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# A Base-Catalyzed H/D Exchange Reaction in the 6-(1-Pyridinio)-6-deoxy Derivatives of Cyclodextrins

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Mono[6-(1-pyridinio)-6-deoxy]-α-cyclodextrin and its analogs exchanged the α-hydrogens of their pyridinio moieties with the deuteriums of solvent $D_2O$ in an alkaline buffer solution via the formation of the pyridinium ylide intermediates. The H/D exchange reaction was facilitated by the hydrophobic cavity of cyclodextrin, as well as by a substitution of the additional pyridinio group for the primary OH group, especially in the adjacent glucopyranose moiety of the cyclodextrin residue. On the other hand, the reaction was moderately retarded by an increase in the ionic strength of the solution, and markedly retarded by inorganic anions, such as $I^-$, $SCN^-$, and $ClO_4^-$, which were strongly bound to the cavity of cyclodextrin.

The substitution of the positively charged pyridinio groups for the primary hydroxyl groups of α-cyclodextrin (α-CD) results in a significant increase in the binding constants for their 1:1 inclusion complexes with $I^-$,[1,2] which also interacts with the pyridinio moieties through charge-transfer interactions to give the characteristic absorption spectra in an aqueous solution.[1] On the other hand, the α-hydrogens of *N*-alkylpyridinium ions, including biologically important nicotinamide-adenine dinucleotide, are known to undergo a base-catalyzed H/D exchange in $D_2O$ by simple deprotonation to give the pyridinium ylide intermediates.[3] In the present study, the H/D exchange reactions in mono[6-(1-pyridinio)-6-deoxy]-α-CD (**1**) and its analogs (**2**—**7**) in alkaline $D_2O$ solutions were examined by $^1$H NMR spectrometry in order to elucidate the effect of the CD moiety on the rate of the exchange reaction (Chart 1). The effect of several inorganic salts on the rate was also examined.

## Experimental

**Materials.** Parent α-, β-, and γ-CD's were kindly supplied by Nihon Shokuhin Kako Co., Ltd. and Ensuiko Seito Co., Ltd. They were dried overnight in vacuo at 110 °C. Reagent-grade pyridine was dried over $CaH_2$ and distilled in the presence of $CaH_2$. Methyl α-D-glucopyranoside, $D_2O$ (Merck, 99.75%), and inorganic salts used were of reagent grade and are commercially available. The $HCO_3^-$ salts of **1**—**7** were prepared by refluxing solutions of the corresponding 6-*O*-arysulfonylated CD's in dry pyridine, followed by elution of the products from a column of CM-cellulose (50×550 mm, Serva) with aqueous $NH_4HCO_3$ as described previously.[1,4]

**Apparatus.** The $^1$H NMR spectra were recorded using a JEOL Model JNM-GX270 FT NMR spectrometer (270 MHz) at 25 °C. Acetonitrile ($\delta$=2.00[5]) were used as an internal reference. Mass spectra were recorded using a Hitachi Model M-80 double-focusing GC-mass spectrometer. The mass (M) of **1** or its reaction product was determined by the SIMS (secondary ion mass spectrometry) technique using glycerol as the matrix. This technique generally gives the $MH^+$ ion with $m/z$=M+1. The pD of each $D_2O$ solution was estimated by adding +0.40 to the reading of an Orion Model 801A digital pH/mV meter at 25 °C.

**Kinetics.** The pyridinio derivatives **1**—**7** were dissolved in $Na_2CO_3$–$NaHCO_3$ buffer solutions in $D_2O$. The concentration of substrates were adjusted to be ca. 9.5 and 4.8 mmol cm$^{-3}$ for the mono- and dipyridinio derivatives, respectively. The buffer solutions were prepared, in most cases, by mixing 0.10 mol dm$^{-3}$ $Na_2CO_3$ in $D_2O$ with 0.10 mol dm$^{-3}$ $NaHCO_3$ in $D_2O$ at various ratios. Samples in NMR tubes were placed in a constant-temperature bath set at 60.0 °C. The tube was removed periodically and the reaction was quenched using a cold water bath. The reactions were followed for about 2 half-lives. First-order rate constants for the exchange at the α-hydrogens were obtained by a curve-fitting analysis of the plots of [α-H]/[β-H] vs. time, where [α-H] and [β-H] refer to the areas of $^1$H NMR signals for α- and β-hydrogens, respectively. A correction for the approach to equilibrium of the hydrogen exchange

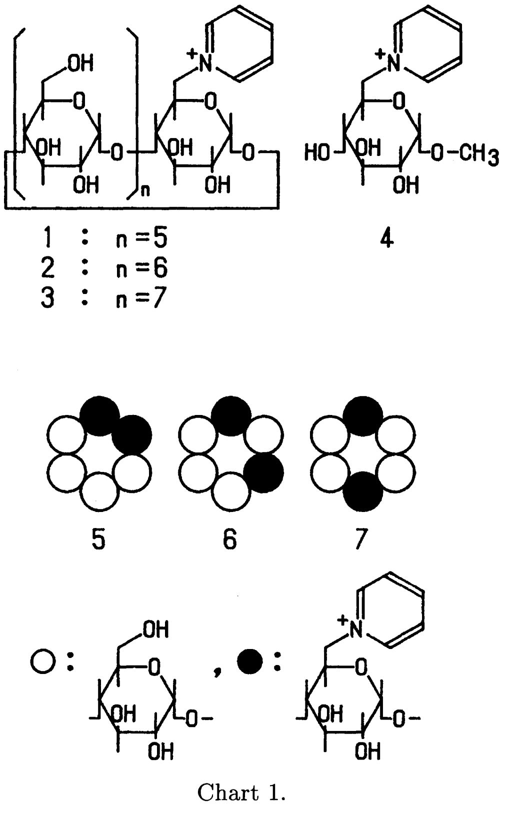


Chart 1.

was not necessary, due to the low concentration of hydrogen isotope present in mixtures.

## Results and Discussion

**Change in $^1$H NMR of 1 with Time.** The $\alpha$-, $\beta$-, and $\gamma$- hydrogens ($\alpha$-, $\beta$-, and $\gamma$-H's, respectively) of the pyridinio group of **1** gave clear $^1$H NMR doublet or triplet signals in the low-field region of $\delta$=8.0—8.9 with a peak area ratio of 2 : 2 : 1 for $\alpha$-H: $\beta$-H: $\gamma$-H (Fig. 1A). Upon heating at 60 °C in a carbonate buffer solution, the peak area of $\alpha$-H relative to that of $\beta$-H decreased with time, and the signal of $\beta$-H changed from triplet to doublet (Figs. 1B and 1C). Virtually no change was observed for the peak area of $\gamma$-H relative to that of $\beta$-H. The $\alpha$-H signal was recovered by treating the reaction product with $H_2O$ at 60 °C for several hours. These facts indicate that the $\alpha$-H's of the pyridinio group of **1** are selectively exchanged by deuteriums in $D_2O$. The H/D exchange was also confirmed by mass spectrometry (Fig. 2). The mass (M) of **1** is 1034, and **1** gave a clear M+1 peak at $m/z$ 1035. On the other hand, the reaction product of **1** gave a clear peak at $m/z$ 1037, indicating that two hydrogen atoms in **1** are replaced by two deuterium atoms.

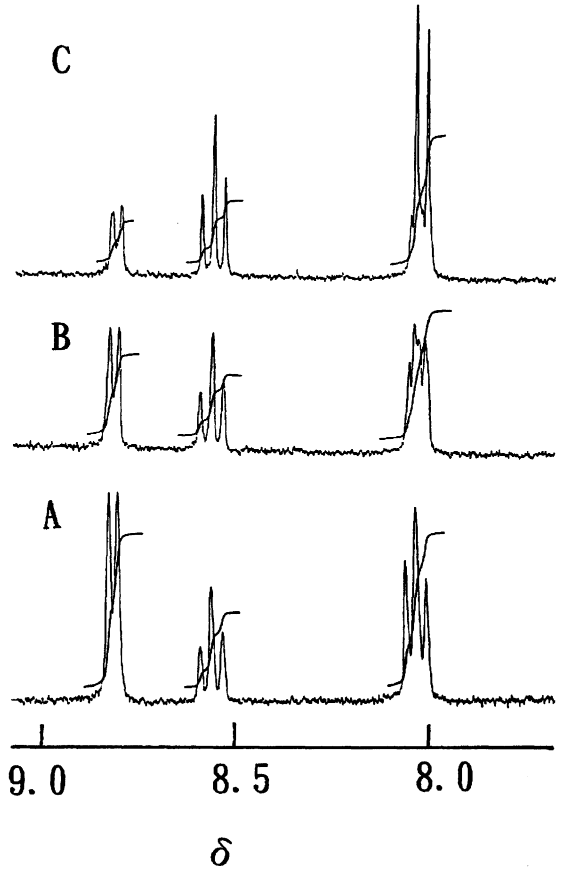


Fig. 1. Change in the $^1$H NMR spectrum of **1** (9.4 mmol dm$^{-3}$) with time in a carbonate buffer (pD 11.0) at 60 °C. A) 0 h, B) 3 h, and C) 7 h.

**Kinetics of the H/D Exchange Reaction.** Figure 3 shows changes in the ratios ($R$) of the $^1$H NMR peak areas of $\alpha$- and $\gamma$-H's to that of $\beta$-H for **1** with time in 0.10 mol dm$^{-3}$ carbonate buffer (pD 11.42) at 60 °C. The $R$ value for $\alpha$-H decreased with time, whereas that for $\gamma$-H was virtually constant. Hydrogens involved in

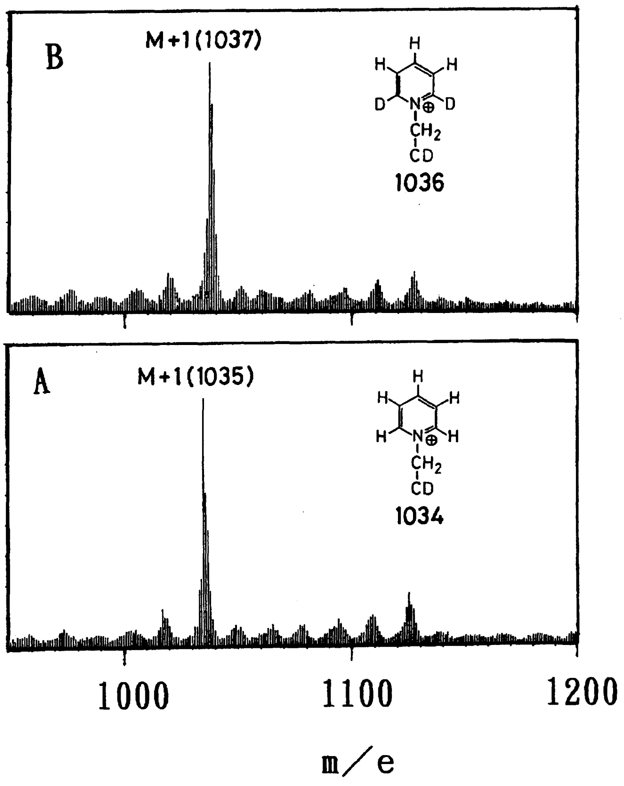


Fig. 2. Mass spectra of **1** (A) and its reaction product (B).

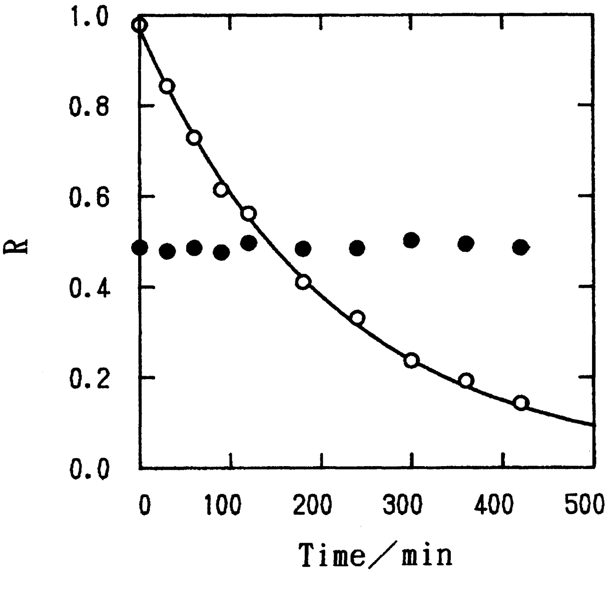


Fig. 3. Changes in $R$ for $\alpha$- (○) and $\gamma$-H's (●) of **1** with time in 0.10 mol dm$^{-3}$ carbonate buffer (pD 11.4) at 60 °C.

the CD moiety of **1** were observed in the higher field region of $\delta$=2.6—5.1,[4] and the $R$ values for them were unchanged with time. The first-order rate constant ($k_1$/min$^{-1}$) for the H/D exchange reaction at $\alpha$-H was determined to be $4.67\times10^{-3}$ min$^{-1}$ by the curve-fitting analysis of the plot. The solid line for $\alpha$-H in Fig. 3 was obtained by the analysis. It was well-fitted to the experimental data.

The $k_1$ value at 60 °C increased with increasing pD of 0.10 mol dm$^{-3}$ carbonate buffer solution at 25 °C (Table 1).[6] The regression analysis of the data gave a linear relationship between log $k_1$ and pD as follows:

$$\log k_1 = -10.64 + 0.725\ \mathrm{pD} \qquad (1)$$
$$n = 5,\ r = 0.9939$$

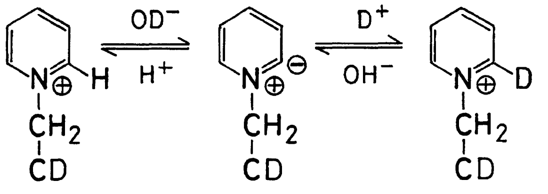
Thus, the H/D exchange reaction is base-catalyzed, and it is reasonable to assume that the reaction proceeds via a pyridinium ylide intermediate (Scheme 1), as had been suggested by Zoltewicz et al.[3b] with regard to the $N$-methylpyridinium ion. The chemical shift for the $\alpha$-H of the pyridinio moiety was not affected by the pD change, suggesting that the electronic structure of **1** in the ground state remains unchanged with the pD change. The slope of Eq. 1 was 0.725, significantly smaller than unity. The deviation of the slope from unity may be due, at least in part, to an increase in the ionic strength ($I$) with increasing pD in 0.10 mol dm$^{-3}$ carbonate buffer solutions. In fact, the $k_1$ value decreased with increasing concentration of the carbonate buffer at a constant pD (Table 2). The plot of log $k_1$ vs. $I^{1/2}$ gave a virtually straight line, as follows:

$$\log k_1 = -1.94 - 0.817\ I^{1/2} \qquad (2)$$
$$n = 4,\ r = 0.9913$$

Table 1. First-Order Rate Constants ($k_1$) for the H/D Exchange Reaction of **1** in 0.10 mol dm$^{-3}$ Carbonate Buffers with Various pD's at 60 °C

| pD[a] | ($k_1$/min$^{-1}$)$\times10^3$ | $\delta$[b] |
|---|---|---|
| 10.20 | 0.50 | 8.844 |
| 10.60 | 1.23 | 8.843 |
| 10.99 | 2.36 | 8.844 |
| 11.42 | 4.67 | 8.843 |
| 11.78 | 7.12 | 8.845 |

a) Determined at 25 °C. b) Chemical shift for the $\alpha$-H of the pyridinio moiety.



Scheme 1.

Table 2. Effect of Carbonate Concentration ($c$/mol dm$^{-3}$) on $k_1$ at 60 °C for 9.5 mmol dm$^{-3}$ **1** in Carbonate Buffers at pD 11.4.

| $c$ | $I$[a] | ($k_1$/min$^{-1}$)$\times10^3$ | $\delta$[b] |
|---|---|---|---|
| 0.05 | 0.346 | 5.75 | 8.846 |
| 0.10 | 0.480 | 4.67 | 8.843 |
| 0.20 | 0.671 | 3.44 | 8.843 |
| 0.30 | 0.819 | 2.32 | 8.840 |

a) Ionic strength. b) Chemical shift for the $\alpha$-H of the pyridinio moiety.

This fact suggests that the activity of the OD$^-$ ion decreases with increasing $I$. The chemical shift for the $\alpha$-H was unaffected by a change in $I$.

Table 3 shows the effect of the addition of 0.20 mol dm$^{-3}$ neutral inorganic salts to 0.1 mol dm$^{-3}$ carbonate/D$_2$O buffer (pD 11.2—11.4) on $k_1$ at 60 °C. Among the anions examined, I$^-$, SCN$^-$, and ClO$_4^-$ are strongly bound to **1** to form inclusion complexes;[1] the other anions form no or labile complexes with **1**. The addition of anions strongly bound to **1** resulted in a marked decrease in $k_1$. These anions are incorporated into the CD cavity of **1**,[1] and may either decrease the local concentration of OD$^-$ around the $\alpha$-H or labilize the pyridinium ylide intermediate, through electrostatically repulsive interactions. The $k_1$ values for the other anions were roughly equal to that in 0.30 mol dm$^{-3}$ carbonate buffer with a similar ionic strength. Thus, the moderate and nonspecific inhibitory effect of these anions was mainly due to an increase in $I$. The chemical shift for the $\alpha$-H was significantly affected by the addition of these anions. However, no relationship was found between $k_1$ and the chemical shift.

**Rate Constants for the Analogs of 1.** Table 4 shows the $k_1$ values for **1**—**7** in a carbonate buffer (pD 11.4) at 60 °C. The $k_1$ values for the monopyridinio derivatives, **1**—**4**, decreased with an increase in size of the CD cavity, and was minimal in **4**, which has no

Table 3. Effect of Several Inorganic Salts[a] on $k_1$ for **1** in 0.10 mol dm$^{-3}$ Carbonate Buffer (pD 11.4) at 60 °C

| Salt | ($k_1$/min$^{-1}$)$\times10^3$ | $\delta$[b] |
|---|---|---|
| None | 4.67 | 8.843 |
| Carbonate[c] | 2.32 | 8.840 |
| KF | 2.43 | 8.846 |
| KCl | 2.29 | 8.852 |
| KBr | 2.26 | 8.913 |
| KI | 0.562 | 9.027 |
| KSCN | 0.215 | 8.909 |
| KNO$_3$ | 2.09 | 8.851 |
| NaClO$_4$ | 0.384 | 8.810 |

a) [Salt]=0.20 mol dm$^{-3}$. b) Chemical shift for the $\alpha$-H of the pyridinio moiety. c) [Carbonate]=0.30 mol dm$^{-3}$.

Table 4. The $k_1$ Values for **1**—**7** in 0.10 mol dm$^{-3}$ Carbonate Buffer (pD 11.4) at 60 °C

| Substrate[a)] | $(k_1/\text{min}^{-1})\times 10^3$ | $\delta$[b)] |
|---|---|---|
| **1** | 4.67 | 8.843 |
| **2** | 3.78 | 8.839 |
| **3** | 3.44 | 8.844 |
| **4** | 2.15 | 8.839 |
| **5** | 22.3 | 8.636 |
|  | 16.9 | 8.952 |
| **6** | 8.18 | 8.815 |
|  | 4.01 | 8.888 |
| **7** | 5.08 | 8.862 |

a) Initial concentrations of substrates were 9.5 and 4.8 mmol dm$^{-3}$ for mono- and dipyridinio derivatives, respectively. b) Chemical shift for the $\alpha$-H's of the pyridinio moiety.

cavity. The hydrophobic cavity of CD may serve, more or less, to stabilize the pyridinium ylide intermediate.

The $k_1$ values for a dipyridinio derivative **5** were about 4—5 times that for **1**. Compound **5** gave two doublet $^1$H NMR signals, due to $\alpha$-H's at $\delta$=8.64 and 8.95. The $k_1$ value for $\alpha$-H at $\delta$=8.64 was larger than that for $\alpha$-H at $\delta$=8.95. A regioisomer **6** also gave two doublet signals at $\delta$=8.82 and 8.89. The $k_1$ value for $\alpha$-H at a higher magnetic field was larger than that for **1** by a factor of about 2. On the other hand, the $k_1$ value for $\alpha$-H at a lower field was roughly equal to that for **1**. Another regioisomer **7** gave a doublet signal due to $\alpha$-H at $\delta$=8.86; the $k_1$ value was roughly equal to that for **1**. These facts indicate that the distances between two pyridinio groups in **5**—**7** play an important role in the H/D exchange reaction. The two positively charged pyridinio groups in **5** are so close to each other as to stabilize the negative charge of the ylide intermediate. It is also possible that the local concentration of OD$^-$ around the reaction site was increased by the closely located positive charges.

In conclusion, the pyridinio derivatives of CD exchange the $\alpha$-H's of the pyridinio moieties with the deuteriums of solvent $D_2O$ in an alkaline solution via the formation of the pyridinium ylide intermediates. The formation of the ylide is interesting in connection with the thiazolium ylide, which plays an important role in the biochemical reactions of thiamine. A pyridinio derivative of CD bears a hydrophobic cavity into which various organic substrates are incorporated. Thus, it is anticipated that a pyridinio derivative of CD specifically catalyzes organic reactions in a similar manner as do thiamine enzymes. The catalytic properties of **1** and **5** are under investigation.

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