© 1999 The Chemical Society of Japan

Transesterification by (Hydroxo)nickel(II) Complex in the Presence of External Alcohol

Masami Ito,* Ken Sakai,† Taro Tsubomura,† and Yu-saku Takita

Research and Development Center, Oita University, Dannoharu 700, Oita 870-1192

†Department of Industrial Chemistry, Seikei University, Kichijoji-Kitamachi, Musashino, Tokyo 180

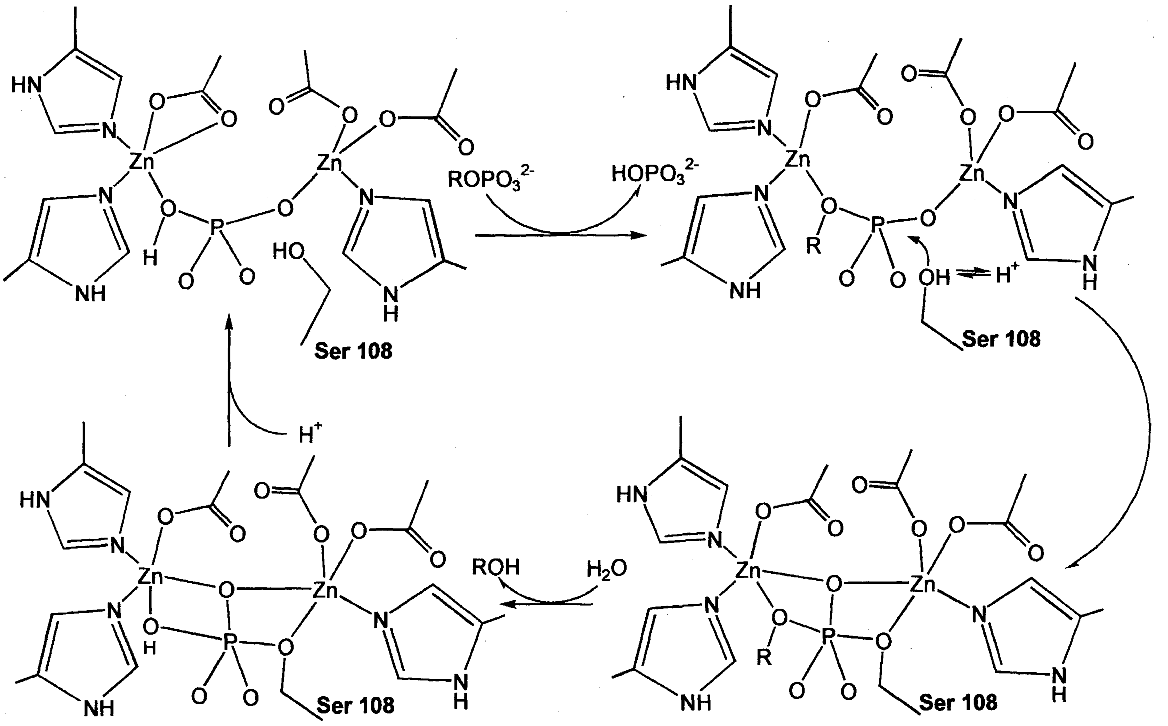
(Received July 6, 1998)

The nickel(II) complexes, $[(\text{tpa})\text{Ni}^{\text{II}}(\mu\text{-OH})_2\text{Ni}^{\text{II}}(\text{tpa})](\text{ClO}_4)_2$ (tpa = tris(2-pyridylmethyl)amine) (**2**), $[\text{Ni}^{\text{II}}(\text{CH}_3\text{CN})(\text{tpa})(\text{H}_2\text{O})](\text{ClO}_4)_2$ (**3**) have been prepared and fully characterized. X-Ray crystallography of complex **2** and its methanol adduct, $[(\text{tpa})\text{Ni}^{\text{II}}(\mu\text{-OH})_2\text{Ni}^{\text{II}}(\text{tpa})](\text{ClO}_4)_2\cdot2\text{MeOH}$ ((**2**)·2MeOH) revealed a molecular structure containing two Ni centers bridged by two OH ligands. The Ni–O distances in **2**·2MeOH are longer than those in **2**. The MeCN ligand in complex **3** is replaced by EtCN to give $[\text{Ni}^{\text{II}}(\text{EtCN})(\text{tpa})(\text{H}_2\text{O})](\text{ClO}_4)_2$ (**4**), which was characterized by X-ray crystallography. The kinetic study of *p*-NPA (*p*-nitrophenyl acetate, 0.1, 0.5, 1 mM) hydrolysis by complex **2** (20, 10, 6 mM) was performed in the presence of ethanol (100 mM) in MeCN solution ($1\ \text{M} = 1\ \text{mol dm}^{-3}$). By following the 400 nm absorption derived from *p*-nitrophenolate ion, we obtained the second-order kinetics; first-order in [**2**] and first-order in [*p*-NPA]. Additon of ethanol resulted in enhancement of the second-order rate constant (from $1.6\times10^{-3}\ \text{M}^{-1}\,\text{s}^{-1}$ to $1.6\times10^{-2}\ \text{M}^{-1}\,\text{s}^{-1}$ at 25 °C) as well as formation of a transesterified product, ethyl acetate. The second-order rate constant of the reaction in the presence of added ethanol ($0.016\ \text{M}^{-1}\,\text{s}^{-1}$) at 25 °C is much larger than that of the reaction without ethanol (0.0016 $\text{M}^{-1}\,\text{s}^{-1}$). Also observed was a transesterified product, ethyl acetate, from the reaction of **2** with *p*-NPA in the presence of ethanol. The $\text{p}K_\text{a}$ value of the ligated water molecule in **3** was estimated to be 10.7 by kinetic measurements in 40% MeCN aqueous solution at 25 °C, $I = 0.3$.

Numerous hydrolytic enzymes are known, but the reaction mechanism of most of these enzymes remains to be elucidated.[1] Among them, alkaline phosphatase (AP) is a hydrolytic enzyme that cleaves phosphomonoesters nonspecifically,[2,3] or transfers the phosphoryl groups to other alcohols.[4] This enzyme has two Zn^{2+} ions and one Mg^{2+} ion in its active site.[5] Although Mg^{2+} is close to the dinuclear Zn site, it does not directly participate in the reaction.[5] This enzyme also contains a serine residue close to the dinuclear Zn site. In contrast with the Mg^{2+} ion, this serine residue is proposed to take part in the catalytic reaction directly. The proposed catalytic reaction mechanism involves the covalent intermediate, phosphorylated serine, as shown in Scheme 1.[5—7] While the alcohol moiety of the serine residue does not react directly with the phosphate ester, the activation of the hydroxyl group in the serine residue is indispensable for the catalytic reaction.[6] However, it is still uncertain how the alkaline phosphatase activates the serine residue close to the dinuclear Zn^{2+} ion site.

Kimura et al. reported the syntheses of several Zn(II) complexes with macrocyclic nitrogen ligands that possessed pendant alcohol groups.[8] The pendant alcohol OH group in the Zn(II) complexes could be activated by Zn(II)-bound OH^- and subsequently the alcohol (or alkoxo) moiety could attack the substrate as a nucleophile.[8] According to their experiments, the nucleophilicity of the "activated" alcohol is much greater than that of the metal-bound hydroxo moiety. They proposed that their system mimicked the catalytic cycle performed by alkaline phosphatase, which had a serine residue close to the active site. Furthermore, their results imply that transesterification may also proceed by nucleophilic attack of external alcohol which possesses hydrogen-bonding to the metal-bound hydroxo moiety, instead of the tethered alcohol held close to the ligated hydroxo group. The series of elegant experiments done by Kimura and his colleague suggest a possible enzymatic mechanism. In the case of alkaline phosphatase, which has a serine residue adjacent to the active site, the metal hydroxo moiety formed by the deprotonation of the Zn(II)-bound water in the active site is a good candidate for the nucleophilic attack on the serine residue. The alcohol OH in the serine residue is activated by the metal-bound hydroxo moiety, and then reacts with the phosphate ester to afford the proposed "phosphorylated serine intermediate." Subsequent hydrolysis of this "phosphorylated serine intermediate" by coordinated hydroxide ion completes the catalytic cycle. While the reaction mechanism of transesterification by AP has not yet been elucidated, their results also imply that transesterification may proceed in the same manner by activation of external alcohol, instead of the serine residue adjacent to the active site. It is known that some hydrolytic enzymes such as phospholipase C[9] and RNase P[10] also perform transesterification in a manner similar to AP. Thus, these enzymes and AP may have a common reaction step in their catalytic reactions.

Here we report transesterification of carboxylic esters promoted by the (hydroxo)nickel(II) complex, [(tpa)-



Scheme 1.

$Ni^{II}(\mu\text{-}OH)_2Ni^{II}(tpa)](ClO_4)_2$ (**2**) (tpa = tris(2-pyridylmethyl)amine). In the reaction of complex **2** with *p*-nitrophenyl acetate (*p*-NPA), it was also observed that the added alcohol had a effect to increase the formation rate of *p*-nitrophenolate. Electronic spectra of the reaction mixture have provided a clue to elucidate the structure of the intermediate, whose d–d transition band is influenced by the change of environement around the metal center. The transesterification mechanism is discussed on the basis of the spectroscopic as well as kinetic results.

Experimental

Instrumentation. IR spectra were obtained on a Hitachi 260-50 using the KBr method. Gas chromatography was performed on an HP-5980 gas chromatograph. Electronic spectra were measured on a Shimadzu UV-240 spectrophotometer. The kinetic studies were carried out on the spectrophotometer equipped with a thermoelectric cell temperature controller. X-Ray data were collected on a Rigaku AFC-5 four-circle diffractometer (AFC program package provided by Rigaku). The structural analyses were carried out with a MICRO-VAX II computer and the teXsan program package provided by Rigaku.

Materials and Methods. Preparation of the Ni(II) complexes was performed using standard Schlenk techniques. Acetonitrile (MeCN) was dried over CaH_2 and distilled under Ar prior to use. Other solvents were used without purification. $tpa(HClO_4)_3$ was prepared by the previously reported method.[11]

Synthesis of $[(tpa)Ni^{II}(\mu\text{-}Cl)_2Ni^{II}(tpa)](ClO_4)_2$ (1). A methanol solution of $tpa(HClO_4)_3$ (3.00 g, 5.01 mmol) and triethylamine (1.52 g, 15.03 mmol) was added to anhydrous nickel(II) chloride ($NiCl_2$) (0.65 g, 5.01 mmol). The yellow solid of $NiCl_2$ was dissolved after 6 h of vigorous stirring. Precipitation of a blue solid from a dark blue-green solution was observed. The solid was collected by filtration and washed with methanol (5 ml×2) to give analytically pure **1** as a blue solid. Yield: 3.98 g (82%). X-Ray quality cubic blue crystals of **1** were obtained from the recrystallization of Et_2O diffusion into a methanol solution of **1**.[12] Anal. Found: C, 44.56; H, 4.02; N, 11.27%. Calcd for **1** $Ni_2C_{36}N_8H_{36}O_8Cl_4$: C, 44.68; H, 3.72; N, 11.57%. IR (KBr), ν (C=C), 1601, ν (ClO_4), 1150 cm^{-1}. UV-vis (ε/$M^{-1}\,cm^{-1}$) 585 (70), 770 (31), 890 nm (43). *Caution! Perchlorate salts of transition metal complexes are potentially explosive. These complexes should be prepared only in small quantities and handled with care.*

Synthesis of $[(tpa)Ni^{II}(\mu\text{-}OH)_2Ni^{II}(tpa)](ClO_4)_2$ (2). An aqueous solution (10 ml) of complex **1** (1.00 g, 1.03 mmol) was slowly added to 1 M NaOH solution (20 ml). A blue precipitate was formed immediately. The solid was filtered and washed with methanol, affording **2** as a blue solid. Yield: 0.73 g (76%). Recrystallization from MeCN gave blue cubic crystals suitable for crystallography. The crystallization of **2** from methanol afforded the methanol adduct of **2**, $[(tpa)Ni^{II}(\mu\text{-}OH)_2Ni^{II}(tpa)](ClO_4)_2\cdot 2MeOH$ ((**2**)·2MeOH). Anal. Found: C, 46.44; H, 4.03; N, 11.81%. Calcd for **2** $Ni_2C_{36}N_8H_{38}O_{10}Cl_2$: C, 46.44; H, 4.08; N, 12.02%. IR (KBr), ν (C=C) 1601, ν (ClO_4) 1150 cm^{-1}. UV-vis (ε/$M^{-1}\,cm^{-1}$) 585 (70), 770 (31), 890 nm (43).

Synthesis of $[Ni^{II}(CH_3CN)(tpa)(H_2O)](ClO_4)_2$ (3). Complex **2** (1.50 g, 1.61 mmol) was treated with perchloric acid (3.20 mmol. 50%aq) in 10 ml of acetonitrile. A color change from blue to purple was noted. The purple acetonitrile solution was cooled to −20 °C overnight, affording **3** as a purple solid. Yield: 1.18 g (65%). Anal. Found: C, 39.11; H, 3.71; N, 11.46%. Calcd for **3** $Ni_1C_{20}N_5H_{23}O_9Cl_2$: C, 39.57; H, 3.79; N, 11.53%. IR (KBr) ν (C=C) 1600, ν (ClO_4) 1150 cm^{-1}. UV-vis (ε/$M^{-1}\,cm^{-1}$) 305 (250), 532 (40), 831 nm (38).

X-Ray Data Collections and Structural Determinations. Single crystals of **2** were grown in an acetonitrile solution at room temperature. Recrystallization of **2** from MeOH yielded single crystals of the MeOH adduct, (**2**·2(MeOH)). Recrystallization of **3** from propionitrile at room temperature afforded the propionitrile-ligated complex formulated as $[Ni^{II}(EtCN)(tpa)(H_2O)](ClO_4)_2$ (**4**). Each crystal was stable when it was sealed in a thin-wall glass capillary, and the diffraction data were recorded at room temperature. A Mo X-ray source equipped with a graphite-monochromater (Mo $K\alpha$, λ = 0.710680 Å) was used in all measurements. Automatic centering and least-squares routines (the AFC program provided by Rigaku) were carried out for all the reflections at 2θ ca. 30°, to determine the cell dimensions. Data collections were performed by the ω–2θ scan method. The data were corrected for Lorentz and polarization effects. Three standard checked reflections were monitored at every 100 reflections. No serious decay was observed for any samples. The cell parameters, data collection details, and refinement results for **2**, **2**·MeOH, **4** are given in Table 1. The positional parameters of the nickel atoms of all complexes were determined by the Patterson method. Subsequent difference Fourier synthesis located all non-hydrogen atoms, which were refined anisotropically. The hydrogen atoms were located by assuming the ideal geometry (d(C–H) 1.0 Å) and included in the structual calculation without further refinement of the positional parameters. The calculations were performed by an IRIS Indigo computer with the teXsan program package provided by Rigaku to minimize the function; $\sum w(|F_o|-|F_c|)^2$ where, $w = 1/\sigma^2(F_o^2)$. The unweighted and weighted factors are $R = \sum w(|F_o|-|F_c|)/\sum|F_o|$ and $R_w = [\sum w(|F_o|-|F_c|)^2/\sum w|F_o|^2]^{1/2}$, respectively. The final R (R_w) value and refinement data are presented in Table 1. Full bond lengths, bond angles, anisotropic thermal parameters for non-hydrogen atoms, hydrogen coordinates, and F_o/F_c tables for **2**, **2**·MeOH, **4** were deposited as Document No. 72002 at the Office of the Editor of Bull. Chem. Soc. Jpn.

Kinetic Experiments for the Carboxylic Acid Ester Cleavage Reaction by 2 in Non-Aqueous Solution. Kinetic measurements for the cleavage reaction of p-nitrophenyl acetate (p-NPA) by complex **2** were performed by following increase of the absorption of p-nitrophenolate ion at 400 nm. The temperature was controlled at 25 °C in all experiments. Acetonitrile solutions containing the substrate, p-NPA (0.1, 0.5, 1 mM), complex **2** (20, 10, 6 mM), and ethanol (100, 60, 40, 0 mM) were prepared. UV data were recorded immediately after the mixing and then followed until formation of ca. 3% of p-nitrophenolate ion. The observed pseudo-first-order rate constant k_{obsd} (s^{-1}) was calculated from the decay slope. The value of k_{obsd}/[OH^- species] gave the second order rate constant ([OH^- species] = 2[**2**]).

Kinetic Experiments for the Carboxylic Acid Ester Cleavage Reaction with 2 in Aqueous-Acetonitrile Solution. The cleavage reaction rate (the formation rate of p-nitrophenolate) of p-NPA was measured by the initial slope method (monitoring the increase in 400 nm absorption of the released p-nitrophenolate anion) at 25 °C. Buffer solutions containing 20 mM Good's buffer (CHES) and NaOH (0—20 mM) were used, and the ionic strengths were adjusted to 0.3 with $NaClO_4$. In the experiments the following conditions were used: p-NPA (1 mM), complex **2** (10 mM), and ethanol (0 mM, 100 mM) were mixed in 40% acetonitrile aqueous buffer solution. The UV absorption spectra were recorded immediately and followed generally until ca. 5% of p-nitrophenolate ion was formed. The log ε values at 400 nm for p-nitrophenolate were 4.26 in the range of pH 8.5—11.0. The observed first order rate constant was calculated from the decay slope.

The GC Analyses for the Transesterified Products Catalyzed by Complex 2. In a typical run, complex **2** (50 mg, 0.05 mmol), phenyl acetate (536 mg, 4 mmol), and ethanol (808 mg, 20 mmol) were dissolved in dry acetonitrile (10 ml) under argon and the reaction mixture was stirred for 20 h. The solution was subjected to gas chromatography and the amount of EtOAc (ethyl acetate) formed was quantified by an internal standard method. Naphthalene was used as internal standard.

Results and Discussion

Synthesis and Characterization of $[(tpa)Ni^{II}(\mu\text{-}OH)_2Ni^{II}(tpa)](ClO_4)_2$ (2). Our initial effort was fo-

Table 1. Selected Crystal Data and Data Collection Detail for **2**, **2**·2MeOH, and **4**

Complex	**2**	**2**·2MeOH	**4**
Formula	$C_{36}H_{38}Ni_2Cl_2O_{10}N_8$	$C_{38}H_{46}Ni_2Cl_2O_{10}N_8$	$Ni_1Cl_2O_9N_5C_{21}H_{25}$
F. W.	931.02	987.07	621.07
Crystal system	Monoclinic	Triclinic	Monoclinic
Space group	$P2_1/a$	$P\bar{1}$	$P2_1/n$
a/Å	16.017(3)	14.679(7)	11.381(3)
b/Å	13.291(2)	14.728(8)	19.278(4)
c/Å	9.564(4)	13.626(8)	12.487(3)
α/°		122.65(3)	
β/°	98.9(2)	111.92(4)	91.73(2)
γ/°		90.0180(5)	
V/Å^2	2020.0(7)	2223(6)	2738(1)
Z	2	2	4
D_c/g cm^{-3}	1.531	1.475	1.506
Crystal size	0.2 × 0.2 × 0.2	0.2 × 0.2 × 0.2	0.50 × 0.38 × 0.15
Radiation	Graphite-monochromatized Mo $K\alpha$ (λ =0.710680 Å)		
μ(Mo $K\alpha$)/cm^{-1}	11.31	5.12	9.60
Scan mode		ω–2θ	
2θ range	3—50	3—50	3.3—50
Octant measured	$\pm h, +k, +l$	$\pm h, \pm k, \pm l$	$h, k, \pm l$
No. of measured reflection	3726	7814	6804
No. of observed data	2833	5882	2605
R	0.078	0.092	0.068
R_w	0.068	0.099	0.089

cused on the synthesis of (hydroxo)nickel(II) tpa complex (tpa = tris(2-pyridylmethyl)amine).[13] We prepared the starting material of (hydroxo)nickel(II) complex, a dinuclear (μ-chloro)nickel(II) complex [(tpa)Ni^{II}(μ-Cl)$_2$Ni^{II}(tpa)](ClO$_4$)$_2$ (**1**), via the reaction of NiCl$_2$ and tpa(HClO$_4$)$_3$ in the presence of triethylamine.[12] The (hydroxo)nickel(II) complex, [(tpa)-Ni^{II}(μ-OH)$_2$Ni^{II}(tpa)](ClO$_4$)$_2$ (**2**) was obtained by treatment of complex **1** with an aqueous NaOH solution. Complex **2** was characterized by elemental analysis, IR and X-ray crystallography. The d–d transition in the UV-vis spectrum indicates its typical high spin nickel(II) character. The crystal structure is shown in Fig. 1(a). The data confirmed the dinuclear structure of **2** with the imposed center of symmetry. The structure contains a slightly distorted octahedral N$_4$O$_2$ ligand set. The selected bond distances and bond angles are given in Table 2. The distances between the bridging hydroxo oxygen and the Ni(II) ion (1.990 and 2.034 Å) are typical of those found in high spin nickel(II) complexes.[14] Gafford et al. reported a chromium bridging hydroxo complex supported by the tpa ligand formulated as [(tpa)Cr^{III}(μ-OH)$_2$Cr^{III}(tpa)]-(ClO$_4$)$_4$.[11] The geometry around the chromium center is comparable to that in **2**.

Crystallization of **2** from methanol gave crystals which contain solvated methanol molecules located close to the ligated hydroxo group as shown in Fig. 1(b). The dinuclear structure of (**2**)·2MeOH sits on an imposed center

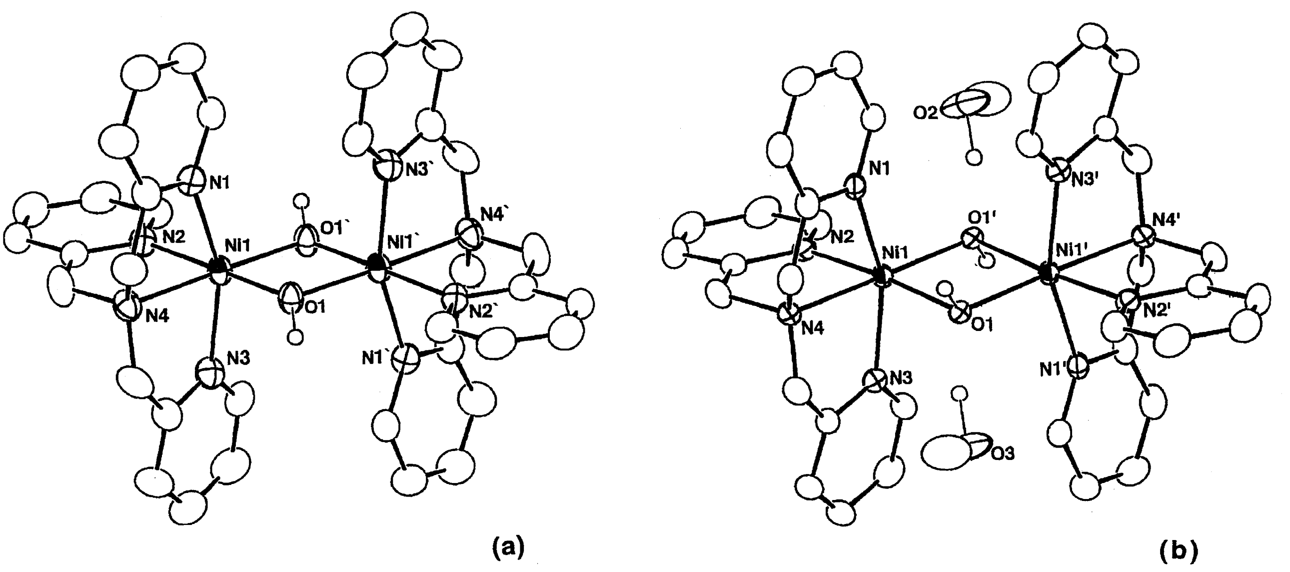


Fig. 1. a) Molecular structure of [tpaNi(II)(μ-OH)$_2$Ni(II)tpa](ClO$_4$)$_2$ (MeOH)$_2$ (**2**). b) Molecular structure of [tpaNi(II)(μ-OH)$_2$Ni-(II)tpa](ClO$_4$)$_2$·2MeOH ((**2**)·2MeOH). Perchlorate ions are omitted for clarity.

Table 2. Selected Bond Distances (Å) and Angles (deg) for [tpaNi(II)(μ-OH)$_2$Ni(II)tpa](ClO$_4$)$_2$ **2** and **2**·2MeOH

Bond distances (Å) and bond angles (deg) for **2**					
Ni(1)–O(1)	2.034(4),	Ni(1)–O(1′)	1.990(4),	Ni(1)–N(1)	2.118(6),
Ni(1)–N(2)	2.090(5),	Ni(1)–N(3)	2.113(5),	Ni(1)–N(4)	2.114(5)
O(1)–Ni(1)–O(1′)	82.1(2),	Ni(1)–N(4)–C(12)	110.3(4),	O(1)–Ni(1)–N(1)	94.5(2),
O(1)–Ni(1)–N(2)	178.2(2),	O(1)–Ni(1)–N(3)	90.4(2),	O(1)–Ni(1)–N(4)	96.5(2),
O(1′)–Ni(1)–N(1)	99.2(2),	O(1′)–Ni(1)–N(2)	99.4(2),	O(1′)–Ni(1)–N(3)	102.6(2)
O(1′)–Ni(1)–N(4)	178.1(2)	N(1)–Ni(1)–N(2)	86.3(2),	N(1)–Ni(1)–N(3)	158.1(2)
N(1)–Ni(1)–N(4)	79.5(2),	N(2)–Ni(1)–N(3)	88.3(2),	N(2)–Ni(1)–N(4)	81.9(2)
N(3)–Ni(1)–N(4)	78.7(2),	Ni(1)–O(1)–Ni(1)	97.9(2).		
Bond distances (Å) and bond angles (deg) for **2**·2MeOH.					
Ni(1)–O(1)	2.091(7),	Ni(1)–O(1′)	2.06(1),	Ni(1)–N(1)	2.12(1),
Ni(1)–N(2)	2.093(9)	Ni(1)–N(3)	2.142(9),	Ni(1)–N(4)	2.14(1),
O(1′)–Ni(1)–O(1)	84.4(3),	O(1)–Ni(1)–N(4)	96.0(4),	O(1)–Ni(1)–N(1)	90.5(3),
O(1)–Ni(1)–N(2)	176.1(4),	O(1)–Ni(1)–N(3)	93.3(3),	O(1′)–Ni(1)–N(4)	178.3(3),
O(1′)–Ni(1)–N(1)	101.4(4),	O(1′)–Ni(1)–N(2)	97.2(4),	O(1′)–Ni(1)–N(3)	99.6(4),
N(1)–Ni(1)–N(4)	80.2(4),	N(2)–Ni(1)–N(4)	82.5(5),	N(3)–Ni(1)–N(4)	78.7(5),
N(1)–Ni(1)–N(2)	85.7(4),	N(1)–Ni(1)–N(3)	158.9(5),	N(3)–Ni(1)–N(2)	90.0(3).

of symmetry.[15] The average bond distance of O1–O2 and O1′–O3 in this compound is 2.693 Å, indicating the existence of a hydrogen-bonding interaction. Furthermore, the bond lengths between the nickel and the oxygen in the hydroxo ligands (Ni1–O1 (2.091(7) Å) and Ni1–O1′ (2.06(1) Å) are longer than the corresponding values in structure **2** (2.034(4) and 1.990(4) Å).[15] Thus, the hydrogen bond weakens the Ni–O bonds. In the hope of getting evidence for the alcohol adduct of **2** in the solution, we attempted ^{1}H NMR measurement in the presence of ethanol. However, the paramagenetism of the complex prevented us from observation of any changes of the OH hydrogen signal caused by the addition of the alcohol.

The Reaction of (Hydroxo)Nickel(II) Complex with Carboxylic Acid Ester in Non-Aqueous Solution. The possible hydrogen bond between the hydroxo group in **2** and external alcohol in solution prompted us to investigate how the alcohol effected the reactivity of complex **2**. The ester, *p*-nitrophenyl acetate (*p*-NPA), was initially used as a substrate. We observed the progress of the reaction of *p*-NPA with complex **2** in MeCN by following the absorption band at 400 nm derived from the *p*-nitrophenolate anion released by the hydrolysis. The pseudo-first-order rate constant ($k_{\text{obsd}}(\text{s}^{-1})$) of the *p*-nitrophenolate formation by the reaction of *p*-NPA (0.1, 0.5, 1 mM) with complex **2** (20, 10, 6 mM) was measured by monitoring the UV-vis band at 400 nm ($\log \varepsilon = 4.30$) at 25 °C.[16] The value of $k_{\text{obsd}}/[\text{OH}^-\text{ species}]$ gave the second order rate constant $k(p\text{-NPA})$ ($\text{M}^{-1}\,\text{s}^{-1}$), as shown in Scheme 2 ($[\text{OH}^-\text{ species}] = 2[\mathbf{2}]$). We obtained the second order rate constant ($k(p\text{-NPA})$) of 0.0016 $\text{M}^{-1}\,\text{s}^{-1}$ for the reaction of *p*-NPA with complex **2** in acetonitrile. These kinetic data indicate that the complex is not dimeric but monomeric. The reaction of *p*-NPA with complex **2** in the presence of ethanol was determined by the same method. The kinetics showed the second-order dependence on the rate constant for the total concentration of the Ni complex (the concentration of the nickel complex was calculated as monomeric form) and [*p*-NPA].

$$\frac{-\mathrm{d}[p\text{-NPA}]}{\mathrm{d}t} = k[p\text{-NPA}][\text{OH}^-\text{ species}]$$

$$[\text{OH}^-\text{ species}] = 2[\text{complex } \mathbf{2}]$$

Scheme 2.

In the presence of excess ethanol with respect to complex **2**, the second-order dependence of the rate constants on the total concentration of the Ni complex (the concentration of the nickel complex was calculated as the monomeric form) and the concentration of *p*-nitrophenyl acetate are consistent with catalysis by a monomeric complex. The *p*-nitrophenolate formation rates by the cleavage of *p*-NPA with **2** in the presence of ethanol were measured under various reaction conditions (solvent: MeCN; temperature = 25 °C; [EtOH] = 100 mM; [*p*-NPA] = 0.1, 0.5, and 1.0 mM; [**2**] = 6.0, 10.0, and 20.0 mM). From the measurements we obtained the second-order kinetics obeying Scheme 2 where $k(p\text{-NPA}) = 0.0160$ $\text{M}^{-1}\,\text{s}^{-1}$.[16] This value is ten times greater than that in the absence of ethanol.

The reaction product of this system was changed by the added alcohol, and transesterification to the external alcohol was observed using complex **2** as a catalyst. The reaction of complex **2** with *p*-NPA in the presence of excess ethanol gave EtOAc, as shown in Table 3.[18] In this case, the amount of *p*-nitrophenolate ion observed by UV-vis spectrum was equal to the quantity of the formed EtOAc observed by the GC analysis. Therefore, almost no hydrolysis occurred in this system. Usually, *p*-NPA is considered to be more "reactive" than other materials, such as phenyl acetate. However, it is striking that the tranesterification from phenyl acetate to ethanol (turnover number (T.N. 29.6)) is larger than that from *p*-NPA (T.N. 3.7) (Table 3). Furthermore, it is notable that we could also observe the transesterification from an amide (EtNHAc). These observations indicated that addition of alcohol to the system resulted in formation of a powerful nucleophile that was stronger than the metal-bound hydroxo moiety.

A remarkable point of this system is the ability to perform catalytic transesterification. It is well known that metal alk-

Table 3. Catalytic Formation of Ethyl Acetate by Complex **2** in the Presence of Ethanol

Substrate	Reaction time h	Turnover number[a)]	Conversion[b)] %
p-Nitrophenyl acetate	2	2.9	7.2
	10	3.7	9.3
	10	3.6	
Phenyl acetate	2	9.4	24.0
	10	29.6	73.7
p-Cresol acetate	2	9.3	23.3
	10	30.6	76.3
N-Ethylacetamide	2	Trace	—
	10	0.3	0.8
	24	1.6	4.0

Complex 0.05 mmol, substrate 2.00 mmol, ethanol 1.80 mmol, solvent acetnitrile 10 ml. a) Based on the amount of complex **2**. b) Based on the added ester.

oxides promote the transesterification, whereas these systems can only perform stoichiometric transesterification and not catalytic transesterification.[19] The details of the proposed reaction mechanism in the present system will be described later.

The Formation of [$Ni^{II}(CH_3CN)(tpa)(H_2O)](ClO_4)_2$ by the Reaction of Complex 2 with a Proton. To elucidate the character of metal-bound hydroxo moiety in complex **2**, spectrophotometric changes of complex **2** were investigated under various pH conditions in 40% MeCN aqueous solution. The UV-vis spectra of complex **2** are shown in Fig. 2.

Under basic conditions, the band at 580 nm is identical with the d–d transition of complex **2** in MeCN, whereas the band at 580 nm shifts to 530 nm at relatively low pH. This shift indicates the change of the coordination environment of the Ni(II) ion in complex **2**. It was strongly suggested that the hydroxo ligand of **2** was protonated under the acidic conditions to give the purple species which has a band at 530 nm. To clarify this point, characterization of this purple species was performed. When complex **2** was made to react with 2 equivalents of $HClO_4$ at room temperature in MeCN, an immediate color change from blue to purple was noted. The solution was concentrated and cooled at −20 °C to yield the purple Ni(II) complex, formulated as [$Ni^{II}(CH_3CN)(tpa)(H_2O)](ClO_4)_2$ (**3**). The composition was identified on the bases of elemental analysis and IR. Owing to problems with crystallization of **3** from acetonitrile, we tried recrystallization of **3** from EtCN. The structure of [$Ni^{II}(EtCN)(tpa)(H_2O)](ClO_4)_2$ (**4**) is shown in Fig. 3.

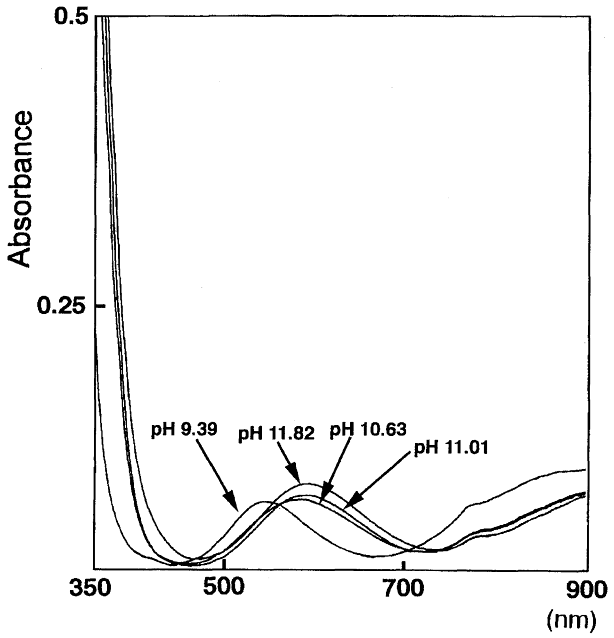


Fig. 2. The UV-vis spectra of complex **2** under various pH conditions in 40% MeCN aqueous solution. Complex **2** 1 mM, CHES 20 mM, and NaOH (0—20 mM).

Complex **4** has a monomeric octahedral structure with the two perchlorate anions. The complex contains a divalent Ni center bonded to a water molecule. The Ni(II)–O distance (2.137 Å) in complex **4** is within the range of already reported Ni(II)–OH_2 bond lengths,[20] and is slightly longer than those in complex **2** (2.034 and 1.990 Å) (Table 4). The UV-vis spectrum of complex **3** exhibits two characteristic bands at 532 and 831 nm. The spectrum of **3** is identical with the UV-vis spectrum of **2** in relatively acidic aqueous solution as shown in Fig. 2. On the basis of these observations, we conclude that complex **2** is converted to complex **3** under acidic conditions, as shown in Scheme 3.

The Reaction of Carboxylic Acid Ester with Complex 2 in MeCN-Aqueous Buffer Solution. To elucidate the details of this reaction system, the dependence of the carboxyester hydrolysis reactivity of **2** on pH was investigated in MeCN-aqueous media. The initial hydrolysis rate in 40% MeCN aqueous solution at 25 °C, $I = 0.3$ and pH 8.0—11.0 was followed by the appearance of *p*-nitrophenolate ion at 400 nm. The pseudo-first-order rate constants k_{obsd} at var-

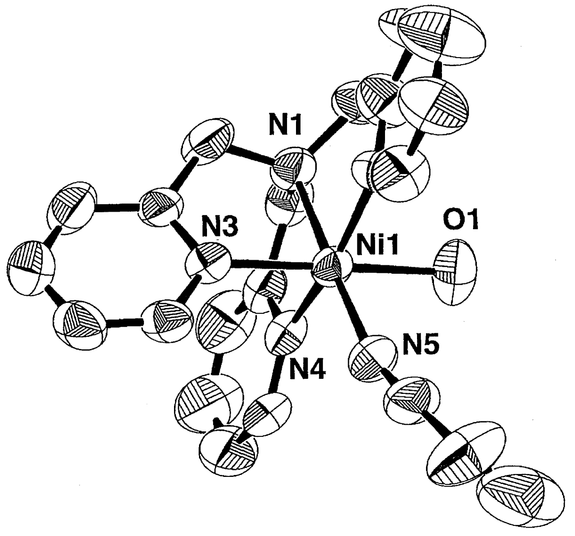


Fig. 3. Molecular structure of [$tpaNi(II)(H_2O)(EtCN)](ClO_4)_2$ (**4**). Perchlorate ions are omitted for clarity.

Table 4. Selected Bond Distances (Å) and Angles (deg) of [$tpaNi(II)(H_2O)(C_2H_5CN)](ClO_4)_2$ (**4**)

Ni(1)–O(1) 2.137(7)	Ni(1)–N(1) 2.089(8)	Ni(1)–N(2) 2.076(10)
Ni(1)–N(3) 2.056(8)	Ni(1)–N(4) 2.057(9)	Ni(1)–N(5) 2.043(9)
O(1)–Ni(1)–O(1) 82.1(2)	O(1)–Ni(1)–N(1) 94.5(2)	O(1)–Ni(1)–N(2) 178.2(2)
O(1)–Ni(1)–N(3) 90.4(2)	O(1)–Ni(1)–N(4) 96.5(2)	O(1)–Ni(1)–N(1) 99.2(2)
O(1)–Ni(1)–N(2) 99.4(2)	O(1)–Ni(1)–N(3) 102.6(2)	O(1)–Ni(1)–N(4) 178.1(2)
N(1)–Ni(1)–N(2) 86.3(2)	N(1)–Ni(1)–N(3) 158.1(2)	N(1)–Ni(1)–N(4) 79.5(2)

$$[\mathrm{tpaNi(II)}(\mu\text{-}\mathrm{OH})_2\mathrm{Ni(II)tpa}]^{2+} \xrightleftharpoons[-2\mathrm{H}^+,\ -2\mathrm{MeCN}]{2\mathrm{H}^+,\ 2\mathrm{MeCN}} 2[\mathrm{tpaNi(II)(H_2O)(CH_3CN)}]^{2+}$$

Scheme 3.

ious pH values were obtained by fitting the absorbance vs. reaction time. Since complex **3** is inert toward hydrolysis of *p*-NPA, the pK_a value was estimated to be 10.7, on the basis of the plot of k_{obsd} vs. pH for the hydrolysis of *p*-NPA by complex **2** in Fig. 4. This value is consistent with the result obtained from the spectrophotometric change shown in Fig. 2, and is slightly larger than the values reported for the (aquo)Zn(II) complexes with cyclic amine ligands reported by Kimura et al.[8] This is presumably due to the strong electron-donating property of the polypyridine ligand.[21] The data shown in Fig. 4 demonstrate that the presence of alcohol accelerates the generation of *p*-nitrophenolate ion from *p*-NPA. The effect of alcohol is less pronounced in aqueous media than in organic media, probably owing to the solvolysis effect of the water.

The pseudo-first-order rate constant of *p*-NPA cleavage with 10 equiv of ethanol toward **2** (0.0052 s^{-1}) is only 1.5 times greater than the value with no ethanol (0.0034 s^{-1}). A GC analysis indicated that transesterification of carboxylate ester was observed in the aqueous MeCN solution, as shown in Table 5. Although the yield of the transesterification products in aqueous solution is lower than that in organic media, the reaction system of **2** and ethanol with a "more reactive substrate" gave larger amounts of the products. The amount of EtOAc formed by using *p*-nitrophenyl acetate as the substrate was larger than that formed by using *p*-methylphenyl acetate or phenyl acetate. While this trend seems to contradict the results observed in MeCN solution shown in Table 4, a plausible mechanism will be discussed below to address this point. The pK_a value of **3** estimated in the presence of ethanol is identical with the value in the absence of ethanol. This indicates that the metal-bound hydroxo moiety in complex **2** will work as a catalyst for transesterification, even in aqueous media.

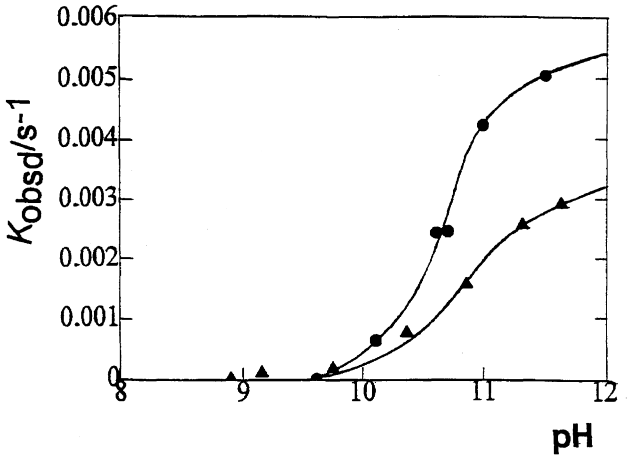


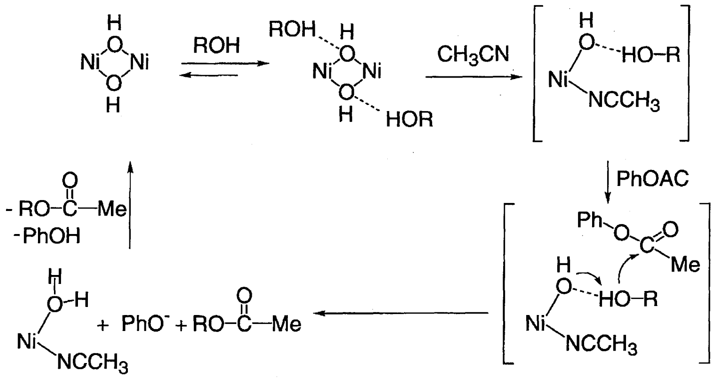
Fig. 4. The rate-pH profile for the first-order rate constants of the *p*-NPA cleavage reaction by **2** in 40% MeCN aqueous solution at 25 °C, and $I = 0.3$ ($\mathrm{NaClO_4}$). *p*-NPA 1 mM, complex **2** 10 mM, CHES (buffer) 20 mM, and NaOH (0—20 mM). Triangle plot: ethanol 0 mM, Circle plot: ethanol 100 mM.

Mechanistic Considerations of This Reaction System. The *p*-nitrophenolate generation by the reaction of complex **2** with *p*-NPA was dramatically accelerated by the addition of alcohol. The transfer of the acetate group in the ester to the added alcohol was observed. A plausible mechanism for this reaction system is shown in Scheme 4. The OH part in complex **2** has an ability to form a hydrogen-bonding interaction with external alcohol as exemplified by the molecular structure of **2**·2MeOH (Fig. 1b). Kinetic experiments indicate the occurrence of dissociation of the dinuclear structure of **2** to a monomeric hydroxo complex. The alcohol that possesses the hydrogen bonding to the metal-bound hydroxo moiety is a candidate for the nucleophile to attack the ester.[8] The metal alkoxide complex generated by the reaction of complex **2** with alcohol such as $[\mathrm{Ni(II)(MeCN)(tpa)OR}]^+$ is also a good nucleophile for the transesterification. But the pK_a value of **3** indicates that formation of such an alkoxide intermediate from **2** is improbable. Furthermore, the X-ray structure (Fig. 1b) revealed that recrystallization of **2** in methanol did not give (alkoxo)Ni(II) complex. The fact also suggests that the basicity of this metal-bound hydroxy group in complex **2** is not sufficient to form an alkoxo complex by the reaction with the alcohol. However, the formation of an alkoxide complex in the solution can not be excluded completely at present, as we do not yet have any direct spectral evidence.

Table 5. Catalytic Formation of Ethyl Acetate by Complex **2** in the Presence of Ethanol

Substrate	Reaction time h	Turnover number[a]	Conversion[b] %
p-Nitrophenyl acetate	10	3.6	9.0
Phenyl acetate	10	2.5	6.3
p-Cresol acetate	10	1.7	4.3

Complex 0.05 mmol, substrate 2.00 mmol, ethanol 1.80 mmol, solvent acetnitrile 40% acetonitrile buffer (CHES 20 mM, pH 10.5) 10 ml. a) Based on the amount of complex **2**. b) Based on the added ester.



Scheme 4.

More facile transesterification of phenylacetate than p-NPA in the present study seems to be inconsistent with the reactivity of p-NPA toward base promoted hydrolysis being higher than that of phenyl acetate. To address this point, it is important to analyze how complex **2** acts in the transesterification process and how the strucuture of complex **2** is changed during the catalytic cycle. The electronic spectra of the acetonitrile solution of the reaction of **2** with phenylacetate in the presence of ethanol exhibit two characteristic bands at 580 and 890 nm (Fig. 5 solid line). This shows excellent accordance with those of complex **2** in acetonitrile. On the other hand, the absorption spectrum of a mixture of **2** and p-NPA in the presence of ethanol contains the two d–d bands at 530 (sh) and 900 nm, in addition to the strong absorption at 400 nm derived from p-nitrophenolate ion (Fig. 5 dotted line). This spectrum is quite different from that of **2**, but closely resembles the spectrum of complex **3**, which is inert toward ester activation. On the basis of these results, we propose that the present transesterification involves proton transfer from the alcohol to the ligated hydroxide in the nickel complex to form the monomeric Ni(II) complex, **3**, as shown in Scheme 4. As complex **3** is inactive toward the ester, other processes such as the proton abstraction from **3** to afford complex **2** should be required for the catalytic cycle. The pK_a value of the ligated water molecule in **3** (10.7) is larger than that of p-nitrophenol (7.3) but close to those of phenol and p-cresol (9.82 and 10.26 respectively). Thus, the proton of the ligated water molecule in complex **3** will be transferred to the phenolate ion generated by the transesterification to regenerate complex **2**, which is the active catalyst in this system. In MeCN, the basicity of the liberated phenolate ion governs the catalytic turnover number. In contrast, when the solvent is an aqueous alkaline solution, the basic conditions regenerate complex **2** from complex **3** spontaneously. Thus, the basicity of the phenolate ion may not be responsible for the catalytic reaction in aqueous media. Therefore, the amount of transesterification product depends on the electrophilicity of the carbonyl group of the substrate (Table 4).

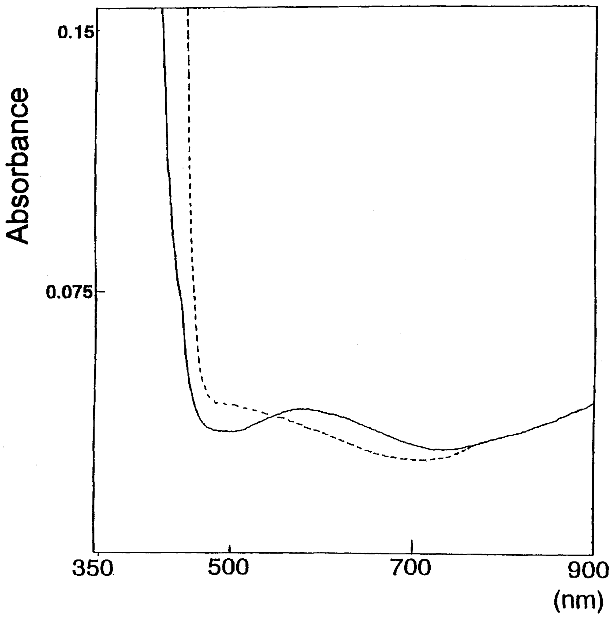


Fig. 5. The UV-vis spectra of the reaction mixture of complex **2** (0.5 mM), ethanol (200 mM), and substrate (40 mM) in MeCN after 5 h. Substrate: p-NPA (dotted line) and phenyl acetate (real line).

Conclusion

Generally speaking, a metal-bound hydroxo species is regarded as a nucleophile. The nucleophilicity of the hydroxo moiety in complex **2** was investigated by kinetic experiments of p-NPA hydrolysis. These experiments indicated that the rate of generation of p-nitrophenolate ion was increased by the addition of alcohol. The effect of the added alcohol was not only a rate enhancement, but also a change in the reaction products to transesterified esters from hydrolyzed products. The recrystallization of the (hydroxo)nickel(II) complex **2** in MeOH gave us a structure containing MeOH molecules hydrogen-bonded with the metal-bound hydroxo moiety (Fig. 2). Kinetic experiments and crystallography suggested that the reactive species for the transesterification is the alcohol interacting with the metal-bound hydroxo moiety in **2**. Furthermore, this hydrogen-bonded alcohol shows a stronger nucleophilicity than the Ni(II)-bound OH^-. However, we have not yet obtained direct spectroscopic evidence for this in the solution. The pK_a value of complex **3** is determined as 10.7 based on the kinetic results, and this value is consistent with the spectrophotomeric changes under various pH conditions. That the pK_a value of complex **3** is higher than other reported values in metal-bound H_2O complexes seems to be related to the electron donating properties of the polypyridine ligand.[21] The d–d transition band of Ni(II) ion in this system allows us to propose the catalytic reaction mechanism shown in Scheme 4.

In summary, we have demonstrated transesterification catalyzed by a Ni(II)OH complex. We believe this system has relevancy to alkaline phosphatase, since this hydrolytic enzyme may also possess a similar reactive species, the metal-bound hydroxo moiety, in the catalytic hydrolysis and transesterification process. The effort to expand this concept to (hydroxo)zinc(II) complex is now in progress.

We are grateful to Prof. M. Akita and Prof. Y. Moro-oka (Tokyo Institute of Technology) for the X-ray measurements. We also appreciate the useful suggestions about the treatment of X-ray data by the editor of B. C. S. J.

References

1) N. W. Lipscomb and N. Sträter, *Chem. Rev.*, **96**, 2375 (1996).

2) D. W. Wilcox, *Chem. Rev.*, **96**, 2435 (1996).

3) a) I. B. Wilson, J. Dayan, and K. Cyr, *J. Biol. Chem.*, **239**, 4182 (1964); b) M. L. Applebury and J. E. Coleman, *J. Biol. Chem.*, **244**, 308 (1969).

4) a) D. M. Blow, J. J. Birktoft, and B. S. Hartley, *Nature*, **221**, 337 (1969); b) P. A. Frey, S. A. Whitt, and J. B. Tobin, *Science*, **264**, 1927 (1994).

5) E. E. Kim and H. W. Wyckoff, *J. Mol. Biol.*, **218**, 449 (1991).

6) J. E. B.-Ranshoff, S. E. Rokita, D. A. Kendall, J. A. Banzon, K. S. Carano, E. T. Kaiser, and A. R. Matlin, *J. Org. Chem.*, **57**, 142 (1992).

7) a) J. E. Coleman and P. Gettins, *Adv. Enzymol. Relat. Areas Mol. Biol.*, **55**, 381 (1983); b) J. E. Coleman, *Annu. Rev. Biophys. Biomol. Struct.*, **21**, 441 (1992); c) H. N. Fernley and P. G. Walker, *Nature*, **12**, 1435 (1966); d) W. N. Albridge, T. E. Barman, and H. Gutfreund, *Biochem. J.*, **92**, 236 (1964); e) S. R. Jones, L. A. Kindman, and J. P. Knowles, *Nature*, **275**, 564 (1978).

8) a) E. Kimura and T. Koike, *Adv. Inorg. Chem.*, **44**, 229 (1997); b) T. Koike and E. Kimura, *J. Am. Chem. Soc.*, **113**, 8935 (1991); c) T. Koike, S. Kajitani, I. Nakamura, E. Kimura, and M. Shiro, *J. Am. Chem. Soc.*, **117**, 1210 (1995); d) E. Kimura, Y. Kodama, T. Koike, and M. Shiro, *J. Am. Chem. Soc.*, **117**, 8304 (1995).

9) K. S. Bruzik, Z. Guan, S. Riddle, and M.-D. Tsai, *J. Am. Chem. Soc.*, **118**, 7980 (1996).

10) T. A. Steitz and J. A. Steitz, *Proc. Natl. Acad. Sci.*, **90**, 6498(1993).

11) B. G. Gafford and R. A. Holwerda, *Inorg. Chem.*, **29**, 60 (1989).

12) The structure of **1** will be reported elsewhere.

13) M. Ito and Y. Takita, *Chem. Lett.*, **1996**, 929.

14) a) I. Murase, G. Vuckovic, M. Kodera, H. Harada, N. Matsumoto, and S. Kida, *Inorg. Chem.*, **30**, 728 (1991); b) T. Tanase, S. Nitta, S. Yoshikawa, K. Kobayashi, T. Sakurai, and S. Yano, *Inorg. Chem.*, **31**, 1058 (1992); c) N. Kitajima, S. Hikichi, M. Tanaka, and Y. Moro-oka, *J. Am. Chem. Soc.*, **115**, 5496 (1993); d) M. R. Churchill, G. Davies, M. A. El-Sayed, M. F. El-Shazly, J. P. Hutchinson, and M. W. Rupich, *Inorg. Chem.*, **18**, 2296 (1976); e) R. Menif, J. Reibenspices, and A. E. Martell, *Inorg. Chem.*, **30**, 3446 (1991); f) R. Alsfasser, S. Trofimenko, A. Looney, G. Parkin, and H. Vahrenkamp, *Inorg. Chem.*, **30**, 4098 (1991).

15) The crystallography of (**2**)·2MeOH revealed the exsistence of two independent molecules. The bond dinstances of Ni–O molecule (2.053 and 2.088 Å) in the other molecule in the cell also indicated the hydrogen bond between the methanol and metal bound hydroxo group; See supplementary material.

16) The amount of *p*-nitrophenolate ion estimated from the absorption at 400 nm was not changed by the addition of a buffer solution (CHES pH 8.2). Therefore, neutral *p*-nitrophenol was not generated in this reaction system.

17) A plot of k(*p*-NPA) vs. the added ethanol concentration gave a straight line. The result indicates a 1 : 1 adduct of alcohol and monomeric (hydroxo)nickel(II) complex is the reasonable nucleophile toward the substrate.

18) Sigman et al. demonstrated transesterification by use of a ternary Zn(II) *N*-(hydroxyethyl)ethylenediamine *p*-nitrophenyl picolinate complex. However the ternary Zn(II) complex was not characterized well. See: D. S. Sigman and C. T. Jorgensen, *J. Am. Chem. Soc.*, **94**, 1724 (1972).

19) a) Y.-J. Kim, K. Osakada, A. Takenaka, and A. Yamamoto, *J. Am. Chem. Soc.*, **112**, 1096 (1990); b) S. R. Sandler and W. Karo, "Organic Functional Group Preparations," Academic Press, New York (1968), Vol. I, Chap. 10; c) J. Otera, T. Yano, A. Kawabata and H. Nozaki, *Tetrahedron Lett.*, **27**, 2383 (1986); d) M. Kubota, T. Yamamoto, and A. Yamamoto, *Bull. Chem. Soc. Jpn.*, **52**, 146 (1979).

20) a) M. A. Halcrow and G. Christou, *Chem. Rev.*, **94**, 2421 (1994), and references therein; b) L. Antolini, L. Menabue, C. C. Pellacani, and G. Marcotrigiano, *J. Chem. Soc., Dalton Trans.*, **1982**, 2541.

21) H. Adams, N. A. Bailey, D. E. Fenton, and Q.-Y. He, *J. Chem. Soc., Dalton Trans.*, **1996**, 2857.