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OXOVANADIUM COMPLEXES OF *N*-SUBSTITUTED 3-HYDROXY-2-METHYL-4(1*H*)-PYRIDINONES: SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION, AND THE INSULIN-MIMETIC ACTIVITY

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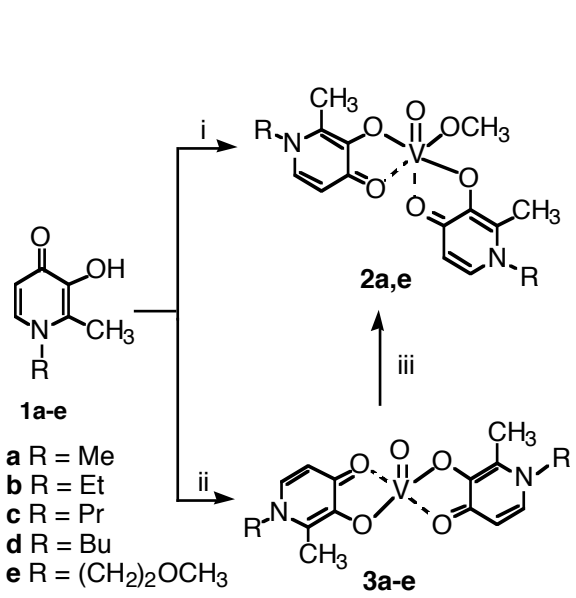
Abstract — Five kinds of *N*-substituted 3-hydroxy-2-methyl-4(1*H*)-pyridinones, and two kinds of tetradentate ligands, *N*,*N*'-[bis(3-hydroxy-1,4-dihydro-2-methyl-4-oxo-1-pyridyl)hexanoyl- and -octanoyl]-1,5-diamino-3-oxapentanes, were synthesized. Treatment of these ligands with $VO(acac)_2$ in CH_3OH exclusively gave CH_3O-coordinated oxovanadium(V) complexes. On the other hand, treatment with $VOSO_4$ in H_2O at around pH 10 afforded oxovanadium(IV) complexes. On the basis of the inhibitory effect of oxovanadium(IV) complexes on free fatty acid (FFA) release from rat adipocytes treated with epinephrine in the presence of 0.1% glucose *in vitro*, it was revealed that some of oxovanadium(IV) complexes show the insulin-mimetic activity.

At the present time, patients with diabetes mellitus (DM) are estimated to be over 140 million in the world. DM results from an absolute or relative deficiency in insulin synthesis on secretion of pancreatic β-cells and a resistance of target tissues to the action of insulin. Patients with DM suffer from a number of secondary complications such as retinopathy, nephropathy, and neuropathy. DM is generally classified into type 1 insulin-dependent DM (IDDM), characterized by hyperglycemia due to absolute deficiency of insulin, and type 2 noninsulin-dependent DM (NIDDM) characterized by hyperglycemia due to resistance of organs to the action of insulin, according to the definition of WHO in 1985.[1] Oral chemotherapeutic agents for type 2 DM have already been developed and are currently available.[2] Patients with type 1 DM can be only treated by daily injections of insulin. However, insulin is a polypeptide with a molecular mass of 5.8 KDa, and thus easily degradated by proteinases in the ingestive systems. Therefore, novel design and synthesis of orally active compounds in place of insulin have been paid much attention.[3]

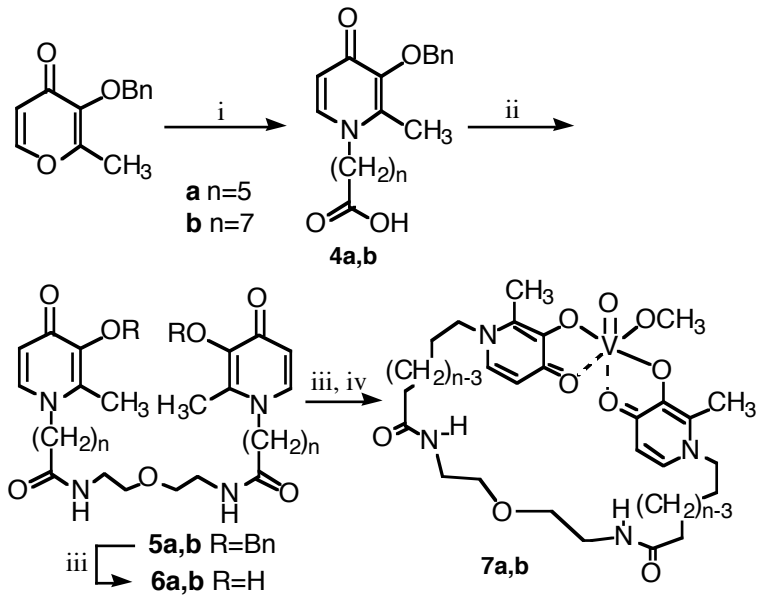
Historically, vanadate (+5 oxidation state of vanadium) ion was found to be a potent inhibitor of Na^+, K^+-ATPase in 1977.[4-6] The interaction of vanadium with ATPases and its expression on cellular, organ, and whole animal levels have been reviewed by Nechay.[7] In 1980's, vanadate ion was demonstrated to show insulin-mimetic action in intracellular insulin receptor or GLUT4.[8,9] Further, it was found that vanadate ion was reduced to vanadyl (+4 oxidation state or oxovanadium(IV)) in organs and organelles when it was administered to animals, and when vanadyl sulfate ($VOSO_4$) and its complexes administered orally or

intravenously to rats with streptozotocin (STZ)-induced diabetes, they normalized the blood glucose.[4,10-13] In addition, the vanadate form exhibited toxicity about 10-15 times higher than the vanadyl one in rats in terms of LD_{50} value.[14] Therefore, research on oxovanadium(IV) complexes has received much attention. A number of orally active oxovanadium(IV) complexes have already been reported to exhibit insulin-mimetic activities *in vivo*.[12,15-21] Recently, it has been reported that the amount of glucose in blood is mutually related to the amount of FFA in blood. Therefore, the insulin-mimetic activity of oxovanadium(IV) complexes has been evaluated by measuring their inhibitory effects on FFA release from isolated rat adipocytes *in vitro*.[16,18,22-25] However, establishment of a clear correlation between chemical structures of oxovanadium(IV) complexes and insulin-mimetic activity is yet very difficult due to absolute lack of available data. Further, only a few papers concerning oxovanadium(IV) complexes including heterocycles as bidentate ligands have been reported.[26]

We have intensively studied on synthesis of hydroxyazine-type heterocycles and their application to chemotherapeutic agents for the iron-overload disease.[27] We, therefore, planed to utilize 3-hydroxy-4(1*H*)-pyridinone as bidentate and tetradentate ligands in synthesizing oxovanadium(IV) complexes with $VO(O_4)$ coordination mode,[12,19,28] because there are some advantages as follows; 1) a facile synthetic procedure,[29-32] 2) an easy introduction of various substituents at *N*-1 position, 3) an effective binding of oxovanadium ion with a tetradentate ligand by the chelate effect, and 4) an easy adjustment of the hydrophilicity or lipophilicity of oxovanadium complex. We describe here synthesis of new heterocyclic oxovanadium(IV) and (V) complexes and characterization of these complexes on the basis of ^{1}H-, ^{13}C-, ^{51}V-NMR, IR, UV-VIS, FAB MS and ESR spectra, cyclic voltammetry (CV), and combustion analysis. Furthermore, we examined whether synthetic oxovanadium(IV) complexes exhibited insulin-mimetic activities with regard to the inhibitory effect of the complexes on FFA release from isolated rat adipocytes *in vitro*.



Scheme 1 *Reagents and conditions*: i) $VO(acac)_2$, MeO-H_2O (1:1), reflux for 2 h; ii) $VOSO_4$, H_2O, pH 10, reflux overnight; iii) CH_3OH, rt.



Scheme 2 *Reagents and conditions* : i) $H_2N(CH_2)_nCO_2Na$, EtOH-H_2O(1:1), reflux for 48 h; ii) $H_2N(CH_2)_2O(CH_2)_2NH_2$, *N*,*N*'-carbonyldiimidazole, dry DMF; iii) H_2/10%Pd-C, CH_3OH, 2 h; iv) $VO(acac)_2$, CH_3OH-H_2O (1:1), reflux for 2 h.

RESULTS AND DISCUSSION

Synthesis and property of bidentate and tetradentate ligands: Bidentate ligands, *N*-substituted 3-hydroxy-2-methyl-4(1*H*)-pyridinones (**1a**-**e**), were prepared *via* three steps from a commercially available maltol (3-hydroxy-2-methyl-4-pyrone) according to the literature method.[29,30] (Scheme 1) The synthetic procedure for tetradentate ligands is also depicted in Scheme 2. 3-Benzyloxy-2-methyl-4-pyrone was allowed to react with Na salt of 6-aminohexanoic acid to give compound (**4a**) in a 88% yield. The structure was determined by ^{1}H NMR and IR spectra. Two characteristic olefinic protons at C-5 and C-6 positions of the pyridinone ring were observed at δ 6.42 and 7.18 ppm, respectively, and the carboxylic proton was observed at δ 10.82 ppm. Two absorption bands due to C=O stretching vibrations of 4(1*H*)-pyridinone and carboxylic acid were observed at 1627 and 1720 cm^{-1}, respectively. Similarly, reaction of 3-benzyloxy-2-methyl-4-pyrone with 8-aminooctanoic acid gave compound (**4b**) in a 90% yield. Compounds (**4a** and **4b**) were treated with *N,N'*-carbonyldiimidazole (CDI), and then coupled with 1,5-diamino-3-oxapentane in dry DMF at room temperature to give compounds (**5a** and **5b**). The absorption bands owing to C=O stretching vibrations of 4(1*H*)-pyridinone and amide bond of compound (**5a**) were observed at 1628 and 1655 cm^{-1}, respectively. Compounds (**5a** and **5b**) were subjected to the hydrogenation for 2 h in the presence of 10% Pd-C to give tetradentate ligands (**6a** and **6b**) in high yields. The pKa value of 3-hydroxy-1-methoxyethyl-2-methyl-4(1*H*)-pyridinone (**1e**) was estimated from the pH titration curve of absorbance at 310 nm at various pH conditions to be 9.8 which is comparable to that[31,32] of 3-hydroxy-1,2-dimethyl-4(1*H*)-pyridinone. The partition coefficient ($C_{octanol}/C_{buffer}$) of ligands (**1a-e**) between KRB (Krebs-Ringer bicarbonate, pH 7.4) buffer,[20] which was used in the bioassay, and octanol was measured by means of UV-VIS spectroscopy,[31,32] and the results are summarized in Table 1.

Table 1 Partition coefficients of *N*-substituted 4(1*H*)-pyridinones

Ligand	**1a**	**1b**	**1c**	**1d**	**1e**
Partition coefficient	0.09	0.27	0.44	1.00	0.16

Among a series of 4(1*H*)-pyridinones (**1a**-**e**), an increase in the methylene chain length at *N*-1 position results in an increase of the solubility in octanol, except for compound (**1e**).

Synthesis of oxovanadium complexes: At first, synthesis of oxovanadium complexes with bidentate ligands (**1a** and **1e**) was carried out using vanadyl acetylacetonate, $VO(acac)_2$. Compound (**1e**) was treated with 0.5 equimolar amount of $VO(acac)_2$ in CH_3OH-H_2O (1:1) mixture.[33] When a solution of $VO(acac)_2$ (green) was added to a solution of compound (**1e**) (colorless), a color of the solution turned into dark purple. After refluxing for 2 h, the solvent was evaporated off, and the crude product was purified by gel-filtration with Sephadex LH-20 to give oxovanadium complex (compound **A**) as dark purple powders. A characteristic absorption band due to V=O stretching vibration was observed at 949 cm^{-1} in IR spectrum.[34] In general, ε value of absorption band of oxovanadium(IV) complex at the visible region was very small,[35] but this complex showed λ_{max} at 503 nm with ε value of 3450. Measurement of NMR spectrum of oxovanadium(IV) complex is impossible because of a paramagnetic character. Compound (**A**), however, showed a diamagnetic character attributable to oxovanadium(V) complex. A signal based on

protons at *cis*-OCH_3 appeared at δ 3.34 ppm, and this chemical shift was completely coincided with that of *cis*-bis(maltolato)methoxooxovanadium(V), *cis*-$VO(OCH_3)(ma)_2$.[36] From these spectral data and combustion analysis, compound (**A**) was assigned to be oxovanadium(V) complex (**2e**). Bidentate (**1a**) and tetradentate ligands (**6a** and **6b**) similarly gave oxovanadium(V) complexes (**2a**, **7a** and **7b**), respectively. Consequently, oxovanadium(IV) complex could not be obtained under the employed conditions.

Synthesis of oxovanadium(IV) complexes was carried out using vanadyl sulfate ($VOSO_4$)[12,36,37] in place of $VO(acac)_2$. Compound (**1e**) was treated with 0.5 equimolar amount of $VOSO_4$ in H_2O. When a solution of $VOSO_4$ (light blue) was added to a solution of compound (**1e**) (colorless), a color of the solution turned into dark purple. The pH of the reaction mixture was adjusted to 10, and then the resulting mixture was refluxed overnight. On raising pH of the aqueous solution, a color turned into dark green, and a blue precipitate came out. The precipitate was collected and washed well with H_2O to give oxovanadium(IV) complex (**3e**) as blue powders. IR spectrum of complex (**3e**) is very similar to that of free ligand (**1e**), except the following points; (i) the absorption band at 1625 cm^{-1} attributable to C=O stretching vibration of free ligand (**1e**) shifted to 1604 cm^{-1}, (ii) the absorption band at 1400 cm^{-1} due to O-H in-plane deformation became small in the intensity, and (iii) a new absorption band owing to V=O stretching vibration appeared at 970 cm^{-1}. The molecular ion peak of complex (**3e**) was observed at m/z 432 ($[M+H]^+$) in FAB MS spectrum. Further, oxovanadium(IV) complexes (**3a** and **3e**) were easily converted into the corresponding oxovanadium(V) ones (**2a** and **2e**) in CH_3OH, It is noteworthy that a facile oxidation of oxovanadium(IV) complexes to oxovanadium(V) ones occurs in CH_3OH even at room temperature when *N*-substituted 3-hydroxy-4(1*H*)-pyridinones were used as bidentate and tetradentate ligands.

Characterization of oxovanadium(V) complexes: On measuring ^1H NMR spectrum of complex (**2e**), the broadening of each signal was observed, and thus the spectra were measured at various temperatures. The shape of signals due to 2-CH_3 and 5-H apparently changed as shown in Figure 1-a. At −30 ℃, two sets of signals were observed for 2-CH_3 and 5-H protons at the range from δ 2.4 to 2.6 and from 6.2 to 6.4 ppm, respectively. On raising temperature up to 30 ℃, the signals coalesced into broad singlets. The similar behavior was observed on the variable temperature ^1H NMR spectral study of *cis*-$VO(OCH_3)(ma)_2$.[36] This result indicated that the equilibrium between the two isomers may exist in CD_3OD solution as shown in Figure 1-b. The activation free energy ($\Delta G^{\neq}$) for the conversion was calculated from the difference ($\Delta\nu$) in frequency between two signals and coalescence temperature (T_c) according to the following equation, and estimated to be roughly 65 KJ mol^{-1}.

$$\Delta G^{\neq} = 19.14\, T_c\, (9.97 + \log T_c / \Delta\nu)$$

UV-VIS spectra of complex (**2e**) were measured in aqueous solution at various pH values (pH 2.1-11.3). The absorption bands due to the ligand-to-metal charge transfer (LMCT) were observed at 530-600 nm in the acidic region. An increase of pH caused the blue-shift of absorption maximum, λ_{max} being 536 nm with ε value of 1380 at pH 4.6. However, in the basic region, the absorption band due to LMCT disappeared, indicating the decomposition of the complex.

^{51}V NMR spectra of complex (**2e**) were measured in D_2O at apparent pD 8, and $VOCl_3$ was used an external standard. Three separated signals, in which one is major and the other two are minor, were observed at δ -480, -503 and -535 ppm, indicating the existence of three oxovanadium(V) species. (Spectrum (i) in Figure 2-a). When an excess of ligand (**1e**) was added to the sample solution, two signals at δ -503 and

–535 ppm completely disappeared. (Specctrum (ii) in Figure 2-a)

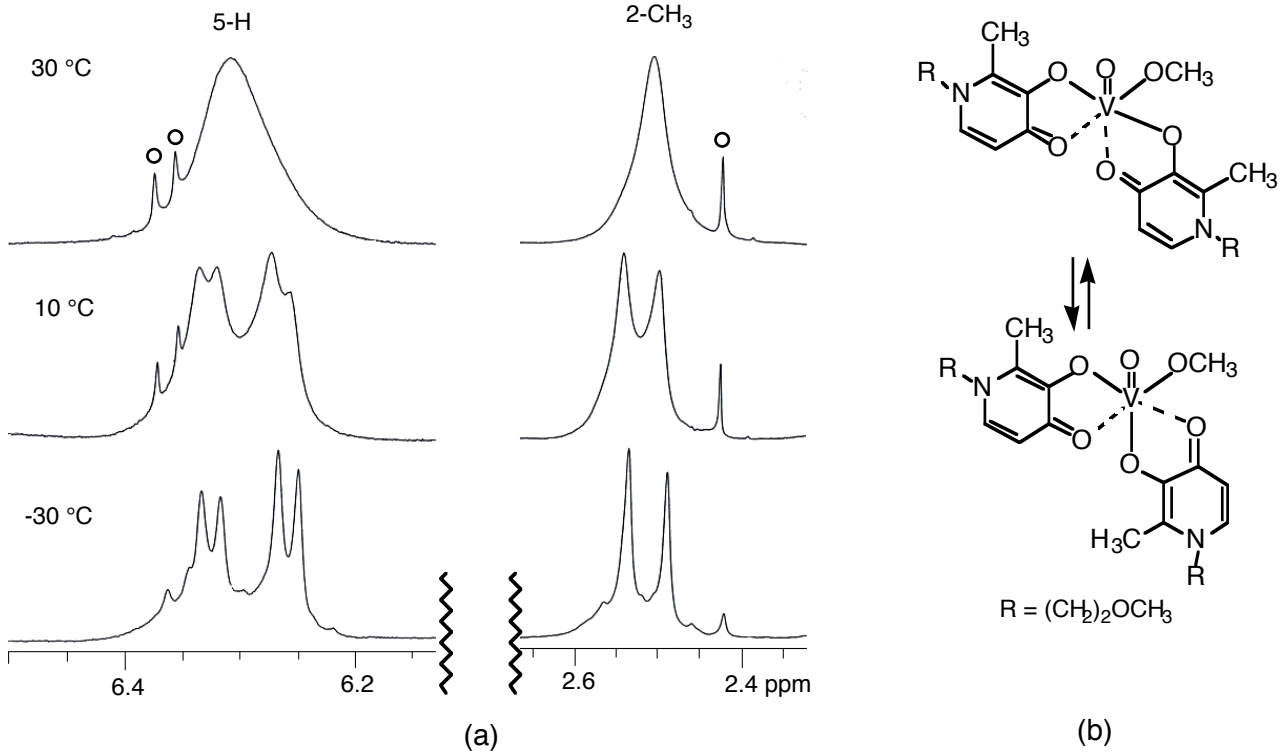


Figure 1 (a) ^{1}H NMR spectra of oxovanadium(V) complex (**2e**) in CD_3OD; ○: free ligand. (b) A possible structure in equilibrium

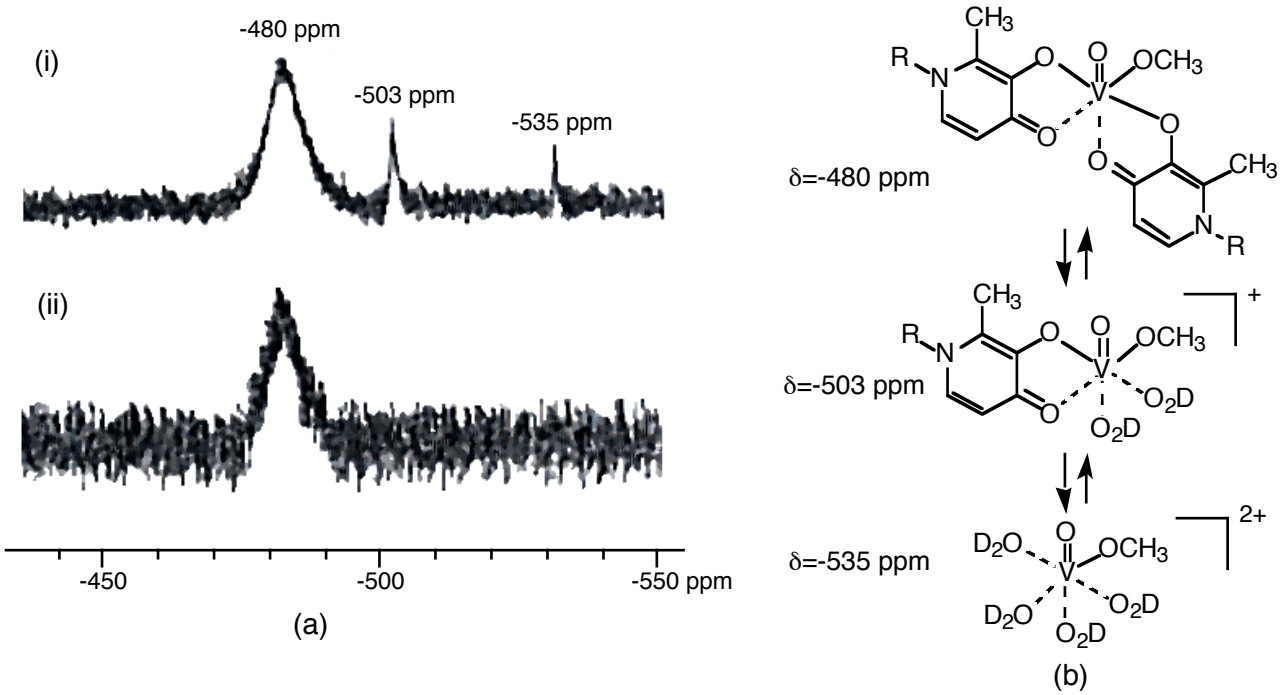


Figure 2 (a) ^{51}V NMR spectra of oxovanadium(V) complex (**2e**) in D_2O at pD 8; i) oxovanadium(V) complex, ii) an additional ligand (**1e**). (b) The tentative assignment of observed three signals.

From these and reported data,[36,37] the two minor signals were tentatively assigned to be the ligand dissociation products, $[VO(L)(D_2O)_2(OCH_3)]^+$ and $[VO(D_2O)_4(OCH_3)]^{2+}$ as shown in Figure 2-b.

Characterization of oxovanadium(IV) complexes: UV-VIS spectra of oxovanadium(IV) complexes were measured both in H_2O and DMSO solutions, and the results are summarized in Table 2. The absorption bands of complexes (**3a**-**e**) at about 600 nm were attributable to d-d transitions. On the other hand, the absorption bands at λ_{max} 535-560 nm apparently increased with time, and these bands corresponded to LMCT bands of oxovanadium(V) complexes, indicating that oxovanadium(IV) complexes were gradually oxidized to the corresponding oxovanadium(V) ones even in aqueous solution.

Table 2 UV-VIS spectral data for oxovanadium(IV) complexes (**3a**-**e**)

Complex	Solvent[a]	λ_{max} / nm		
3a	H_2O	599	538	394
	DMSO	613	555	403
3b	H_2O	605	536	400
	DMSO	607	556	401
3c	H_2O	602	541	401
	DMSO	610	561	404
3d	H_2O	598	548	402
	DMSO	607	552	401
3e	H_2O	606	541	395
	DMSO	607	556	401

[a] in H_2O, 1.0 mM; in DMSO, 5.0 mM

Since oxovanadium(IV) ion, which has an unpaired electron, is paramagnetic, ESR spectrum and magnetic susceptibility are useful tools for investigation of electronic structure of oxovanadium(IV) complexes.[10,28,34]

Table 3 ESR parameters of oxovanadium(IV) complexes (**3a**-**e**) in DMSO

	g-value			A-value ($x10^{-4}$ cm^{-1})		
Complex	g_0[a]	$g_{//}$	$g_\perp$	A_0[b]	$A_{//}$	$A_\perp$
3a[c]	1.979	1.947	1.995	79.43	153.9	42.12
3b[c]	1.977	1.953	1.989	80.14	153.2	43.61
3c[d]	1.971	1.950	1.982	80.58	159.9	40.91
3d[d]	1.983	1.951	1.999	81.12	162.9	40.19
3e[d]	1.979	1.950	1.994	79.59	162.9	37.94

[a] $g_0 = (g_{//} + 2\,g_\perp)/3$; [b] $A_0 = (A_{//} + 2A_\perp)/3$; [c] c 3.0 mM; [d] c 5.0 mM.

Complexes (**3a**-**e**) showed typical isotopic eight line ESR spectra at room and liquid nitrogen temperature, suggesting that they exist in a single isomer. The ESR parameters, universal constants (g-values) and hyperfine coupling constants (A-values) were calculated, and the results are summarized in Table 3. These values were consistent with those of oxovanadium(IV) complexes with $VO(O_4)$ coordination mode.[10]

Further, oxovanadium(IV) complex with maltol, which is strucurally very close to the present 4(1*H*)-pyridinone, has been proven to be the *trans* isomer by X-Ray crystallographic analysis.[36] From these data, the obtained oxovanadium(IV) complexes probably exist in the *trans* form. The measurement of magnetic susceptibility of the oxovanadium(IV) complex (**3e**) was carried out by using Evans' method.[38] A number of unpaired electron (n) was calculated to be 1.15, indicating the formation of oxovanadium(IV) complex with an unpaired electron.

The electrochemical behavior of oxovanadium(IV) complexes in aqueous solution was evaluated by means of CV with a glassy carbon electrode, and a typical cyclic voltammogram of complex (**3a**) is shown in Figure 3. The electron transfer processes of complexes (**3a**,**3b**,**3e**) were quasi-reversible, because i_{pa}/i_{pc} ratios (0.91 for **3a**, 0.88 for **3b**, and 0.92 for **3e**) were close to unity, although values of the peak-to-peak separation (ΔE_p) (110 mV for **3a**, 90 mV for **3b**, and 115 mV for **3e**) were greater than 60 mV. It is noteworthy that $E_{1/2}$ vs Ag/AgCl values (315 mV for **3a**, 320 mV for **3b**, and 300 mV for **3e**) are approximately 130-150 mV lower than that of bis(maltolato)oxovanadium(IV), $VO(ma)_2$,[36] indicating that these complexes are less stable towards the oxidation. As ΔE_p values were larger than 60 mV, cyclic voltammgrams were measured at various scan rates (ν). A plot of i_{pc} and $\nu^{1/2}$ gave a straight line, indicating that the electron transfer proceeds at the diffusion control.

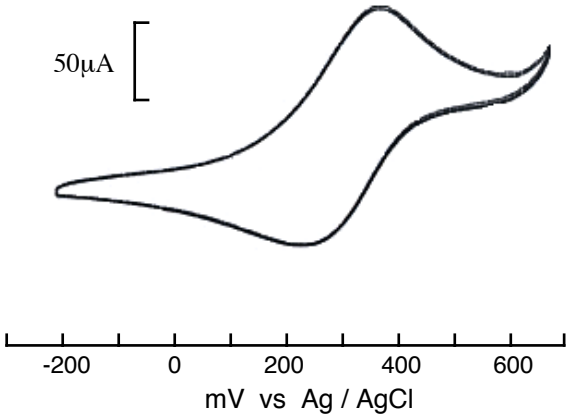


Figure 3 Cyclic voltammogram of oxovanadium(IV) complex (**3a**) in H_2O: 1.0 mM complex; 0.1 M NaCl; scan rate 100 mV s^{-1}; a working electrode: glassy carbon; an auxiliary electrode: Pt wire; a reference electrode: Ag/AgCl

The inhibitory effect of oxovanadium complexes on FFA release from rat adipocytes: It is well recognized that the hydrophobicity of oxovanadium(IV) complexes is one of the most important factors for predicting the insulin-mimetic activity.[20]

Table 4 Partition coefficients of oxovanadium(IV) complexes

Ligand	**3a**	**3b**	**3c**	**3d**	**3e**
Partition coefficient	a)	0.06	0.96	1.85	0.07

a) not measurable due to high water solubility.

Thus, the partition coefficient of oxovanadium(IV) complexes (**3a-e**) was measured as the same manner described above, and the results are summarized in Table 4. Among a series of complexes, an increase in the methylene chain length results in an increase of the solubility in octanol. Complexes (**3c** and **3d**)

showed higher solubility in octanol than the corresponding free ligands (**1c** and **1d**).

Previously, we have demonstrated that the FFA release from isolated rat adipocytes is a good *in vitro* evaluation system to find a compound which shows an insulin mimetic action.[13] Therefore, the insulin-mimetic activity of oxovanadium(IV) complexes was evaluated by *in vitro* experiments, in which the inhibition of release of FFA from isolated rat adipocytes treated with epinephrine, was estimated in the presence of 0.1% glucose. The obtained IC_{50} values are summarized in Table 5, when IC_{50} value of $VOSO_4$, as a positive control, was calibrated to be 1.0 mM.

Table 5 IC_{50} values of oxovanadium(IV) complexes (**3a-e**) in the presence of 0.1% glucose

Complex	**3a**	**3b**	**3c**	**3d**	**3e**	**$VOSO_4$**	**$VO(ma)_2$**
IC_{50} / mM	*	*	2.5	2.3	1560	1.0	2.5

* not measurable due to the low activity in the *in vitro* evaluation system.

Complexes (**3a** and **3b**) did not show measurable activity, while complexes (**3c** and **3d**) with relatively high partition coefficients (see Table 4) exhibited comparable activities to $VO(ma)_2$, although these values were lower than $VOSO_4$. As seen in Table 5, $VO(ma)_2$ exhibited higher IC_{50} value than $VOSO_4$, when the complexes were tested under the same experimental conditions. However, $VO(ma)_2$ was found to treat DM in experimental animals when it was given by daily intravenous injections or through drinking water.[12,39-41] Therefore, the present complexes (**3c** and **3d**) might be possible to reduce the high blood glucose level of experimental diabetic animals.

EXPERIMENTAL

Melting points were measured on a Mel-Temp apparatus in open capillaries and are uncorrected. IR and UV-VIS spectra were recorded on a JASCO FT/IR-230 infrared and on a JASCO Ubest V-550 spectrophotometers, respectively. ^{1}H NMR spectra were obtained on JEOL GX-270 and JEOL JNM-LA400D NMR spectrometers, and chemical shifts were expressed in ppm (δ) downfield from internal TMS. ^{51}V NMR spectra were obtained on a JEOL JNM-LA400D spectrometer, and chemical shifts were reported in ppm (δ) downfield from external $VOCl_3$. FAB MS spectra were taken on a JEOL DX303 with a DA 5000 data system by using a Xe beam and a glycerol matrix. ESR spectra were recorded on a JEOL RE1X spectrometer (X band) with a 100 KHz modulation. The magnetic field was calibrated by Mn(II) doped in MgO powder. Measurement were carried out at 298 K in solution and at 77 K in liquid nitrogen at the frozen state. Thin layer chromatography (TLC) was performed on silica gel 60F-254 with a 0.2 mm layer thickness. Column chromatography was carried out with Merck Kieselgel 60 (230-400 mesh). Combustion analysis was performed on a PERKIN ELMER series II CHNS/O analyzer 2400. Cyclic voltammograms were collected using a HUSO Electro Chemical System (HECS) 315 B Cyclic Voltammograph. *N*-Substituted 3-hydroxy-2-methyl-4(1*H*)-pyridinones (**1a-e**) were prepared according to the literature methods.[29,30]

Bis(1,4-dihydro-1,2-dimethyl-4-oxo-3-pyridinolato)methoxooxovanadium(V)(2a)

To a solution of compound (**1a**) (122 mg, 0.88 mmol) in CH_3OH-H_2O (1:1) (8 mL) mixture was added a

solution of $VO(acac)_2$ (106 mg, 0.4 mmol) in CH_3OH-H_2O (1:1) (8 mL) mixture, and then the mixture was refluxed for 2 h. Removal of the solvent gave the crude product, which was purified by gel-filtration with Sephadex LH-20 with MeOH as an eluent to give the product (**2a**) (53 mg, 39%) as deep purple powders: IR (KBr): 949 cm^{-1} ($\nu_{V=O}$); $\lambda_{max}(CH_3OH)$: 503 nm (ε 3420). *Anal.* Calcd for $C_{15}H_{19}N_2O_6V$: C, 47.00; H, 5.26; N, 7.31. Found: C, 47.01; H, 5.09; N, 7.38.

Bis(1,4-dihydro-1-methoxyethyl-2-methyl-4-oxo-3-pyridinolato)methoxooxovanadium (V)(2e) 60 mg (23%); IR (KBr): 1606 ($\nu_{C=O}$) and 948 cm^{-1} ($\nu_{V=O}$); $\lambda_{max}(CH_3OH)$: 503 nm (ε 3450); ^1H NMR (δ, 400 MHz; CD_3OD at 30 ℃): 2.49 (6H, br s, 2-CH_3), 3.30 (6H, s, -CH_2-O-CH_3), 3.34 (3H, s, -OCH_3), 3.67 (4H, br s, N-CH_2-), 4.30 (4H, br s -O-CH_2-), 6.29 (2H, br s, 5-H), 7.59 (2H, d, J =7.3 Hz, 6-H); ^{51}V NMR (δ, 400 MHz; in solvent): -374 ($CDCl_3$), -390 (CD_3OD), and -410 ppm (D_2O). *Anal.* Calcd for $C_{19}H_{27}N_2O_8V$: C, 49.36; H, 5.89; N, 6.06. Found: C, 49.64; H, 6.02; N, 6.14.

Bis(1,4-dihydro-1,2-dimethyl-4-oxo-3-pyridinolato)oxovanadium(IV)(3a)
To a solution of compound (**1a**) (1.0 g, 7.2 mmol) in H_2O (10 mL) was added dropwise $VOSO_4 \cdot 3.6H_2O$ (0.82 g, 3.6 mmol) in H_2O (5 mL). The pH of the reaction mixture was adjusted to 10 with 10 M KOH, and then the resulting mixture was refluxed overnight. After cooling to rt, the precipitate was collected by filtration and washed several times with H_2O, and then dried over anhydrous P_2O_5 to give the product (**3a**) (1.04 g, 84%) as blue solids: IR (KBr): 1606 ($\nu_{C=O}$) and 967 cm^{-1} ($\nu_{V=O}$). *Anal.* Calcd for $C_{14}H_{16}N_2O_5V$: C, 48.99; H, 4.70; N, 8.16. Found: C, 48.70; H, 4.53; N, 7.81.

Bis(1,4-dihydro-1-ethyl-2-methyl-4-oxo-3-pyridinolato)oxovanadium(IV)(3b)
yield: 66 %; IR (KBr): 1603 ($\nu_{C=O}$) and 964 cm^{-1} ($\nu_{V=O}$). *Anal.* Calcd for $C_{16}H_{20}N_2O_5V$: C, 51.76; H, 5.43; N, 7.54. Found: C, 51.50; H, 5.45; N, 7.63.

Bis(1,4-dihydro-2-methyl-4-oxo-1-propyl-3-pyridinolato)oxovanadium(IV)(3c)
yield: 72%; IR (KBr): 1603 ($\nu_{C=O}$) and 979 cm^{-1} ($\nu_{V=O}$). *Anal.* Calcd for $C_{18}H_{24}N_2O_5V$: C, 54.14; H, 6.06; N, 7.01. Found: C, 54.30; H, 6.06; N, 7.17.

Bis(1-butyl-1,4-dihydro-2-methyl-4-oxo-3-pyridinolato)oxovanadium(IV)(3d)
yield: 72%; IR (KBr): 1605 ($\nu_{C=O}$) and 976 cm^{-1} ($\nu_{V=O}$). *Anal.* Calcd for $C_{20}H_{28}N_2O_5V \cdot 0.5H_2O$: C, 55.04; H, 6.70; N, 6.42. Found: C, 55.02; H, 6.80; N, 6.38.

Bis(1,4-dihydro-2-methyl-1-methoxyethyl-4-oxo-3-pyridinolato)oxovanadium(IV)(3e)
yield: 73%; IR (KBr): 1604 ($\nu_{C=O}$) and 979 cm^{-1} ($\nu_{V=O}$); FAB MS: m/z 432 ($[M+H]^+$). *Anal.* Calcd for $C_{18}H_{24}N_2O_7V$: C, 50.12; H, 5.61; N, 6.49. Found: C, 50.16; H, 5.83; N, 6.49.

3-Benzyloxy-1-carboxypentyl-2-methyl-4(1H)-pyridinone(4a)
To a mixture of 6-aminohexanoic acid (1.31 g, 10 mmol) and 3-benzyloxy-2-methyl-4-pyrone (1.18 g, 5.5 mmol) in EtOH-H_2O (1:1) mixture (8 mL) was added 1 M NaOH (10 mL, 10 mmol), and then the reaction mixture was refluxed for 48 h. The pH of the reaction mixture was adjusted to 8 with 6 M HCl. After concentration to a half volume, the aqueous layer was washed with $CHCl_3$ (200 mL) to remove 3-

benzyloxy-2-methyl-4-pyrone. The aqueous layer was adjusted to pH 4 with 10% citric acid, and the resulting solid was dissolved in $CHCl_3$ (300 mL). The organic layer was washed with saturated NaCl solution (100 mL), and then dried over anhydrous Na_2SO_4. Removal of the solvent afforded the product (**4a**) (1.81 g, 88%) as pale brown solids; mp: 106-109 ℃; IR (KBr): 3260-2760 (ν_{O-H}, br), 1720 ($\nu_{C=O}$), 1627 ($\nu_{C=O}$), 752 and 706 cm^{-1} (δ_{C-H}); ^1H NMR (δ, 270 MHz; $CDCl_3$): 1.31 (2H, m, -CH_2-), 1.62 (4H, m, -CH_2-x2), 2.10 (3H, s, -CH_3), 2.32 (2H, t, *J* =7.1 Hz, -CH_2-CO-), 3.80 (2H, t, *J* =7.0 Hz, N-CH_2-), 5.14 (2H, s, -O-CH_2-), 6.65 (1H, d, *J* =7.6 Hz, 5-H), 7.28-7.39 (6H, m, 6-H, -Ph), and 10.82 ppm (1H, br s, -OH). *Anal.* Calcd for $C_{19}H_{23}NO_4 \cdot 0.5H_2O$: C, 67.43; H, 6.99; N, 4.13. Found: C, 67.38; H, 7.08; N, 4.16.

3-Benzyloxy-1-carboxyheptyl-2-methyl-4(1*H*)-pyridinone(4b)

2.67 g (93%); mp: 135-137℃; IR (KBr): 1717 ($\nu_{C=O}$), 1623 ($\nu_{C=O}$), 752 and 706 cm^{-1} (δ_{C-H}); ^1H NMR (δ, 270 MHz; $CDCl_3$): 1.31 (6H, m, -CH_2-x3), 1.60 (4H, m, -CH_2-x2), 2.09 (3H, s, -CH_3), 2.35 (2H, t, *J* =7.3 Hz, -CH_2-CO-), 3.75 (2H, t, *J* =7.3 Hz, N-CH_2-), 5.20 (2H, s, -O-CH_2-), 6.63 (1H, d, *J* =7.6 Hz, 5-H), 7.23 (1H, d, *J* =7.6 Hz, 6-H), 7.29-7.38 (5H, m, -Ph). *Anal.* Calcd for $C_{21}H_{27}NO_4$: C, 70.56; H, 7.61; N, 3.92. Found: C, 70.34; H, 7.74; N, 3.63.

***N*,*N*'-[Bis(3-benzyloxy-1,4-dihydro-2-methyl-4-oxo-1-pyridyl)hexanoyl]-1,5-diamino-3-oxapentane(5a)**

N,*N*'-Carbonyldiimidazole (165 mg, 1 mmol) was added to a solution of compound (**4a**) (336 mg, 1 mmol) in dry DMF (10 mL), and the mixture was stirred at rt. After 1 h, 1,5-diamino-3-oxapentane (47 mg, 0.45 mmol) was added to the mixture, and then the reaction mixture was stirred for 120 h at 40℃. After removal of the solvent, the oily residue was dissolved in $CHCl_3$ (80 mL). The organic layer was successively washed with H_2O (40 mL), 5% citric acid (40 mL), 5% $NaHCO_3$ (40 mL), H_2O (40 mL), saturated NaCl solution (40 mL), and then dried over anhydrous Na_2SO_4. Removal of the solvent gave a crude product, which was purified by column chromatography on silica gel with $CHCl_3$:CH_3OH (5:1) mixture as an eluent to give the product (**5a**)(146 mg, 44%) as pale brown amorphous solids: IR (KBr): 3270 (ν_{N-H}), 1655 ($\nu_{C=O}$), 1628 ($\nu_{C=O}$), 752 and 702 cm^{-1} (δ_{C-H}); ^1H NMR (δ, 270 MHz; $CDCl_3$): 1.22 (4H, m, -CH_2-x2), 1.58 (8H, m, -CH_2-x4), 2.08 (6H, s, -CH_3x2), 2.21 (4H, t, *J*=7.1 Hz, -CH_2-CO-x2), 3.38 (4H, m, -CH_2-O-x2), 3.47 (4H, m, -C*H*$_2$-N*H*-x2), 3.72 (4H, t, *J* =7.1 Hz, N-CH_2-x2), 5.14 (4H, s, -*CH*$_2$-Phx2), 6.37 (2H, d, *J* =7.3 Hz, 5-Hx2), 7.24 (2H, d, *J* =7.3 Hz, 6-Hx2), 7.28-7.34 (10H, m, -Phx2). *Anal.* Calcd for $C_{42}H_{54}N_4O_7 \cdot 0.5H_2O$: C, 68.54; H, 7.53; N, 7.61. Found: C, 68.20; H, 7.55; N, 7.44.

***N*,*N*'-[Bis(3-benzyloxy-1,4-dihydro-2-methyl-4-oxo-1-pyridyl)octanoyl]-1,5-diamono-3-oxapentane(5b)**

yield: 52%; IR (KBr): 3270 (ν_{N-H}), 1625 ($\nu_{c=o}$), 752 and 702 cm^{-1} (δ_{C-H}); ^1H NMR (δ, 270 MHz; $CDCl_3$): 1.24 (12H, m, -$(CH_2)_2$-(C*H*$_2$)$_3$-$(CH_2)_2$-x2), 1.59 (8H, m, N-CH_2-C*H*$_2$-x2, -C*H*$_2$-CH_2-CO-x2), 2.08 (6H, s, -CH_3x2), 2.17 (4H, t, *J* =7.3 Hz, -CH_2-CO-x2), 3.43 (4H, t, *J* =7.3 Hz, -CH_2-O-x2), 3.52 (4H, m, -C*H*$_2$-NHx2), 3.72 (4H, t, *J* =7.3 Hz, N-CH_2-x2), 5.19 (4H, s, -C*H*$_2$-Phx2), 6.40 (2H, d, *J* =7.7 Hz, 5-Hx2), 6.75 (2H, t, *J* =5.2 Hz, -NH-x2), 7.20 (2H, d, *J* =7.7 Hz, 6-Hx2), 7.35-7.41 (10H, m, -Phx2). *Anal.* Calcd for $C_{46}H_{62}N_4O_7 \cdot 0.5H_2O$: C, 69.96; H, 8.02; N 7.07. Found: C, 69.68; H, 8.08; N, 7.36.

***N*,*N*′-[Bis(3-hydroxy-1,4-dihydro-2-methyl-4-oxo-1-pyridyl)hexanoyl]-1,5-diamino-3-oxapentane(6a)**

A suspension of 10% Pd-C (10 mg) in CH_3OH (10 mL) was prehydrogenated with H_2 for 30 min. To this suspension was added a solution of compound (**5a**)(90 mg, 0.12 mmol) in CH_3OH (5 mL). The mixture was stirred for 2 h under hydrogen atmosphere. After removal of the catalyst by filtration, the filtrate was evaporated off to give the product (**6a**)(59 mg, 88%) as colorless amorphous solids: IR (neat): 1660 ($\nu_{C=O}$) and 1627 cm^{-1} ($\nu_{C=O}$); ^1H NMR (δ, 270 MHz; CD_3OD): 1.35 (4H, m, -CH_2-CH_2-CH_2-x2), 1.64 (4H, m, N-CH_2-CH_2-x2), 1.76 (4H, m, -CH_2-CH_2-CO-x2), 2.22 (4H, t, *J* =7.1 Hz, -CH_2-CO-x2), 2.44 (6H, s, -CH_3x2), 3.33 (4H, m, -NH-CH_2-x2), 3.50 (4H, t, *J* =6.5 Hz, -CH_2-O-x2), 3.92 (4H, t, *J* =6.8 Hz, N-CH_2-x2), 6.47 (2H, d, *J* =6.6 Hz, 5-Hx2), 7.18 (2H, d, *J* =6.6 Hz, 6-Hx2). *Anal.* Calcd for $C_{28}H_{42}N_4O_7 \cdot 4H_2O$: C, 54.32; H, 8.14; N, 9.04. Found: C, 54.40; H, 8.19; N, 8.88.

***N*,*N*′-[Bis(3-hydroxy-1,4-dihydro-2-methyl-4-oxo-1-pyridyl)octanoyl]-1,5-diamino-3-oxapentane(6b)**

yield: 83%; IR (neat): 1660 ($\nu_{C=O}$) and 1627 cm^{-1} ($\nu_{c=o}$); ^1H NMR (δ, 270 MHz; CD_3OD): 1.36 (12H, m, -$(CH_2)_2$-$(CH_2)_3$-$(CH_2)_2$-x2), 1.59 (4H, m, N-CH_2-CH_2-x2), 1.75 (4H, m, -CH_2-CH_2-CO-x2), 2.18 (4H, t, *J* =7.3 Hz, -CH_2-CO-x2), 2.46 (6H, s, -CH_3x2), 3.31 (4H, m, -NH-CH_2-x2), 3.51 (4H, t, *J* =6.6 Hz, -CH_2-O-x2), 4.09 (4H, t, *J* =7.7 Hz, N-CH_2-x2), 6.50 (2H, d, *J*=7.0 Hz, 5-Hx2), 7.70 (2H, d, *J* =7.0 Hz, 6-Hx2). *Anal*. Calcd for $C_{32}H_{50}N_4O_7 \cdot 2.5H_2O$: C, 59.31; H, 8.55; N, 8.64. Found: C, 59.51; H, 8.62; N, 8.29.

***N*,*N*'-[Bis(3-hydroxy-1,4-dihydro-2-methyl-4-oxo-1-pyridyl)hexanoyl]-1,5-diamino-3-oxapentanomethoxooxovanadium(V)(7a)**

To a solution of compound (**6a**)(186 mg, 0.33 mmol) in CH_3OH-H_2O (1:1) mixture (5 mL) was added a solution of $VO(acac)_2$ (80 mg, 0.30 mmol) in CH_3OH-H_2O (1:1) mixture (10 mL), and then the mixture was refluxed for 2 h. Removal of the solvent and subsequent gel-filtration with Sephadex LH-20 with distilled CH_3OH as an eluent afforded the product (**7a**)(42 mg, 22%) as deep purple powders: IR (KBr): 949 cm^{-1} ($\nu_{V=O}$); λ_{max}(10% aqueous CH_3OH): 527 nm (ε 2840). *Anal.* Calcd for $C_{29}H_{43}N_4O_9V \cdot 2H_2O$: C, 51.31; H, 6.98; N, 7.86. Found: C, 51.61; H, 7.03; N, 8.08.

***N*,*N*'-[Bis(3-hydroxy-1,4-dihydro-2-methyl-4-oxo-1-pyridyl)octanoyl]-1,5-diamino-3-oxapentanomethoxooxovanadium(V)(7b)**

30 mg (23%); IR (KBr): 953 cm^{-1} ($\nu_{V=O}$); λ_{max}(30% aqueous CH_3OH): 527 nm (ε 1870). *Anal.* Calcd for $C_{33}H_{51}N_4O_9V \cdot 0.8H_2O$: C, 55.57; H, 7.43; N, 7.86. Found: C, 55.65; H, 7.64; N, 7.74.

Measurement of the partition coefficient

To a solution of sample (5 mL) in KRB buffer solution (120 mM NaCl, 1.27 mM $CaCl_2$, 1.2 mM $MgSO_4$, 4.75 mM KCl, 1.2 mM KH_2PO_4, and 24 mM $NaHCO_3$: pH 7.4) (ligand : 1.0×10^{-4} M ; complex: 1.0×10^{-5} M) was added octanol (5 mL). The mixture was stirred at 600 rpm for 4 h with a magnetic stirrer, and then centrifuged at 4000 rpm for 20 min with a centrifugal separator. The concentrations of a ligand and its complex in each phase were measured from absorbance. The partition coefficient was calculated from the following equation ; $P = C_{octanol}/C_{buffer}$.

Measurement of cyclic voltammetry

To a solution of each oxovanadium(IV) complex (1 mM) in H_2O was added NaCl (0.1 M) as a supporting electrolyte. Cyclic voltammetry was carried out by generating triangular waves at an ambient temperature. A carbon electrode was used together with an Ag/AgCl electrode as a reference and a platinum wire as an auxiliary electrode. Current-voltage curves were recorded on an X-Y recorder.

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