Inhibitory Effects of a Novel Water-Soluble Cyclophane on the Hydrolysis Reactions of Aromatic Esters

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The effects of a novel water-soluble cyclophane (TGCP 44 ,1) on the hydrolysis reactions of aromatic esters have been studied. TGCP 44 (1) was found to inhibit the hydrolysis of three aromatic esters, *p*-nitrophenyl chloroacetate (2), glycine *p*-nitrophenyl ester hydrobromide (3) and 1-nitro-2-naphthyl chloroacetate (4). The hydrolysis rates of the aromatic esters were retarded by 3.8—7.65 fold relative to the spontaneous rates.

Keywords water-soluble cyclophane; hydrolysis; aromatic ester; inhibitory effect; Lineweaver–Burk type plot

Molecular recognition by host–guest complex formation is known to play an important role in biological processes, such as enzyme catalysis and inhibition and immunological response. Extensive investigations have already been reported on the utility of cyclophanes as host compounds to hydrolyze *p*-nitrophenyl esters in aqueous solution.[1] In a previous paper we reported that water-soluble cyclophane (TGCP 44 (**1**)) having two diphenylmethane units connected with two bridging chains *via* four oxygens is an excellent inclusion host for cationic, anionic and neutral aromatic guests.[2] In the present work, we studied on the hydrolysis of aromatic esters in the presence or absence of TGCP 44 in order to investigate the molecular recognition efficiency of TGCP 44.

Hydrolysis was initiated by adding 4 μl of 2×10^{-2} M *p*-nitrophenyl chloroacetate (**2**) in CH_3CN or glycine *p*-nitrophenyl ester hydrobromide (**3**) in EtOH to 1.0 ml of a phosphate (1/15 M) buffer solution in a cell which was thermostated at 25°C and equipped with an ultraviolet absorption spectrometer. The reaction was followed by monitoring the liberation of *p*-nitrophenol in terms of the absorbance at 400 nm. In the case of 1-nitro-2-naphthyl chloroacetate (**4**), the substrate was dissolved in CH_3CN (4×10^{-2} M) and the hydrolysis reaction was monitored by measuring the absorbance of 1-nitro-2-naphthol at 434 nm at 37 °C. The kinetic data were analyzed in terms of the Michaelis–Menten treatment based on the reaction pathway given by Eq. 1, where E, S, and P stand for catalyst, substrate, and hydrolysis products, respectively. ES is the Michaelis-type inclusion complex.

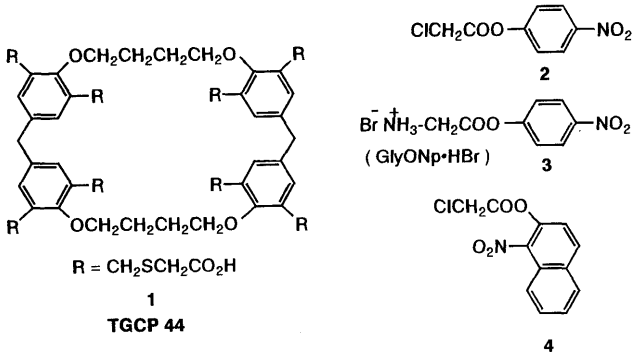

Chart 1

$$E+S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E+P \qquad (1)$$
$$ES \xrightarrow{k_0} P$$

The dependence of the pseudo-first-order rate constant on the TGCP 44 concentration (4 or 5 points) was determined by the use of the Lineweaver–Burk type plot (Eq. 2),[3] where K_m is the Michaelis constant, k_{obs} is the observed rate constant and k_0 is the spontaneous hydrolysis rate constant. By plotting $1/(k_{obs}-k_0)$ *vs.* $1/[C]$, a straight line was obtained as shown in Fig. 1. This result confirms that the hydrolysis reactions involve a pseudo-first-order mechanism.

$$1/(k_{obs}-k_0)=1/(k_2-k_0)(K_m/[\text{TGCP 44}])+1/(k_2-k_0) \qquad (2)$$

The rates of intracomplex hydrolysis reactions of all of the aromatic esters were retarded by 3.8—7.65 fold relative to the spontaneous hydrolysis rate. A significant difference of reaction rate was observed between the hydrolysis of *p*-nitrophenyl ester and that of 1-nitro-2-naphthyl ester

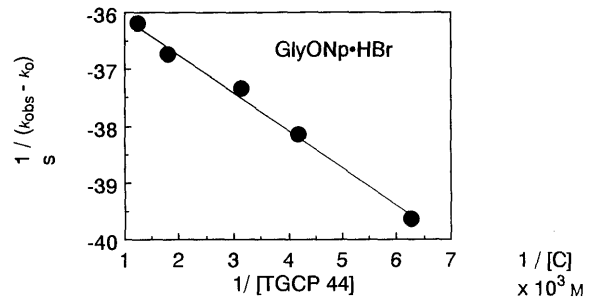

Fig. 1. Lineweaver–Burk Type Plot

TABLE I. Inhibitory Effects of TGCP 44 on the Hydrolysis of Aromatic Esters[a]

Substrate[b]	pH	Temp. °C	k_0[c] $\times10^{-3}$ s^{-1}	k_2 $\times10^{-3}$ s^{-1}	K_m mM	k_2/k_0	k_0/k_2
2	7.0	25	37.49	4.9	0.019	0.13	7.65
3	7.0	25	33.65	5.38	0.019	0.16	6.25
4	7.0	37	13.74	3.61	0.153	0.26	3.8

a) TGCP 44 concentration: 2.4×10^{-4}—1.6×10^{-3} M. *b*) Substrate concentration: [**2**] = 8.0×10^{-5} M; [**3**] = 8.0×10^{-5} M; [**4**] = 1.6×10^{-4} M. *c*) Uncatalyzed hydrolysis rate constant.

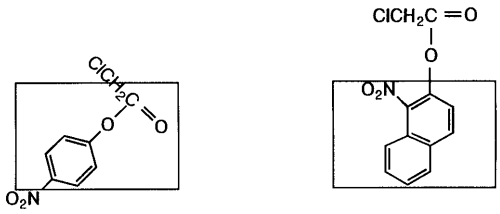

Fig. 2. Possible Geometries of the Complex

(Table I). K_m values for the hydrolysis of **2**, **3**, and **4** by TGCP 44 were calculated to be 0.019, 0.019, and 0.153 mM, respectively, as shown in Table I. From a consideration of Corey-Pauling-Koltun (CPK) models, it is postulated that the *p*-nitrophenyl esters (**2**, **3**) can be incorporated more deeply into the cavity of TGCP 44 compared with the bulky naphthyl ester (**4**), as shown in Fig. 2, and consequently the ester carbonyl groups of **2** and **3** are shielded more effectively from the attack of an external anion in the case of **4**. Another reason for retardation of the hydrolysis reaction might be electrostatic repulsion between the carboxylate anion in TGCP 44 and the external anion. Further studies on hydrolysis reactions using functionalized cyclophanes are in progress.

Experimental

***p*-Nitrophenyl Chloroacetate (2) and Glycine *p*-Nitrophenyl Ester Hydrobromide (3)** *p*-Nitrophenyl chloroacetate (**2**) and glycine *p*-nitrophenyl ester hydrobromide (**3**) were prepared according to the reported procedures.[4,5] mp **2**, 94—95 °C (reported,[4] 94 °C); **3**, 213—214 °C (dec.) (reported,[5] 213—215 °C (dec.)).

1-Nitro-2-naphthyl Chloroacetate (4) A solution of chloroacetic acid (47.3 mg, 0.5 mmol), *N*,*N*′-dicyclohexylcarbodiimide (DCC) (206 mg, 1 mmol), and 1-nitro-2-naphthol (94.3 mg, 0.5 mmol) in AcOEt (5 mmol) was stirred for 24 h at room temperature and precipitated *N*,*N*′-dicyclohexylurea (DCU) was removed by filtration. The filtrate was concentrated under reduced pressure. The residue was chromatographed on silica gel (Et_2O–hexane, 1 : 2) to give **4**. Recrystallization from $CHCl_3$–hexane gave the pure ester **4** (60 mg, 45%) as colorless needles, mp 114—115 °C. MS *m/z*: 265 (M^+). ^{1}H-NMR ($CDCl_3$ 90 MHz) δ: 4.37 (2H, s),7.39 (1H, d, J=9.2 Hz), 7.59—8.00 (4H, m), 8.06 (1H, d, J=9.2 Hz).

Kinetic Measurements of Hydrolysis Each hydrolysis was initiated by adding *p*-nitrophenyl chloroacetate **2** (4 μl of 2×10^{-2} M) or 1-nitro-2-naphthyl chloroacetate **4** (4 μl of 4×10^{-2} M) in CH_3CN or glycine *p*-nitrophenyl acetate **3** (4 μl of 2×10^{-2} M) in EtOH to 1.0 ml of a reaction medium containing TGCP 44 (2.4×10^{-4} M to 1.6×10^{-3} M in 1/15 M phosphate buffer solution) in a cell which was thermostated at 25 °C or 37 °C. The liberation rates of *p*-nitrophenol or 1-nitro-2-naphthol were followed at 400 or 434 nm, respectively, with a Shimadzu UV-160 spectrometer. The pseudo-first-order rate constants were obtained in the presence of TGCP 44 at 4—5 concentrations. Calculations were carried out using Lineweaver–Burk type plots.

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References

1) *a*) Y. Murakami, J. Sunamoto, K. Kano, *Chem. Lett.*, **1973**, 223; *b*) I. Tabushi, Y. Kimura, K. Yamamura, *J. Am. Chem. Soc.*, **100**, 1304 (1978); *c*) Y. Murakami, Y.Aoyama, M. Kida, A. Nakano, *Bull. Chem. Soc. Jpn.*, **50**, 3365 (1977); *d*) Y. Murakami, A. Nakano, R. Miyata, Y. Matsuda, *J. Chem. Soc., Perkin Trans. 1*, **1979**, 1669; *e*) Y. Murakami, Y. Aoyama, M. Kida, *J. Chem. Soc., Perkin Trans. 2*, **1980**, 1665; *f*) I. Tabushi, Y. Kimura, K. Yamamura, *J. Am. Chem. Soc.*, **103**, 6486 (1981).
2) *a*) M. Miyake, M. Kirisawa, K. Koga, *Chem. Pharm. Bull.*, **40**, 3124 (1992); *b*) *Idem*, *Heterocycles*, **36**, 1845 (1993).
3) R. L. Van Etten, J. F. Sebastian, G. A. Clowes, M. L. Bender, *J. Am. Chem. Soc.*, **89**, 3242 (1967).
4) K. V. Auwers, H. Baum, H. Lorenz, *J. Prakt. Chem.*, **115**, 103 (1927).
5) M. Goodman, K. C. Stueben, *J. Am. Chem. Soc.*, **81**, 3980 (1959).