,6^D$-, $6^A,6^B,6^F$-, and $6^A,6^C,6^E$-trisulfonates, or a mixture of $6^A,6^B,6^D$-, $6^A,6^B,6^F$-, $6^A,6^C,6^E$-, and $6^A,6^B,6^E$-trisulfonates, respectively.

The results of the reverse-phase HPLC analysis of the mixture obtained from the additional sulfonylation of each disulfonate are summarized in Scheme 1. From this Scheme, the trisulfonates **1**a-e are assigned to $6^A,6^B,6^C$-, $6^A,6^B,6^D$- (or $6^A,6^B,6^F$-), $6^A,6^B,6^F$- (or $6^A,6^B,6^D$-), $6^A,6^C,6^E$-, and $6^A,6^B,6^E$-isomers, respectively. However, in order to make this assignment complete, we must develop a novel method for differentiating the $6^A,6^B,6^D$-isomer from the $6^A,6^B,6^F$-isomer because the extended Körner method can not do it theoretically as shown in Scheme 1. A direct Taka amylase A-catalyzed hydrolysis of each trisulfonate is not expected to be helpful for this purpose since it will give a mixture of 6',6"-di-O-(p-toluenesulfonyl)-maltotriose, 6'-O-(p-toluenesulfonyl)-maltose, and glucose from each trisulfonate **1**b,c.[5]

Recently, Fujita and coworkers reported preparation of $3^A,6^A$-anhydro-β-cyclodextrins from the reaction of 6-O-(p-toluenesulfonyl)-β-cyclodextrin with

aqueous alkali.[6] They also showed that the enzymatic hydrolysis of $3^A,6^A$-anhydro-β-cyclodextrin by Taka amylase A gave 3",6"-anhydromaltotetraose and glucose selectively.[6] This method is applied to the present case. By treatment with aqueous $Ba(OH)_2$, the trisulfonates **1**b and **1**c were converted to the anhydrides **3** and **4**, respectively. Their FABMS showed the correct molecular ions. The Taka amylase A-catalyzed hydrolysis of **3** or **4** gave the 3,6-tri-anhydromaltoheptaose **5** or **6**, respectively, whose FABMS showed the correct molecular ions.

The anhydromaltoheptaose **5** or **6** was reduced with aqueous $NaBH_4$ to the corresponding glucitol derivative **7** or **8**, which was completely acetylated with acetic anhydride in pyridine to give the octadecaacetate **9** or **10**, respectively. The acetates **9** and **10** were analyzed by FABMS to determine the positions of the 3,6-anhydroglucose parts. The results (Fig. 1) demonstrate that the three 3,6-anhydroglucose units of **5** or **6** are located at the second, the fourth, and the fifth units, or the second, the third, and the fifth units from the non-reducing unit, respectively, and therefore that the original trisulfonates **1**b and **1**c are the $6^A,6^B,6^D$-isomer and the $6^A,6^B,6^F$-isomer, respectively.

In conclusion, all regioisomeric $6^A,6^X,6^Y$-trisulfonates of β-cyclodextrin were isolated and their structures were assigned through a combination of the extended Körner method, the 3,6-anhydration, and the specific enzymatic hydrolysis method. This method of structure determination will be applicable for more complex pattern of substitution including 6-O-sulfonylations. From the isomers described here, artificial enzymes or receptors which have three functional groups at the given positions will be prepared.

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References

1) I. Tabushi, Y. Kuroda, K. Yokota, and L. C. Yuan, J. Am. Chem. Soc., 103, 711 (1981); I. Tabushi and L. C. Yuan, ibid., 103, 3574 (1981); I. Tabushi, T. Nabeshima, K. Fujita, A. Matsunaga, and T. Imoto, J. Org. Chem., 50, 2638 (1985).

2) a) K. Fujita, A. Matsunaga, and T. Imoto, J. Am. Chem. Soc., 106, 5740 (1984); b) K. Fujita, H. Yamamura, A. Matsunaga, and T. Imoto, ibid., 108, 4509 (1986); c) K. Fujita, A. Matsunaga, and T. Imoto, Tetrahedron Lett., 25, 5533 (1984); d) K. Fujita, H. Yamamura, and T. Imoto, J. Org. Chem., 53, 1943 (1988).

3) Körner assigned disubstituted benzenes by their conversion to the tri-substituted benzenes. G. Körner, Gazz. Chim. Ital., 4, 305 (1874).

4) J. Boger and J. R. Knowles, J. Am. Chem. Soc., 101, 7631 (1979).

5) 6-Substituted cyclodextrins gave 6'-substituted maltose and glucose. L. D. Melton and K. N. Slessor, Can. J. Chem., 51, 327 (1973). See also Refs. 1c, 2a, and 2c. $6^A,6^C$-Disubstituted or $6^A,6^D$-disubstituted cyclodextrins gave 6'-substituted maltose and glucose. See Refs. 1c and 2c.

6) K. Fujita, H. Yamamura, T. Imoto, and I. Tabushi, Chem. Lett., 1988, 543.

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