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^{1}H NMR Study of the Regioisomers of Primary *O*-Di- and Trisubstituted α-Cyclodextrins

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The regioisomers of bis- and tris[C(6)-*O*-sulfonylated] α-cyclodextrins were converted in satisfactory yields to the corresponding C(6)-pyridinio derivatives, which afforded the characteristic splitting patterns of ^{1}H NMR signals available for a regioisomer determination. Electrostatic repulsive interactions between the positively charged pyridinio groups play an important role in the remarkable splitting of the ^{1}H NMR signals observed for some regioisomers.

O-Arylsulfonylated cyclodextrins are the key intermediates from which a number of other cyclodextrin derivatives have been prepared.[1] In particular, primary *O*-bis- or poly(arylsulfonylated) cyclodextrins have attracted much attention regarding the construction of refined artificial enzymes[2] or artificial receptors.[3] Polysubstituted cyclodextrins generally involve a fair number of regioisomers. For example, bis- and tris[C(6)-*O*-sulfonylated] α-cyclodextrins involve three and four regioisomers, respectively. They are designated as 6A,6B-, 6A,6C-, and 6A,6D-isomers for disubstituted α-cyclodextrins and 6A,6B,6C-, 6A,6B,6D-, 6A,6B,6E-, and 6A,6C,6E-isomers for trisubstituted α-cyclodextrins. Recently, Fujita et al.[4] have succeeded in separating a mixture of the regioisomers of C(6) polysulfonylated α-cyclodextrins from one another and determining the position of the substituents in them. In this regioisomer determination, however, ^{1}H NMR spectroscopy played only a minor role, owing to the similarity and/or complexity of the spectra.

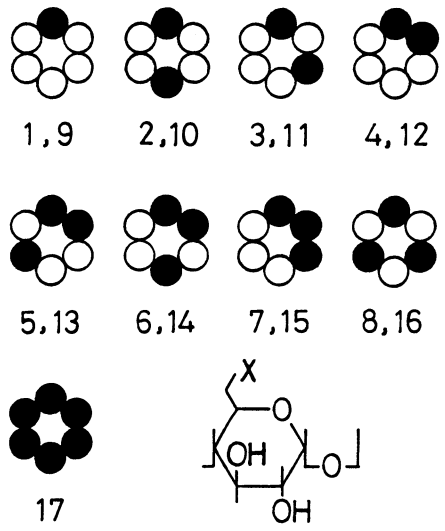

Fig. 1. α-Cyclodextrin derivatives examined.
○: X=-OH (**1**—**16**)
●: X= -OSO$_2$-(Me, Me) (**1**—**8**)
●: X= -N$^+$ (**9**—**17**)

In the course of an investigation of charged cyclodextrins,[5] we recently found that the regioisomers of bis- and tris[C(6)-*O*-arylsulfonylated] α-cyclodextrins are converted in satisfactory yields to the corresponding pyridinio derivatives, the ^{1}H NMR spectra of which afford more abundant structural information than those of the *O*-arylsulfonylated α-cyclodextrins. The present article deals with the structural information obtained from the ^{1}H NMR spectra of the hydrogencarbonate salts of mono-, bis-, and tris[6-(1-pyridinio)-6-deoxy]-α-cyclodextrins (**9**—**16**) as well as the ^{1}H NMR spectra of mono-, bis-, and tris(6-*O*-mesitylsulfonyl)-α-cyclodextrins (**1**—**8**), as shown in Fig. 1. The ^{1}H NMR spectra of the hydrogencarbonate salts of hexakis[6-(1-pyridinio)-6-deoxy]-α-cyclodextrins (**17**) and methyl 6-(1-pyridinio)-6-deoxy-α-D-glucopyranoside (**18**) were also examined for the sake of comparison. Brief ^{1}H and ^{13}C NMR data for **2**—**8** have been reported by Fujita et al.[4b]

Experimental

Materials. α-Cyclodextrin was kindly supplied by Nihon Shokuhin Kako Co., Ltd., and was dried overnight in vacuo at 110 °C. Reagent-grade pyridine was dried over calcium hydride and distilled in the presence of fresh calcium hydride just before use. Reagent-grade mesitylenesulfonyl chloride was used without further purification. Deuterium oxide (Merck, 99.75%), dimethyl-d_6 sulfoxide (DMSO-d_6, CEA, 99.8%), tetramethylsilane (TMS), and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) were available for NMR use.

Apparatus. ^{1}H NMR spectra were recorded using a JEOL Model JNM-GX270 FT NMR spectrometer (270 MHz). Absorption spectra and optical rotation were measured with a Hitachi Model 220 spectrophotometer and a Union Giken Model PM-101 polarimeter, respectively. High-performance liquid chromatography (HPLC) was performed on a Hitachi Model 683-56 chromatograph.

Preparation of 1—8. *O*-Mesitylsulfonylated α-cyclodextrins **1**—**8** were prepared and separated from one another according to the method of Fujita et al.[4] A prepacked column (Merck, Lober column LiChroprep RP-8, 25×310 mm) was used for reversed-phase column chromatography to separate

a mixture of **1**—**8** from one another. Aqueous methanol solutions were used as eluents. A silica-gel column (Wako, C-200, 50×350 mm) was used for a further separation of **8** from **7**.[4b] The eluates of these column chromatographies were assayed by UV spectrophotometry at 275 nm. In addition, the main fractions were analyzed by HPLC with a LiChrosorb RP-18 column (Merck, 4×250 mm) and aqueous acetonitrile solutions as eluents. Typical chromatograms are shown in Fig. 2. All the peaks, except peaks 5 and 6, have been assigned in terms of their regiochemistries:[4] Peak 1 corresponds to monosulfonylated α-cyclodextrin **1**, peaks 2, 3, and 4 to disulfonates **2**, **3**, and **4** respectively, and peaks 7 and 8 to trisulfonates **7** and **8**, respectively. Peaks 5 and 6 have been attributed to the trisulfonylated regioisomers, **5** and **6**, but a discrimination between them has not been accomplished. As will be mentioned later, the ^{1}H NMR spectra of tripyridinio-α-cyclodextrins **13** and **14** derived from **5** and **6**, respectively, suggested that **5** is a 6A,6B,6E-isomer and **6** is a 6A,6B,6D-isomer. Here, we tentatively assume that peak 5 corresponds to regioisomer **5** and peak 6 to **6**. Purified samples of **5**—**8** were obtained by a repeated collection of the main peak fractions in the HPLC, followed by an evaporation of the fractions to dryness.

Preparation of 9—16. A typical run was as follows: A purified sample of **2** (140 mg, 0.105 mmol) was dissolved in dry pyridine (10 cm^3) and the resulting solution was refluxed with stirring for 5 h. After removing pyridine by evaporation in vacuo at 40 °C, a mixed solvent of acetone–water [9 : 1 (v/v)] was added to the residue. A resulting precipitate (156 mg) was separated by filtration, dissolved in water (2 cm^3), and chromatographed on a 15×130 mm CM-cellulose (Serva) column with the stepwise elution of 0.05, 0.10, 0.25, and 0.50 mol dm^{-3} aqueous ammonium hydrogencarbonate solutions. The elution was followed by UV-spectrophotometry at 270 nm and polarimetry at 589 nm. The eluates of 0.10 mol dm^{-3} ammonium hydrogencarbonate were combined and evaporated to dryness at 40 °C to give **10** (113 mg, 0.093 mmol, 89% yield). Likewise, **9** was obtained from the eluates of 0.05 mol dm^{-3} ammonium hydrogencarbonate in 80% yield, **11** and **12** from the eluates of 0.10 mol dm^{-3} ammonium hydrogencarbonate in 88 and 86% yields, respectively, and **13**, **14**, **15**, and **16** from the eluates of 0.25 mol dm^{-3} ammonium hydrogencarbonate in 82, 85, 73, and 23% yields, respectively. Each product gave a virtually single peak in HPLC on a TSKgel CM-5PW column (Toyo Soda, 7.5×75 mm) with 0.40 or 0.50 mol dm^{-3} ammonium hydrogencarbonate as an eluent.

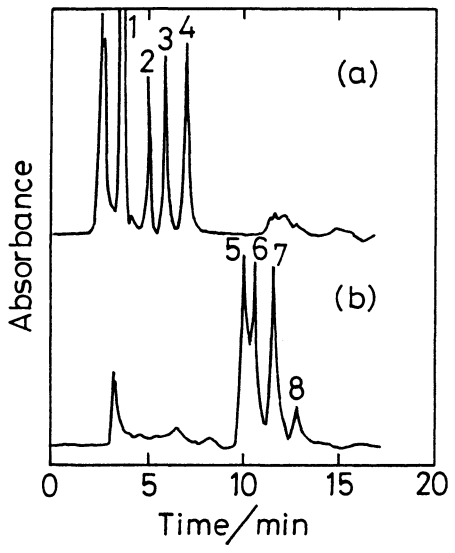

Fig. 2. The HPLC of **1**—**8** with a LiChrosorb RP-18 column and 40% (a) or 50% (b) aqueous acetonitrile as an eluent. The eluates were assayed by UV spectrophotometer at 275 nm.

Preparation of 17. Hexakis(2,3-di-*O*-acetyl-6-bromo-6-deoxy)-α-cyclodextrin (**19**) was prepared by the method of Takeo et al.[6] A solution of **19** (5.00 g, 2.7 mmol) in dry pyridine (50 cm^3) was refluxed with stirring for 30 h. A tar-like material was separated out from the solution during refluxing and was solidified upon cooling to room temperature. The solid was separated by filtration, dissolved in 25% aqueous ammonia solution, and allowed to stand for 0.5 h at room temperature to complete deacetylation. The solution was evaporated to dryness in vacuo to give a dark-brown residue (4.47 g). An aliquot (1.20 g) of the residue was chromatographed on a 20×280 mm Toyopearl CM 650S column (Toyo Soda). A stepwise elution was applied with 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, and 0.50 mol dm^{-3} aqueous ammonium hydrogencarbonate solutions. Each fraction (10 cm^3) was assayed by UV spectrophotometry at 270 nm and polarimetry at 589 nm. Eluates given by 0.20 mol dm^{-3} ammonium hydrogencarbonate were combined and evaporated to dryness in vacuo at 40 °C to afford crude **17** (0.92 g, 0.54 mmol, 74% yield based on **19**). The HPLC of the product with a TSKgel CM-5PW column and 0.40 mol dm^{-3} hydrogencarbonate as an eluent gave a high main peak, though a few additional low peaks were also observed before and after the main peak. A purified sample of **17** was obtained by a repeated collection of a main peak fraction in the HPLC; then, the fraction was evaporated to dryness.

Preparation of 18. Methyl 6-*O*-(*p*-tolylsulfonyl)-α-D-glucopyranoside (5.00 g, 14.4 mmol) was dissolved in dry pyridine (100 cm^3) and heated at 100 °C for 5 h. After removal of pyridine by evaporation in vacuo at 40 °C, the residue was dissolved in 50 cm^3 of distilled water. To the solution was added Amberlite IR-120B (Na form, 15 cm^3); the mixture was stirred overnight at room temperature. The ion-exchange resin was separated by filtration, washed well with distilled water, and then added to 0.5 mol dm^{-3} aqueous ammonium hydrogencarbonate solution (100 cm^3). The mixture was stirred overnight at room temperature. After removal of the ion-exchange resin by filtration, the filtrate was evaporated to dryness in vacuo at 40 °C to give a crude product (4.23 g, 92% yield) of **18**. A purified sample of **18** was obtained by column chromatography with a 45×430 mm CM-cellulose (Serva) column and 0.05 mol dm^{-3} ammonium hydrogencarbonate as an eluent.

Results and Discussion

^{1}H NMR Spectra of 1—8. The ^{1}H NMR spectrum of the 6A,6B,6C-trisulfonylated isomer **7** (43 mmol dm^{-3}) in DMSO-d_6 at 50 °C is shown in Fig. 3 as being typical. In the spectrum, the absorptions at about δ=7.1, 2.5, and 2.3 were assigned to the meta-H's, ortho-methyl-H's, and para-methyl-H's, respectively, of the mesityl groups on the basis of a comparison with the spectrum of sodium mesitylenesulfonate.

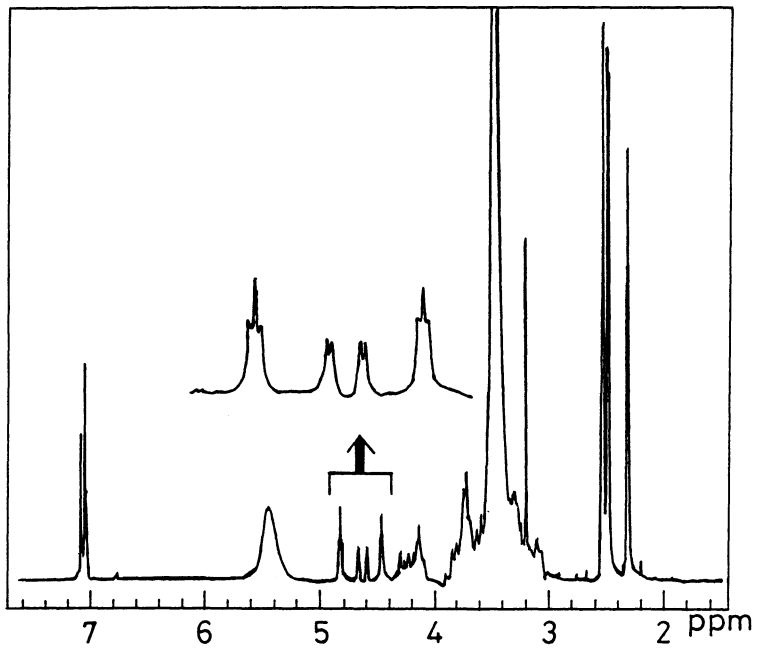

Fig. 3. 270 MHz ^{1}H NMR spectrum of **7** in DMSO-d_6 at 50 °C.

Table 1. ^{1}H NMR Data for **1**—**8**[a)]

Comound	meta-H[b)]	para-Me[b)]	C(1)-H[c)]		
			Region 1[d)]	Region 2[e)]	Region 3[f)]
1	7.09(2H)	2.29(3H)		4.64(1H)	4.79(2H)
				4.68(1H)	4.81(2H)
2	7.07(4H)	2.29(6H)		4.67(2H)	4.81(2H)
				4.70(2H)	
3	7.05(2H)	2.29(3H)		4.65(1H)	4.80(1H)
	7.09(2H)	2.30(3H)		4.66(1H)	4.82(1H)
				4.68(1H)	
				4.70(1H)	
4	7.07(2H)	2.29(6H)	4.48(1H)	4.57(1H)	4.81(3H)
	7.09(2H)			4.64(1H)	
5	7.01(2H)	2.28(6H)	4.52(1H)	4.64(1H)	4.83(1H)
	7.02(2H)	2.30(3H)		4.69(1H)	
	7.09(2H)			4.72(1H)	
				4.74(1H)	
6	7.05(4H)	2.29(9H)	4.51(1H)	4.63(1H)	4.82(1H)
	7.06(2H)			4.68(1H)	
				4.70(1H)	
				4.73(1H)	
7	7.04(4H)	2.29(6H)	4.45(1H)	4.58(1H)	4.81(1H)
	7.07(2H)	2.30(3H)	4.46(1H)	4.66(1H)	4.82(1H)
8	7.08(6H)	2.30(9H)		4.64(3H)	
				4.70(3H)	

a) Chemical shift (δ/ppm from TMS) in DMSO-d_6 at 50 °C. The number of protons in a molecule were evaluated by the integration of peak area and were shown in parentheses. b) Singlet signal. c) Doublet signal with J=3.20±0.03 Hz. d) Higher field than δ=4.55. e) δ=4.55—4.75. f) Lower field than δ=4.75.

Protons involved in the glucopyranose (GP) moieties gave complex signals at δ=3—5. Among them, doublet signals at δ=4.4—4.9 (J=2.8—3.4 Hz) were relatively distinct and were assigned to anomeric C(1)-H's.

Table 1 shows the chemical shifts (δ) and the numbers of protons for the singlets of meta-H's and para-Me-H's, together with those for the doublets of C(1)-H's, in **1**—**8**. The δ values for ortho-Me-H's were ambiguous in some cases, owing to an overlapping of the signals with solvent signals; thus, data for the signals are omitted from the table. The effect of sample concentrations on the NMR spectra, examined for **1**—**4**, indicated that the δ values shown in the table were virtually independent, within a standard error of 0.01 ppm, of sample concentrations ranging from 10 to 44 mol dm^{-3}.[7)] The A,D-isomer **2** or A,C,E-isomer **8** gave

only a singlet at δ=ca. 7.1 for meta-H's and a singlet at δ=ca. 2.3 for para-Me-H's, suggesting that two mesityl groups of **2** or three mesityl groups of **8** are equivalent (isochronous). On the ^{1}H NMR time scale, the molecular structures of **2** and **8** in solution are regarded as being symmetrical, as would be expected from their structural formula. On the other hand, essentially asymmetrical molecules, such as **3**—**7**, gave two or three signals for meta-H's and one or two signals for para-Me-H's (within the resolution limit of the apparatus). The splitting of the signals indicates that the mesityl groups in the compounds are not equivalent. However, the width of the splitting was too narrow to obtain more information regarding their regiochemistries.

More detailed information was available from the splitting patterns of the doublets due to C(1)-H's. All the C(1)-H's of **1**—**8** gave doublets in the region of δ=4.45—4.85, which could be subdivided into three regions, i.e., higher field than δ=4.55 (Region 1), δ=4.55—4.75 (Region 2), and lower field than δ=4.75 (Region 3). It was found that the numbers (m) of C(1)-H's giving doublets in Regions 1—3 reflect the structural characteristics of **1**—**8**: Thus, the ratio of the m's in Regions 1, 2, and 3 was equal to 0:2:4 for the monosulfonate **1**, 0:4:2 for the disulfonates **2** and **3**, 1:2:3 for another disulfonate **4**, 1:4:1 for the trisulfonates **5** and **6**, 2:2:2 for **7**, and 0:6:0 for **8**. These characteristic splitting patterns are well explained on the basis of the following assumptions:

1) The C(1)-H's of unsubstituted GP rings generally give doublets in Region 3.[8]

2) If the A ring [GP(A)] of the GP's in α-cyclodextrin is sulfonylated at C(6)-OH, the C(1)-H of GP(A) is subject to significant shielding by the sulfonylation to give a doublet in Region 2. Furthermore, one of the C(1)-H's involved in GP's adjacent to GP(A) is also significantly shielded by a remote substituent effect to give another doublet in Region 2. The C(1)-H, thus shielded, may belong to GP(B), but not to GP(F), since the substituent is favorably oriented to GP(B) and not to GP(F).[9]

3) If both of vicinal GP(A) and GP(B) are sulfonylated, the C(1)-H of GP(B) is subject to strong shielding by both of the substituents in GP(A) and GP(B) to give a doublet in Region 1. The C(1)-H of GP(A) is significantly shielded by the substituent of GP(A), but little by that of GP(B), and give a doublet in Region 2.

Based on these assumptions, we estimated the number (n) of C(1)-H's belonging to each δ region for **1**—**8** (Table 2). For example, the GP's of A,C,E-isomer **8** are alternatingly sulfonylated, so that all the C(1)-H's should give doublets in Region 2. The A,B,C-isomer **7** bears three sulfonylated-GP's which are bound in series. Then, two C(1)-H's of GP(B) and GP(C) should give doublets in Region 1, those of GP(A) and GP(D) in Region 2, and those of GP(E) and GP(F) in Region 3. The n values, thus estimated, agreed entirely with those observed (Table 1), indicating that the above assumptions are reliable. These results also mean that the regioisomer determination by Fujita et al.[4] is substantiated by ^{1}H NMR spectroscopy, though a distinction between A,B,E-isomer **5** and A,B,D-isomer **6** still remained impossible owing to the similarity of their splitting patterns.

^{1}H NMR Spectra of 9—18. Upon refluxing pyridine solutions containing **1**—**8**, the mesitylenesulfo-

Table 2. The Estimated Number (n) of C(1)-H's Giving Doublets in Regions 1—3

Compound	Region 1[a]	Region 2[b]	Region 3[a]
1	0	2	4
2	0	4	2
3	0	4	2
4	1	2	3
5	1	4	1
6	1	4	1
7	2	2	2
8	0	6	0

a) Higher field than δ=4.55. b) δ=4.55—4.75. c) Lower field than δ=4.75.

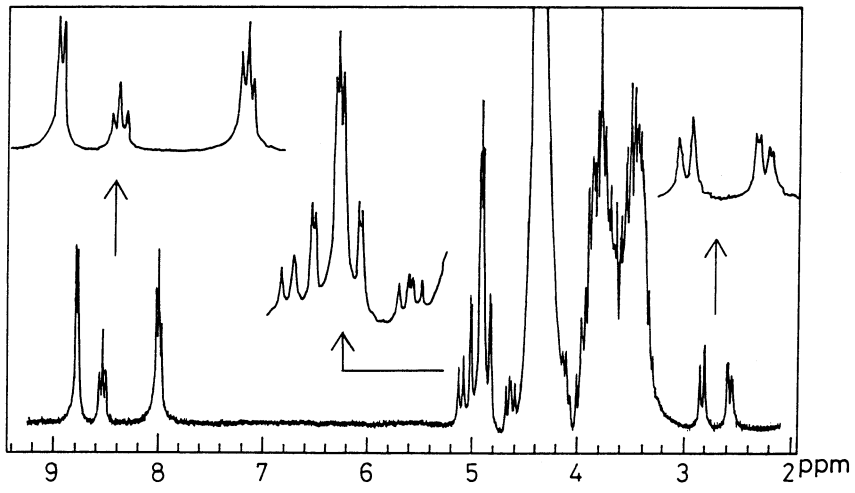

Fig. 4. 270 MHz ^{1}H NMR spectrum of **9** in D_2O at 50°C.

Table 3. ^{1}H NMR Data for **17**, **18**, and Unsubstituted α-Cyclodextrin (**20**)[a)]

Proton	**17**	**18**	**20**
C(1)-H	5.16 (6H, d, J=3.2)	4.77 (1H, d, J=3.7)	4.94(1H, d, J=3.2)
C(2)-H	3.59 (6H, dd, J=10.0, 3.2)	3.55 (1H, dd, J=9.8, 3.9)	3.51(1H, dd, J=9.9, 3.3)
C(3)-H	4.01 (6H, t, J=9.2)	3.69 (1H, t, J=9.3)	3.87(6H, t, J=9.3)
C(4)-H	3.44 (6H, t, J=9.0)	3.30 (1H, dd, J=10.0, 8.8)	3.48 (6H, t, J=9.5)
C(5)-H	4.82 (6H, m)	4.00 (1H, m)	ca. 3.8 (6H, m)
C(6)-H(a)	4.70 (6H, d, J=13.9)	5.06 (1H, dd, J=13.9, 2.7)	3.7—3.9 (6H, m)
C(6)-H(b)	4.23 (6H, dd, J=13.9, 6.6)	4.78 (1H, dd, J=13.8, 8.2)	3.7—3.9 (6H, m)
ortho-H	8.73 (12H, d, J=5.9)	8.89 (2H, d, J=5.4)	
meta-H	8.09 (12H, t, J=7.0)	8.12 (2H, t, J=7.2)	
para-H	8.46 (6H, t, J=7.7)	8.63 (1H, t, J=7.9)	
OMe-H		3.05 (3H, s)	

a) Chemical shift (δ/ppm from DSS) in D_2O at 50 °C. The unit of J is Hz.

nyloxy groups were replaced by the positively charged pyridinio groups to give **9**—**16** in satisfactory yields. Figure 4 illustrates the ^{1}H NMR spectrum of the monopyridinio derivative **9** (14 mmol dm^{-3}) in D_2O at 50 °C.[10] The ortho-, meta-, and para-protons of the pyridinio group gave a doublet at δ=8.76 (2H, J=5.6 Hz), a triplet at δ=7.99 (2H, J=7.0 Hz), and a triplet at δ=8.52 (1H, J=7.4 Hz), respectively. The protons of the GP moieties gave complex absorptions at δ=2.5—5.1. Among them, the following absorptions were relatively distinct: Doublets at δ=4.8—5.0 (6H, J=ca. 3.2 Hz) due to anomeric C(1)-H's, a pair of a double doublet signal (dd-signal) (1H, δ=4.64, J=13.3 and 9.6 Hz) and an apparently doublet signal (1H, δ=5.12, J=12.9 Hz) due to diastereotopic geminal C(6)-H's, and a pair of a dd-signal (1H, δ=2.54, J=12.0 and 2.9 Hz) and an apparently doublet signal (1H, δ=2.81, J=12.0 Hz) due to another diastereotopic geminal C(6)-H's. The pairing of the absorptions was confirmed by spin-spin decoupling experiments. The dd-signals reflect spin-spin coupling between the geminal C(6)-H's (J=12—13 Hz), together with that between vicinal C(5)-H and C(6)-H(trans) (J=3—10 Hz). The magnitude of coupling constants between vicinal C(5)-H and C(6)-H(gauche) is too small to be detected with the apparatus used, so that another C(6)-H apparently gives a doublet signal. Upon assigning the GP rings involving these C(6)-H's, the ^{1}H NMR spectra of **17**, **18**, and unsubstituted α-cyclodextrin (**20**) were instructive. They gave clear signals, most of which could be assigned (Table 3).[10] The geminal C(6)-H's of **20** gave signals around δ=3.7—3.9, whereas those of **17** or **18** gave a pair of signals in the lower-field region of δ=4—5, but no signal in the higher-field region of δ=2.5—2.8. Based on these data, we assigned the lower-field pair of **9** to the C(6)-H's of the substituted GP ring [GP(A)] and the higher-field pair to those of the unsubstituted GP [GP(B)] adjacent to GP(A). A molecular model study showed that the pyridinium ring is located in the close vicinity of the C(6)-H's of GP(B), when the C(5)-C(6) bond of GP(A) is held in the gauche-trans conformation, as illustrated in Fig. 5.[11] The striking upfield shift of the absorptions for

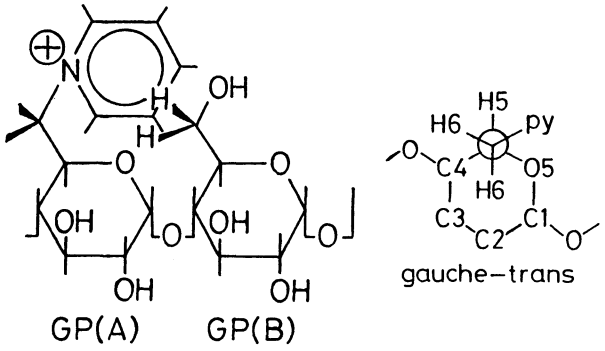

Fig. 5. The schematic diagram for the orientation of the pyridinio group in **9**.

the C(6)-H's of GP(B) in **9** compared with those for the C(6)-H's of **20** is attributable to the strong shielding by the ring current of the adjacent pyridinio group. On the other hand, the remarkable downfield shift observed for the C(6)-H's of GP(A) in **9** may be due to the deshielding effect of the positively charged pyridinio group directly bound to the C(6)-carbon. The vicinal coupling constant between C(5)-H and C(6)-H (trans) is larger in GP(A) (J=9.6 Hz) than in GP(B) (J=2.9 Hz), indicating that the rotation about the C(5)-C(6) bond is significantly restricted to a gauche-trans conformation in GP(A), but little in GP(B).[11]

Characteristic splitting patterns were observed for ^{1}H NMR signals due to the protons involved in the pyridinio groups of the di- and trisubstituted regioisomers of **10**—**16** (Table 4).[10] The A,D-isomer **10** gave the signals similar to those of **9**: A doublet at δ=8.75 for ortho-H's, a triplet at δ=7.97 for meta-H's, and a triplet at δ=8.49 for para-H's. The A,C-isomer **11** and A,B-isomers **12** gave more complex signals than **10**. Signals for ortho- and para-H's of **11** were split into two doublets at δ=8.79 and 8.70 (Δδ=0.09) and two triplets at δ=8.49 and 8.46 (Δδ=0.03) respectively, though meta-H's gave only a triplet at δ=7.96. The splittings of the signals for ortho- and para-H's were markedly enlarged in **12** to give two doublets at δ=8.79 and 8.46 (Δδ=0.33) and two triplets at δ=8.47 and 8.12 (Δδ=0.35). Furthermore, signals for meta-H's were

Table 4. ^{1}H NMR Data for Ortho-, Meta-, and Para-H's of the Pyridinio Groups in **9**—**17**[a)]

Compound	Ortho-H	Meta-H	Para-H
9	8.76 (2H, d, J=5.6)	7.99 (2H, t, J=7.0)	8.52 (1H, t, J=7.4)
10	8.75 (4H, d, J=6.1)	7.97 (4H, t, J=6.8)	8.49 (2H, t, J=7.8)
11	8.79 (2H, d, J=6.1)	7.96 (4H, t, J=6.3)	8.49 (1H, t, J=8.3)
	8.70 (2H, d, J=6.1)		8.46 (1H, t, J=8.1)
12	8.79 (2H, d, J=6.1)	7.94 (2H, t, J=6.2)	8.47 (1H, t, J=7.7)
	8.46 (2H, d, J=7.1)	7.80 (2H, t, J=6.4)	8.12 (1H, t, J=7.6)
13	8.92 (2H, d, J=6.4)	8.11 (4H, t, J=6.8)	8.63 (1H, t, J=7.8)
	8.90 (2H, d, J=7.8)	7.96 (2H, t, J=6.5)	8.60 (1H, t, J=7.6)
	8.70 (2H, d, J=5.9)		8.27 (1H, t, J=7.8)
14	9.01 (2H, d, J=5.6)	8.16 (2H, t, J=6.7)	8.66 (1H, t, J=7.3)
	8.97 (2H, d, J=5.6)	8.11 (2H, t, J=7.2)	8.62 (1H, t, J=7.9)
	8.75 (2H, d, J=5.6)	8.01 (2H, t, J=7.2)	8.29 (1H, t, J=7.8)
15	9.05 (2H, d, J=6.1)	8.12 (2H, t, J=7.0)	8.64 (1H, t, J=7.7)
	8.66 (4H, d, J=5.4)	8.05 (2H, t, J=8.1)	8.40 (1H, t, J=6.8)
		7.99 (2H, t, J=7.2)	8.30 (1H, t, J=6.8)
16	8.94 (6H, d, J=6.1)	8.13 (6H, t, J=6.5)	8.62 (3H, t, J=7.7)
17	8.73 (12H, d, J=5.9)	8.09 (12H, t, J=7.0)	8.46 (6H, t, J=7.7)

a) Chemical shift (δ/ppm from DSS) in D_2O at 50 °C. The unit of J is Hz.

also split into two triplets at δ=7.94 and 7.80 ($\Delta\delta$=0.14) in **12**. Somewhat similar splittings of signals have been observed for the regioisomers of disulfonylated α-cyclodextrins **2**—**4**, as described above. However, there is a large difference in the splitting mode of the signals between the pyridinio and mesitylsulfonyl derivatives. Thus, the splitting in **11** and **12** are much larger than those in **3** and **4** ($\Delta\delta$ less than 0.04), indicating that interactions between two pyridinio groups are much stronger than those between two mesitylsulfonyl groups. Moreover, the width of the splitting in the pyridinio derivatives increased with decreasing distance between the substituents, whereas that in the mesitylsulfonyl derivatives was not parallel to the distance. The splitting in the former may be caused mainly by strong electrostatic repulsive interactions between the positively charged pyridinio groups, which enlarge differences in the electronic and/or stereochemical structures between two pyridinio groups in a molecule.

The splitting patterns of the signals due to the protons involved in the pyridinio groups of trisubstituted regioisomers **13**—**16** were also characteristic. For example, the para-H's of A,C,E-isomer **16** gave only a triplet at δ=8.62, those of A,B,E-isomer **13** and A,B,D-isomer **14**, two triplets at δ=8.6—8.7 and one triplet at δ=8.3, and those of A,B,C-isomer **15**, one triplet at δ=8.64 and two triplets at δ=8.3—8.4. These results indicate that, if the C(6)-OH's of vicinal GP's [GP(A) and GP(B)] are displaced by the pyridinio groups, one of the para-H's of the pyridinio groups is subject to significant shielding to give a triplet in the higher-field region of δ=8.3—8.4, though another para-H is little affected to give a triplet at δ=8.6—8.7. In A,B,C-isomer **15**, two para-H's of the pyridinio groups are significantly shielded to give two triplets at the higher field-region. Similar splittings of the signals were also observed for the ortho-H's of **13**—**16**. These characteristic splitting patterns serve to distinguish the regioisomers from one another, though the distinction between A,B,E- and A,B,D-isomers are still impossible.

Table 5. ^{1}H NMR Data for the C(6)-H's of **9**—**17** Giving Double Doublet Signals in the High-Field Region of δ=2.4—2.7

Compound	δ[a)]
9	2.54 (1H, dd, J=12.0 and 2.9 Hz)
10	2.63 (2H, dd, J=13.0 and 4.6 Hz)
11	2.60 (1H, dd, J=12.9 and 4.7 Hz)[b)]
12	2.42 (1H, dd, J=11.6 and 4.3 Hz)
13	2.50 (1H, dd, J=12.4 and 4.3 Hz)[c)]
14	2.73 (1H, dd, J=13.2 and 4.6 Hz)[b)]
15	2.63 (1H, dd, J=12.6 and 4.1 Hz)
16	No Signal[d)]
17	No Signal

a) Chemical shift (ppm from DSS) in D_2O at 50 °C. b) Another C(6)-H gave a double doublet signal at δ=ca. 2.9. Details were ambiguous owing to overlapping with the other signals. c) Another C(6)-H gave a signal at δ=2.99 (1H, dd, J=11.7 and 5.3 Hz). d) The C(6)-H gave a signal at δ=2.99 (3H, dd, J=12.8 and 6.1 Hz).

There are another signals which are available for a regioisomer determination. They are the dd-signals appearing in the upfield region of δ=2.4—2.7 (Table 5). As described above, the dd-signal for **9** is attributable to one of the geminal C(6)-H's involved in an unsubstituted GP [GP(B)] adjacent to a substituted GP [GP(A)]. Similar dd-signals were observed for **10**—**15**, but not for **16** and **17**. It is reasonable that **17** gives no such dd-signal, since **17** has no unsubstituted GP. The A,C,E-isomer **16** gave no dd-signal in this region, though a dd-signal was found at a significantly lower field (δ=2.99). The A,C-isomer **11** also gave a dd-signal in the lower-field region (δ=ca. 2.9), together with a dd-signal in the higher-field region (δ=2.60). On the other hand, either A,D-isomer **10** or A,B-isomer **12** gave a dd-signal only in the higher-field region and

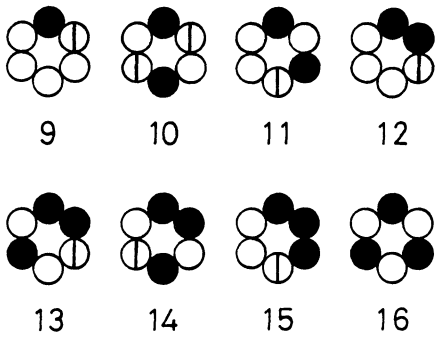

Fig. 6. Assigned glucopyranose rings (Ⓓ) which involve the C(6)-H's affording the high-field dd-signals at δ=2.4—2.7.

no dd-signal in the lower-field region. These facts indicate that the dd-signal of **11** in the lower-field region is due to the C(6)-H of GP(B) which is interposed between two substituted GP's [GP(A) and GP(C)]. The downfield shift of the dd-signal may be caused by electrostatic repulsive interactions between the pyridinio groups in GP(A) and GP(C) which compel the pyridinium ring of GP(A) to move away from the C(6)-H's of GP(B) and to weaken the ring current shielding effect. Upon this assumption, we assigned the GP rings which involve the C(6)-H's affording the upfield dd-signals (Fig. 6). The number of the GP's assigned was two for **10**, one for **9** and **11**—**15**, and zero for **16** and **17**, which agreed well with those observed (Table 5). This assignment also affords a clue to the distinction between regioisomers **13** and **14**. As Fig. 6 shows, GP(C) in **13** and GP(E) in **14** are responsible for the dd-signals in the higher-field region. It is worthwhile to point out that the intramolecular environments of GP(C) in **13** and GP(E) in **14** are significantly different from each other: The former is adjacent to two vicinal substituted-GP's [GP(A) and GP(B)] and the intramolecular environment is very similar to the GP(C) in A,B-isomer **12**. On the other hand, the latter is linked to an isolated substituted-GP [GP(D)] similar to the GP(E) in A,D-isomer **10**. If the intramolecular environments of GP(C) in **13** is similar to those of GP(C) in **12** and those of GP(E) in **14** to those of GP(E) in **10**, it is anticipated that the GP(C) of **13** gives the dd-signal at higher field than that for the GP(E) of **14**, since the dd-signal for the GP(C) of **12** was found at higher field by 0.21 ppm than that for the GP(E) of **10**. The upfield shift of the dd-signal for **12** compared with that for **10** may be attributed to strong electrostatic repulsive interactions between the pyridinio groups of the vicinal GP's [GP(A) and GP(B)], which force the pyridinium ring of GP(B) to come close to the C(6)-H's of GP(C) and strengthen the shielding by the ring current of the pyridinio group. Thus, the regioisomers which gave dd-signals at δ=2.50 (**13**) and δ=2.78 (**14**) are ascribable to the A,B,E- and A,B,D-isomers, respectively. The basis of the assignment described here is not necessarily rigid for a definite regioisomer determination. However, it must be one clue in solving this puzzling problem.

All the pyridinio derivatives **9**—**17** also gave distinct doublet signals at δ=4.7—5.3 due to C(1)-H's. The splitting patterns of the doublets reflected the structural features of the derivatives. However, details are omitted here, since they offer no further information concerning the regiochemistries than that which is described above.

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7) This fact suggests that no aggregation of host molecules occurs in solution. The size of the mesityl group may be too large to be deeply included in a cavity of another host molecule. The relatively apolar solvent DMSO-d_6 may also contribute to weakening the hydrophobic interactions between the host molecules.

8) The unsubstituted α-cyclodextrin gives a doublet at δ=4.81 (J=3.0 Hz) due to C(1)-H's in DMSO-d_6: B. Casu and M. Reggiani, *Tetrahedron*, **22**, 3061 (1966).

9) A study with the CPK molecular model suggested that the orientation of the mesitylsulfonyl group to GP(F) is unfavorable due to steric hindrance from the C(5)-oxygen of GP(F).

10) The effect of sample concentrations on the NMR spectra was examined for **9**—**12**. The δ values obtained were virtually independent, within the standard error of 0.02 ppm, of the sample concentrations ranging from 10 to 60 mmol dm^{-3}. This fact indicates that no aggregation of the host molecules occurs in solution. Electrostatic repulsive interactions between positively charged host molecules may be responsible for the repression of aggregation. The δ values for **13**—**17** were determined in dilute solutions of 2—3 mmol dm^{-3}, where the aggregation hardly occurs.

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