tational laboratory. We also wish to acknowledge the support of USPS Grant AM 07300 to E. R. Blout.

Registry No. I, 62150-58-7; II, 84498-65-7.

Supplementary Material Available: Thermal parameters for non-hydrogen atoms (Table S1 for I, Table S4 for II), hydrogen atom positions and thermal parameters (Table S2 for I, Table S5 for II), and a listing of observed and calculated structure factors (Table S3 for I, Table S6 for II) (19 pages). Ordering information is given on any current masthead page.

Multifunctional Micellar Catalysis as a Model of Enzyme Action

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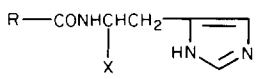
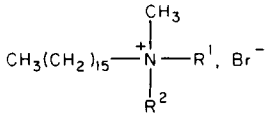
Abstract: The rate constants of both the acylation and deacylation processes in the hydrolyses of *p*-nitrophenyl acetate (PNPA) and hexanoate (PNPH) by imidazole catalysts (**1**) in the presence of surfactant micelles (**2**) have been directly determined under single turnover conditions at pH 7.30 in 0.02 M phosphate buffer and 25 °C. The major course of catalysis was the acylation followed by deacylation at the imidazole group. The kinetic analysis suggests that a multifunctional mode of action is involved in the catalytic ester hydrolysis; the acylation and deacylation rates are accelerated by carboxylate ion in the catalyst and by surfactant hydroxyl group, respectively.

The active site of α-chymotrypsin consists of the imidazole (His-57), hydroxyl (Ser-195), and carboxylate (Asp-102) groups. These three functional groups act as an esterolytic catalyst with mutual cooperation.[3] Many micellar enzyme models have been investigated in order to gain further insight into the nature of enzyme reactions[4] and, particularly, micellar bifunctional catalysts have been utilized successfully to elucidate certain aspects of the estrolytic enzyme reaction.[5]

The present study describes a kinetic analysis of multifunctional catalysis during the hydrolysis of *p*-nitrophenyl acetate (PNPA) and hexanoate (PNPH) by *N*-acylhistidine (**1**) in the presence of surfactant micelles (**2**). These micellar catalytic systems involve three functional groups and one can anticipate multifunctional interactions between them. The results obtained in this investigation demonstrate the first example of micellar catalysis with a triad of catalytic groups.





1a, R = CH_3; X = CO_2H
b, R = $CH_3(CH_2)_8$; X = CO_2H
c, R = $CH_3(CH_2)_8$; X = CO_2CH_3

2a, $R^1 = R^2 = CH_3$
b, $R^1 = CH_3$; $R^2 = (CH_2)_2OH$
c, $R^1 = R^2 = (CH_2)_2OH$
d, $R^1 = CH_3$; $R^2 = (CH_2)_3OH$
e, $R^1 = CH_3$; $R^2 = CH(OH)CH_3$

Although there have been some investigations of the catalytic hydrolysis of *p*-nitrophenyl carboxylates by similar micellar catalytic systems, these investigations followed only the acylation process[6a] or acyl transfer during the reactions.[5a,b,6b] In this study, we directly measured deacylation rate constants of an acylated intermediate, as well as acylation rate constants during the course of the hydrolysis reactions.

Experimental Section

Materials. *p*-Nitrophenyl acetate (PNPA) and hexanoate (PNPH) were obtained from Tokyo Kasei Organic Chemicals. PNPA was purified by recrystallization from cyclohexane before use. Commercial *N*-acetylimidazole (Nakarai Chemicals Co.) was used without further purification. Functional surfactants (**2b–e**) were prepared by reaction of cetyl bromide and the corresponding tertiary amines in refluxing ethanol:[7] **2b**, mp 212–213 °C (lit.[7a] mp 208–210 °C); **2c**, mp 86–87 °C (lit.[7b] mp 85 °C); **2d**, mp 80–82 °C (lit.[7b] mp 82 °C); **2e**, mp 116–117 °C. Anal. ($C_{21}H_{46}O_2NBr$) C, H, N. Other materials have been described elsewhere.[8]

Kinetic Measurements. Reactions were generally monitored on a Hitachi 200 spectrophotometer or a Shimazu 140 spectrophotometer with a thermostated cell holder at 25 °C. In the general procedure, a solution (25 μL) of substrate in acetonitrile was added to a buffer solution (3.00 mL) containing the catalyst and surfactant at the desired concentrations. Details of the kinetic techniques and conditions may be found in the Results and Discussion. The spectra–time study of the reactions was monitored on a Hitachi 200 spectrophotometer with a wavelength program attachment.

Results and Discussion

Acyl Intermediates and the Reaction Traces. The spectra vs. time study of the reaction of PNPA with **1b** in the presence of **2a** was followed in the wavelength range 240–320 nm as shown in Figure 1. The spectra in Figure 1 consist of two phases: a fast reaction (acylation process) followed by a considerably slower reaction (deacylation process). We also observed good isosbestic points at 254 nm during the course of the acylation reaction. This reaction trace at a fixed wavelength (245 nm) is shown in Figure 2. However, although the reactions of PNPA with **1b** in the presence of **2a** or **2b** show different spectra–time behavior, we can

(1) Yamaguchi Women's University
(2) University of Osaka Prefecture.
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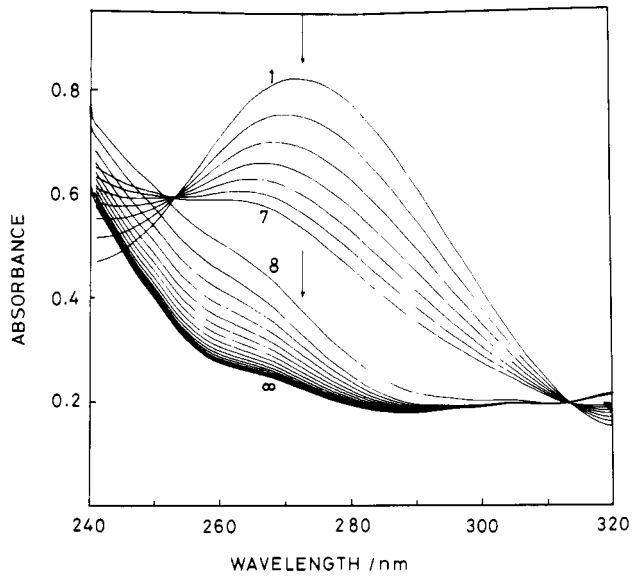


Figure 1. Spectral time study for the hydrolysis of PNPA by **1b** in the presence of **2a** at pH 7.30, 0.02 M phosphate buffer, and 25 °C: [PNPA] = 1.2×10^{-4} M; [**1b**] = 1.0×10^{-3} M; [**2a**] = 1.00×10^{-2} M. Spectral curves 1–7 and 8–∞ show repeated scans and scans after a 10-min interval, at 480 nm/min, respectively.

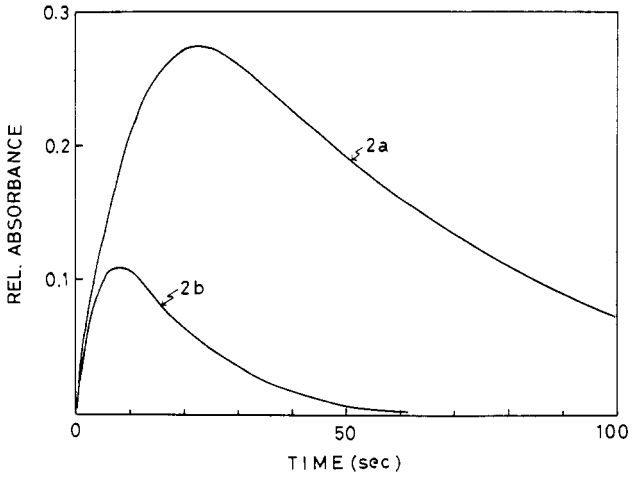


Figure 2. Relative absorbances at 245 nm during the reaction of PNPA with **1b** in the presence of surfactants, **2a** or **2b**, at pH 7.30, 0.02 M phosphate buffer, and 25 °C: [PNPA] = 1.0×10^{-4} M; [**1b**] = 1.00×10^{-3} M; [**2a** or **2b**] = 1.00×10^{-2} M.

observe buildups of the acylated intermediates during the reaction spectrophotometrically at 245 nm. The trace for **2b** indicates a slow acylation followed by a rapid, hydroxyl-mediated deacylation. This kinetic behavior has also been confirmed by Tagaki.[6b]

Kinetics. The catalytic process for the hydrolysis of substrates by **1** can be described by eq 1, where C_{Im} designates the imidazole

$$C_{Im} + AcONp \xrightarrow{k_a} Ac\text{-}C_{Im} \xrightarrow{k_d} C_{Im} + AcOH \quad (1)$$
$$+$$
$$p\text{-nitrophenol}$$

catalyst, AcONp is the substrate, Ac–C_{Im} is the acylated intermediate, and k_a and k_d represent the rate constants for acylation and deacylation processes, respectively.

The kinetics were studied under single turnover conditions at pH 7.30, 0.02 M phosphate buffer, and 25 °C. Under single turnover conditions, [surfactant] > [catalyst] > [substrate], the rate constants k_a and k_d depicted in eq 1 were independently determined. The acylation rate constants (k_a) were followed by monitoring the release of *p*-nitrophenolate ion spectrophotometrically at 410 nm. The deacylation rate constants (k_d) were directly measured by the following kinetic techniques: (a) in the case where $k_a \gg k_d$, the correlation obtained from the time dependence of the slow decrease in absorption at 245 nm (Figure 1, in the case of **2a**) was analyzed to obtain the deacylation rate constants, (b) in the case where $k_a < k_d$, we independently prepared an acylimidazole intermediate in the presence of **2a**[9] and this intermediate was then quickly injected into a buffered micellar solution to measure the deacylation rate constants.[10] In both cases, the kinetics were first order and good least-squares rate constants were obtained ($r > 0.999$).

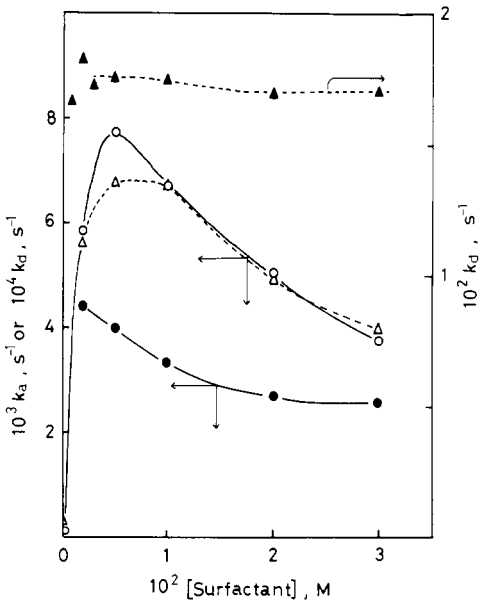


Figure 3. Effect of surfactant concentration on the rate constants at pH 7.30, 0.02 M phosphate buffer, and 25 °C: [**1b**] = 1.00×10^{-3} M; [PNPA] = 1.0×10^{-4} M; (○, △) acylation rate constants in the presence of **2a** and **2b**; (●, ▲) deacylation rate constants in the presence of **2a** and **2b**.

We also carried out some experiments under "burst" conditions, [surfactant] > [substrate] > [catalyst]. The kinetic treatment was that of Bender and has been used by others.[11] If the initial concentrations of catalyst and substrate are $[C_0]$ and $[S_0]$, respectively, π is the absorbance under steady-state conditions, extrapolated to time zero, (t_0), and ΔA is the difference between the observed and extrapolated absorbance at time t, we obtain

$$\Delta A = \pi e^{-bt} \quad (2)$$

$$k_a = \frac{b(\pi)^{1/2}}{[S_0]([C_0])^{1/2}} \quad (3)$$

$$k_d = b - k_a[S_0] \quad (4)$$

Although the "burst" kinetics were observed for the reaction of **1b** with PNPA and PNPH in the presence of **2a**,[12] this kinetic treatment did not in our systems give good values of k_d, which is a small difference between two larger numbers. In the presence of **2b** "burst kinetics" could not be observed, probably due to faster deacylation ($k_a[S_0] < k_d$).

Effect of Surfactant Concentration. The catalytic rates of PNPA hydrolysis by **1b** were measured at different surfactant concentrations ($2.0–30 \times 10^{-3}$ M) at pH 7.30, 0.02 M phosphate

(9) Conditions: pH 7.30, 0.02 M phosphate buffer, 25 °C, 12% v/v CH_3CN, [**1b**] = 5.0×10^{-3} M, [**2a**] = 1.0×10^{-2} M, [PNPA or PNPH] = 2.4×10^{-3} M.

(10) In preliminary experiments, **1b**, under anhydrous conditions, was treated with acetic anhydride and 2 equiv of triethylamine in dichloromethane. After the solvent was removed in vacuo, the residual oil was used to measure the deacylation rate in the presence of surfactant micelles. The deacylation rate constants obtained from this method and method b in the text agreed within experimental error.

(11) (a) Bender, M. L.; Marshall, T. H. *J. Am. Chem. Soc.* **1968**, *90*, 201. (b) Kunitake, T.; Okahata, Y. *Bioorg. Chem.* **1975**, *4*, 136. (c) Bunton, C. A.; Ihara, Y. *J. Org. Chem.* **1977**, *42*, 2865. (d) Reference 5d.

(12) Conditions: pH 7.30, 0.02 M phosphate buffer, 25 °C, [**1b**] = $2.00–4.00 \times 10^{-5}$ M, [**2a**] = 1.00×10^{-2} M, [PNPA or PNPH] = $0.5–5.0 \times 10^{-4}$ M.

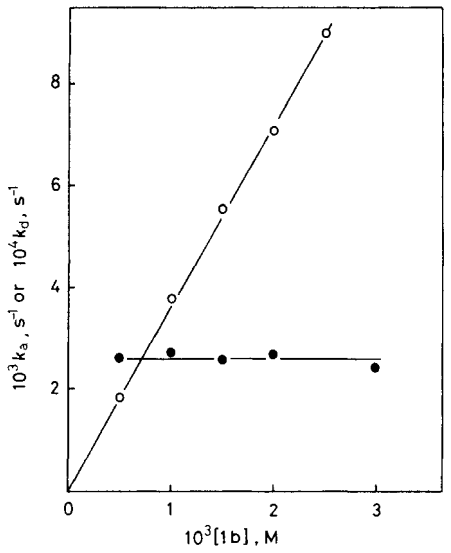


Figure 4. Effect of catalyst concentration on the rate constants in the presence of **2a** at pH 7.30, 0.02 M phosphate buffer, and 25 °C: [PNPA] = 1.0×10^{-4} M; [**2a**] = 3.00×10^{-2} M; (○) acylation rate constants; (●) deacylation rate constants.

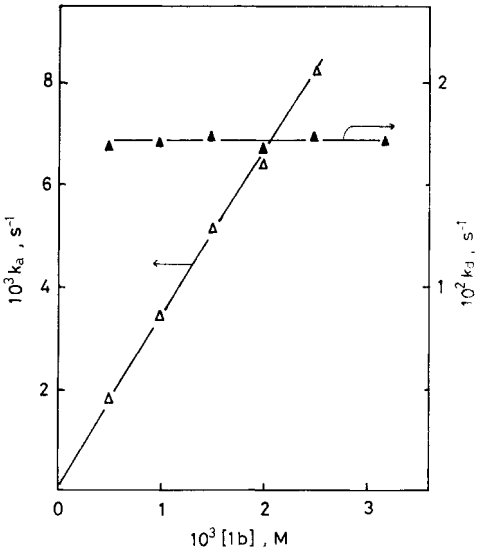


Figure 5. Effect of catalyst concentration on the rate constants in the presence of **2b** at pH 7.30, 0.02 M phosphate buffer, and 25 °C: [PNPA] = 1.0×10^{-4} M; [**2b**] = 3.00×10^{-2} M; (△) acylation rate constants; (▲) deacylation rate constants.

buffer, and 25 °C and are shown in Figure 3. The k_a values were sensitive to the surfactant concentrations. The maxima were observed at 5.0×10^{-3} M **2a** or **2b** for k_a. However, k_d slightly decreased (**2a**), or almost reached a constant value (**2b**), as the concentration of surfactant increased. Note that k_a for both micellar systems, **2a** or **2b**, was essentially the same and was mainly determined by the reactivity of **1b**. On the other hand, k_d for the micellar systems showed contrasting values.

Effect of Catalyst Concentration. The catalytic hydrolysis was also carried out with varying catalyst concentrations at a fixed surfactant concentration. Typical rate–catalyst concentration profiles are shown in Figures 4 and 5. Although the kinetics of the acylation process were obviously first order in catalyst, those of the deacylation process did not depend on the catalyst concentration over the range of concentration followed. This indicates that the deacylation is pseudo-zero-order in imidazole catalyst. The acceleration of deacylation by intermolecular imidazole catalysis could not be observed in this study, but Jencks and Carrioulo[13] reported that a high concentration of imidazole can catalyze the deacylation of acetylimidazole.

(13) Jencks, W. P.; Carrioulo, J. *J. Biol. Chem.* **1959**, *234*, 1272, 1280.

Table I. Rate Constants for the Hydrolysis of PNPA and PNPH in the Presence of Surfactant Micelles[a]

case	catalyst (substrate)	surfactant	10^3k_a, s^{-1}	10^3k_d, s^{-1}	rel k_d
1	1a (PNPA)	2a	0.278	0.178	1
2		2b	0.270	1.35	7.58
3	1b (PNPA)	2a	6.61	0.337	1
4		2b	6.58	16.9	50.1
5		2c	4.94	51.3	152
6		2d	5.72	3.09	9.08
7		2e	6.08	1.66	4.93
8	1b (PNPH)	2a	9.31	0.196	1
9		2b	9.07	5.33	27.2
10		2c	8.16	14.5	74.0
11		2d	7.18	0.976	4.98
12		2e	8.71	0.633	3.23
13	1c (PNPA)	2a	3.72	0.885	
14	1c (PNPH)	2a	5.08	0.559	

[a] At pH 7.30, 0.02 M phosphate buffer, and 25 °C; [catalyst] = 1.00×10^{-3} M; [surfactant] = 1.00×10^{-2} M; [PNPA or PNPH] = 1.0×10^{-4} M. From three or more independent experiments, we estimate that the rate constants are reproducible to ±4%.

Table II. Deacylation Rate Constants of *N*-Acetylimidazole in the Presence of Surfactants[a]

surfactant	10^3k_d, s^{-1}	rel k_d
none	0.375	
2a	0.428	1
2b	1.86	4.35
2c	3.65	8.53
2d	0.697	1.63
2e	0.527	1.23
SDS[b]	0.349	
2a[c]	0.377	1
2b[c]	2.91	7.76

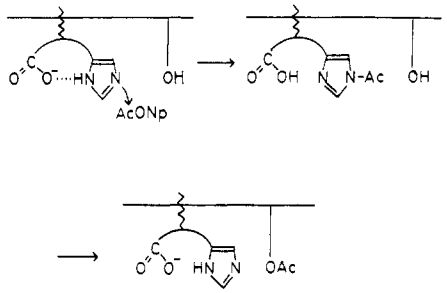
[a] At pH 7.30, 0.02 M phosphate buffer, and 25 °C; [surfactant] = 1.00×10^{-2} M; [*N*-acetylimidazole] = 1.0×10^{-4} M, unless specified otherwise. [b] Sodium dodecyl sulfate. [c] [Surfactant] = 3.00×10^{-2} M.

Catalytic Efficiency and the Mechanism of Catalysis. Results obtained at a fixed [surfactant] of 1.00×10^{-2} M with various surfactant micelles are summarized in Table I. For comparison, analogous results for the reaction of *N*-acetylimidazole are shown in Table II.

In micellar **2a**, the deacylation rate constants for catalyzed hydrolyses of PNPA and PNPH by **1b** are about 20 times (case 3) and 50 times (case 8) slower than the corresponding acylation rate constants. This indicates that the acylimidazole intermediate is very stable in the presence of **2a** and that deacylation is rate limiting. The functional surfactants (**2b–e**), however, enhance the deacylation rate constants; especially large enhancements are observed in micellar **2b** and **2c** (cases 4, 5, 9, and 10). The acylation rate constants, however, are almost independent of the surfactant used. Note that the deacylation rate constants are greater than the acylation rate constants and that acyl intermediates do not accumulate in micellar **2c** (PNPA and PNPH, cases 5 and 10) or **2b** (PNPA, case 4). These large deacylation enhancements can be ascribed to intermolecular acyl transfer from the catalyst imidazole to the surfactant hydroxyl groups. This observation has been made in several laboratories.[5a,b,6b]

The deacylation rate constants for catalytic hydrolysis of PNPA and PNPH with **1b** fall in the order **2c** > **2b** ≫ **2d** > **2e** > **2a**. This indicates that the deacylation step is very sensitive to the surfactant structure. Similar behavior is observed in the catalytic hydrolysis of PNPA by **1a** (cases 1 and 2) and the reaction of *N*-acetylimidazole (Table II), but the latter deacylation rate constants are much smaller than those associated with reactions catalyzed by **1b**. More importantly, acetyl is uniformly more reactive than hexanoyl in the deacylation process, in contrast to their reversed reactivities in the acylation process. Clearly, the attack of micellar hydroxyl groups is rather more favorable on

Scheme I



N-acetyl-**1b** than *N*-hexanoyl-**1b**.

These results suggest that the acyl transfer reaction is sensitive to the catalytic activity of the hydroxyl functions on the surfactants. This probably reflects the steric environment, acidity, and number (for **2c**) of surfactant hydroxyls. This behavior is associated with the incorporation of the acylimidazole intermediates on to the surface of the micelles, leading to an effective orientation for the attack of the hydroxyl groups. Thus, the reactions with **2c** give the largest deacylation rate enhancements: the relative deacylation rate constant ratio, based on **2a**, is 152 (PNPA, case 5) and 74 (PNPH, case 10).

Comparisons of the catalytic reactivities of **1b** and **1c** show that the acylation rates for the catalytic hydrolyses of PNPA and PNPH by **1b** in the presence of **2a** are greater than those by **1c** (cases 3 and 13, 8 and 14). This is consistent with intramolecular assistance provided by the carboxyl group to the imidazolyl group in **1b**.[14] However, the rate effect is small and the assistance is not definitively established by the data. It is also seen that the deacylation rate constants with **1b** are smaller than those with **1c**, in contrast to the opposite order of their acylation rate constants. The carboxylate anion of **1b** may stabilize the acylimidazolium group of the intermediate. In our previous papers, we also suggested that the carboxylate ion of **1b** enhanced the reactivity of the imidazolyl group in the catalytic enantioselective ester hydrolysis.[15] Recently, Murakami and co-workers showed that the carboxyl group of cationic peptide surfactants bearing both histidyl and aspartyl residues intramolecularly enhances the reactivity of the imidazolyl group.[16]

It is thus clear that the present functional micellar systems operate with nucleophilic acylation of the imidazolyl group, following which the hydroxyl group acts as an effective catalyst in the deacylation process. It is also suggested that the carboxylate group of **1b** interacts with the imidazolyl group so as to enhance the reactivity of the latter. All of the results are consistent with the mechanism given in Scheme I. Although, the esterolytic efficiency of these micellar catalytic systems is much lower than that of α-chymotrypsin, we find that the three functional groups are involved in the catalytic cycle of ester hydrolysis. This mode of action should be of considerable interest in connection with studies on the enzyme reaction since a key feature of the α-chymotrypsin-catalyzed hydrolysiss is basic activation by the Asp-Ser-His triad catalytic system.[17]

Acknowledgment. We are grateful to Professor Robert A. Moss for helpful comments.

Registry No. 1a, 2497-02-1; **1b**, 55258-10-1; **1c**, 78829-12-6; **2a**, 57-09-0; **2b**, 20317-32-2; **2c**, 42474-90-8; **2d**, 63989-29-7; **2e**, 84174-13-0; PNPA, 830-03-5; PNPH, 956-75-2; esterase, 9013-79-0; chymotrypsin, 9004-07-3.

(14) The acylation rate constants were measured at several concentrations of **2a** (2.0–30 × 10^{-3} M). The catalytic effects of both **1b** and **1c** were sensitive to **2a** concentration, but the ratios of the rates for **1b** compared to **1c** were essentially unchanged, suggesting that there was no significant structural difference between the mixed micelles of **1b** and **1c**. See also ref 15.

(15) Ihara, Y.; Hosako, R.; Nango, M.; Kuroki, N. *J. Chem. Soc., Chem. Commun.* **1981**, 393; *J. Chem. Soc., Perkin Trans. 2*, in press.

(16) Murakami, Y.; Nakano, A.; Yoshimatsu, A.; Matsumoto, K. *J. Am. Chem. Soc.* **1981**, *103*, 2750.

(17) The hydrolysis of peptides catalyzed by α-chymotrypsin involves nucleophilic attack by the Ser-195 hydroxyl moiety.[3] However, for the hydrolysis of nonspecific substrates, Kirsch and Hubbard suggested that acylation of the enzyme would involve nucleophilic attack by the His-57 imidazole, followed by fast acyl transfer to the Ser-195 hydroxyl function: Kirsch, J. F.; Hubbard, C. D. *Biochemistry* **1972**, *11*, 2483.

Total Syntheses of *dl*-Gephyrotoxin and *dl*-Dihydrogephyrotoxin

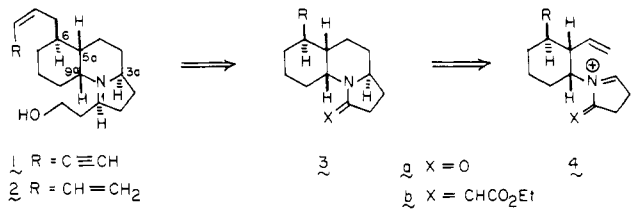
David J. Hart*† and Ken-ichi Kanai

Contribution from the Department of Chemistry, The Ohio State University, Columbus, Ohio 43210. *Received July 6, 1982*

Abstract: A total synthesis of the Dendrobatid alkaloid *dl*-gephyrotoxin (**1**) has been achieved in 23 steps, using cyclohexenone, 1,3-butadiene, succinimide, ethyl bromoacetate, and propyne as carbon sources. A total synthesis of *dl*-dihydrogephyrotoxin (**2**), a structure tentatively assigned to a minor Dendrobatid alkaloid, is also described.

A number of alkaloids that possess interesting pharmacological properties have been isolated in minute quantities from skin extracts of frogs belonging to the Dendrobatid family.[1,2] These alkaloids have stimulated numerous synthetic studies,[3-10] and work on the synthesis of these natural products continues in laboratories throughout the world. Our interest in the Dendrobatid alkaloids has focused on gephyrotoxin (**1**), a muscarinic antagonist whose structure was determined by X-ray crystallographic analysis of its *p*-bromobenzoate.[11] This paper describes the details of studies that have culminated in a total synthesis of **1** and the related compound dihydrogephyrotoxin (**2**).[11]

Scheme I



† This paper is dedicated to my father and mother, Prof. Harold Hart and Geraldine Hart, on the occasions of their 60th birthdays and 40th wedding anniversary.

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