Insulin-Enhancing Vanadium(III) Complexes

Marco Melchior,[†] Steven J. Rettig,[†] Barry D. Liboiron,[†] Katherine H. Thompson,[†] Violet G. Yuen,[‡] John H. McNeill,[‡] and Chris Orvig*[,†]

Medicinal Inorganic Chemistry Group, Department of Chemistry and Faculty of Pharmaceutical Sciences, University of British Columbia, 2036 Main Mall, Vancouver, BC V6T 1Z1, Canada

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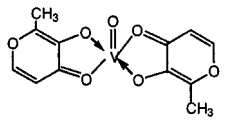
Simple, high-yield, large-scale syntheses of the V(III) complexes tris(maltolato)vanadium(III), $V(ma)_3$, tris(ethylmaltolato)vanadium(III), $V(ema)_3$, tris(kojato)vanadium(III) monohydrate, $V(koj)_3 \cdot H_2O$, and tris(1,2-dimethyl-3-hydroxy-4-pyridinonato)vanadium(III) dodecahydrate, $V(dpp)_3 \cdot 12H_2O$, are described; the characterization of these complexes by various methods and, in the case of $V(dpp)_3 \cdot 12H_2O$, by an X-ray crystal structure determination, is reported. The ability of these complexes to normalize glucose levels in the STZ-diabetic rat model has been examined and compared with that of the benchmark compound BMOV (bis(maltolato)oxovanadium(IV)), an established insulin-enhancing agent.

Introduction

Mononuclear coordination complexes of V(III) are generally six-coordinate, hydrolytically stable, and axially symmetric with an octahedral or pseudo-octahedral geometry.[1] They are not usually considered for therapeutic applications[2,3] because of rapid oxidation to V(IV/V) in aqueous solutions of pH >3.[4,5] Physiologically relevant oxidation states of vanadium are thought traditionally to be V(IV), as vanadyl, and V(V), often as vanadate.[2] There are examples of V(III) in biological systems; however, these are not very common.[6] To our knowledge, no V(III) complex has been examined previously for insulin-enhancing activity in vivo or in vitro.

Inorganic vanadyl and vanadate, as well as some vanadium(IV) and vanadium(V) complexes, enhance insulin action when administered orally.[7−12] The first chelated vanadium complex designed and tested chronically in vivo as an insulin mimetic agent (bis(maltolato)oxovanadium(IV), BMOV) has been thoroughly characterized chemically[13,14] and tested biologically.[15,16] BMOV has proven to be an excellent "benchmark" compound against which to test more recently synthesized complexes,[10,11,17−19] as was done in this study as well. A new vanadium(V) insulin mimetic coordination complex, dipicolinatooxovanadium(V), VO_2dipic^-, has proven to have significant glucose-lowering ability, though its rapid decomplexation and reduction in vivo is unquestioned.[20]



BMOV

The existence of strongly reductive V(III) compounds at high concentrations (up to 1 M) in marine organisms, e.g., sea squirts such as *Ascidia ceratodes* and *A. nigra*,[21] has engendered considerable speculation about the type of coordination environment that would be adequately protective of this oxidation state in an aerobic, predominately neutral pH medium.[22,23] Strongly

* To whom correspondence should be addressed. Tel: 604-822-4449. Fax: 604-822-2847. E-mail: orvig@chem.ubc.ca.
[†] Department of Chemistry.
[‡] Faculty of Pharmaceutical Sciences.

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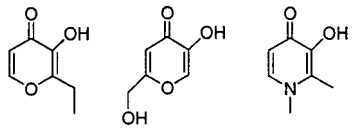
chelating agents such as catecholates[23] or Schiff bases such as salicylideneiminate (SALEN)[24,25] have been used successfully in the synthesis of relatively stable V(III) complexes and may yield useful clues to this conundrum.[22,26]

For the design of vanadium complexes appropriate for use as insulin-enhancing agents,[12] ligands of intermediate binding strength are likely more appropriate. Possessing neither high intrinsic bioactivity nor appreciable toxicity, maltol (Hma, a food additive approved in many countries) is an excellent spectator ligand in biological applications; Hma forms stable neutrally charged metal complexes with an optimum combination of water solubility, reasonable hydrolytic stability, and significant lipophilicity.[27,28] Ligands related to maltol include ethylmaltol (Hema), also a food additive, kojic acid (Hkoj), and Hdpp (1,2-dimethyl-3-hydroxy-4-pyridinone); all can be used to alter selectively the water solubility, hydrolytic stability, and lipophilicity of a metal complex.[29,30]



The biological chemistry of ML_3 complexes of these ligands is not new. Many years ago, we ourselves introduced $Al(ma)_3$ as an aluminum source for neurotoxicological studies.[31,32] The coordination chemistry of Fe(III) with 3-hydroxy-4-pyrones and 3-hydroxy-4-pyridinones has been explored,[33] with the former complexes having potential as iron supplementation agents[34−36] and the latter ligands as orally active scavenging agents for iron overload, particularly Hdpp (also known as L1 or deferiprone).[37,38] $VO(koj)_2$ has also been the subject of solution and insulin-enhancing biological studies; it is insulin-mimetic but is somewhat less potent than $VO(ma)_2$.[19] By contrast, *cis*-$[VO_2(ma)_2]^-$, a dioxovanadium(V) analogue of BMOV was pharmacologically less active than BMOV (each at 0.55 mmol kg^{-1}).[19]

In this paper, we examine V(III) ligation to several representative hydroxypyrones and pyridinones and assess their potential as insulin-enhancing agents. Oxovanadium(IV) and (V) complexes of these ligands have been characterized chemically and biologically previously; however, this is the first report of V(III) maltol (and analogues) as candidate antidiabetic agents. As with other metal chelates of these ligands, we anticipated reasonable hydrolytic and thermodynamic stability; the relative air stability of $V(ma)_3$ was an unexpected advantage.

Experimental Section

Materials. All chemicals were reagent grade and were used as received without further purification: $VOSO_4 \cdot 3H_2O$ (Aldrich), ethylmaltol (Pfizer), maltol (Pfizer), kojic acid (Lancaster), sodium dithionite (Fisher), 40% aqueous methylamine (Aldrich). Water was distilled (Barnstead D8902 and D8904 Cartridges) and deionized (Corning MP-1 Megapure still) before use.

Instrumentation. Infrared spectra were recorded as KBr disks in the range 4000−600 cm^{-1} on a Mattson Galaxy 5000 spectrophotometer and referenced to polystyrene. Mass spectra were obtained with a Kratos Concept II H32Q (Cs^+, LSIMS) or a Kratos M50 (EIMS) spectrometer. The UV−vis spectra of samples in 10 mm quartz cells were recorded with a Shimadzu UV-2100 spectrophotometer equipped with a Julabo UC circulating bath (25.0 ± 0.1 °C) or an HP 8453 spectrophotometer connected to a Fisher ISOTEMP 1016D circulating bath (25.0 ± 0.1 °C). Analyses for C, H, and N were performed in this department by Mr. Peter Borda. Room-temperature magnetic susceptibilities were measured on a Johnson Matthey magnetic susceptibility balance; diamagnetic corrections were based on Pascal's constants.[4]

Syntheses of the Complexes. Tris(maltolato)vanadium(III), V(ma)$_3$. Vanadyl sulfate trihydrate (1.03 g, 4.7 mmol) and maltol (1.80 g, 14.3 mmol) were added to a small flask, which was subsequently purged with Ar. Sodium dithionite (2.62 g, 15.0 mmol) was dissolved in 25 mL of degassed (Ar stream) water. This solution was filtered anaerobically such that the filtrate was collected in the reaction flask containing the vanadyl sulfate and maltol. The black-colored reaction mixture was stirred magnetically under a positive Ar pressure at 40 °C for 2 h, during which time the color of the mixture changed to a dark red. The dark red precipitate was collected by suction filtration and washed with a 3 × 20 mL portion of water followed by 3 × 20 mL portions of diethyl ether. The powder was dried overnight in vacuo over P_2O_5 and stored in an inert atmosphere glovebox. Yield: 1.39 g, 71% based on V. Anal. Calcd (found) for $C_{18}H_{15}O_9V$ (426.55): C, 50.72 (50.43); H, 3.55 (3.63). Mass spectrum (EI): *m*/*z* 426 (M^+), 301 ($[V(ma)_2]^+$). IR (cm^{-1}): 1606, 1571, 1507, 1464 (pyrone ring vibrations), 722 (ν_{V-O}). Magnetic moment: μ_{eff} = 2.7 BM.

Tris(ethylmaltolato)vanadium(III), V(ema)$_3$. a. [VO(ema)$_2$(Hema)]·H$_2$O. Vanadyl sulfate trihydrate (19.70 g, 90.72 mmol) and ethylmaltol (25.43 g, 182.0 mmol) were suspended in 300 mL of water at 45 °C. Following an induction period of 15−20 min, a fine yellow powder precipitated rapidly, and the suspension (pH ∼1) was cooled to room temperature. The solid was collected by filtration and air-dried (21.90 g, 43.51 mmol, 72% yield based on Hema). Anal. Calcd (found) for $C_{21}H_{24}O_{11}V$ (503.36): C, 50.11 (50.09); H, 4.73 (4.81). Mass spectrum (LSIMS): *m*/*z* 329 ($[V(ema)_2]^+$, 100%). IR (cm^{-1}): 1634, 1581 (Hema ring vibrations) 1596, 1562 (ema^- ring vibrations), 971 ($\nu_{V=O}$). Magnetic moment: μ_{eff} = 1.71 BM.

b. Reduction of [VO(ema)$_2$(Hema)]·H$_2$O. Yellow $[VO(ema)_2(Hema)]\cdot H_2O$ (0.287 g, 0.573 mmol) was dissolved in 50 mL of water at 65 °C, and excess sodium dithionite (0.200 g) was added under Ar. A fine air-sensitive red powder precipitated; the reaction mixture was left stirring overnight and allowed to cool to room temperature over that period. The precipitate was collected by filtration and dried in vacuo to yield 0.246 g (92% based on V). Anal. Calcd (found) for $C_{21}H_{21}O_9V$ (468.33): C, 53.86 (53.31); H, 4.56 (4.46). IR (cm^{-1}): 1600, 1567, 1502, 1470 (pyrone ring vibrations), 715 (ν_{V-O}). Mass spectrum (LSIMS): *m*/*z* 329 ($[V(ema)_2]^+$, 100%). Magnetic moment: μ_{eff} = 2.6 BM.

Tris(kojato)vanadium(III) Monohydrate, V(koj)$_3$·H$_2$O. Vanadyl sulfate trihydrate (2.13 g, 9.81 mmol) and kojic acid (4.26 g, 29.9 mmol) were dissolved with stirring in 50 mL of water at 55 °C under Ar. Addition of excess sodium dithionite (5.40 g) yielded, upon cooling, a

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Table 1. Selected Crystallographic Data for $V(dpp)_3 \cdot 12H_2O$

empirical formula	$C_{21}H_{48}N_3O_{18}V$
fw	681.56
space group	$P\bar{3}$ (No. 147)
a, Å	16.6167(9)
c, Å	6.8101(2)
V, Å^3	1628.45(11)
Z	2
ρ_{calc}, g cm^{-3}	1.390
μ(Mo Kα), cm^{-1}	3.82
total reflections	15 267
unique reflections	3038
R^a	0.058
$R_w{}^a$	0.064

^{a} $R = \sum||F_o| - |F_c||/\sum|F_o|$; $R_w = (\sum[w(F_o^2 - F_c^2)^2]/\sum[wF_o^4])^{1/2}$.

moderately air-stable orange powder that was collected by filtration, air-dried, and subsequently dried in vacuo to yield 3.60 g (75% based on V). Anal. Calcd (found) for $C_{18}H_{15}O_{12}V \cdot H_2O$ (492.27): C, 43.97 (43.92); H, 3.57 (3.48). IR (cm^{-1}): 1613, 1556, 1514, 1471 (pyrone ring vibrations), 758 (ν_{V-O}). Mass spectrum (LSIMS): m/z 333 ([V(koj)$_2$]$^+$, 100%). Magnetic moment: $\mu_{eff} = 2.7$ BM.

Tris(1,2-dimethyl-3-hydroxy-4-pyridinonato)vanadium(III) Dodecahydrate, $V(dpp)_3 \cdot 12H_2O$. Vanadyl sulfate trihydrate (10.8 g, 49.9 mmol) and maltol (18.8 g, 149 mmol) were dissolved with stirring at 65 °C in 300 mL of water under Ar. Aqueous 40% methylamine (40 mL, 573 mmol) was added, at which time all reagents dissolved. Sodium dithionite (10.0 g) was added as a solid, and the reaction mixture was stirred overnight. The reaction was cooled to room temperature, and the resulting brown crystalline solid was isolated by filtration and dried in a stream of Ar gas to yield 15.6 g (45% based on V). Anal. Calcd (found) for $C_{21}H_{24}N_3O_6V \cdot 12H_2O$ (681.56): C, 37.01 (37.19); H, 7.10 (6.98); N, 6.17 (6.03). IR (cm^{-1}): 1606, 1550, 1501, 1461 (pyridinone ring vibrations), 706 (ν_{V-O}). Mass spectrum (LSIMS): m/z 327 ([V(dpp)$_2$]$^+$, 100%). Magnetic moment: $\mu_{eff} = 2.5$ BM.

X-ray Crystallographic Analysis of $V(dpp)_3 \cdot 12H_2O$. All measurements were performed on a Rigaku/ADSC CCD area detector with graphite-monochromated Mo Kα radiation. The data were collected at a temperature of 180 ± 1 K to a maximum 2θ value of 60.1° in 0.50° oscillations with 70.0 s exposures. A sweep of data was performed using ϕ oscillations from 0.0 to 190.0° at $\chi = -90°$, and a second sweep was performed using ω oscillations between −23.0 and 18.0° at $\chi = -90°$. The structure was solved by direct methods[39] and expanded using Fourier techniques.[40] The non-hydrogen atoms were refined anisotropically; the hydrogen atoms were refined isotropically. Neutral atom scattering factors, anomalous dispersion corrections, values of Δf and $\Delta f''$, and those for the mass attenuation coefficients were taken from *International Tables for X-ray Crystallography*.[41] All calculations were performed using the teXsan crystallographic software package.[42] Selected crystallographic data are shown in Table 1 with distances and angles displayed in Table 2.

Oxidative Stability Analysis. Red $V(ma)_3$ (10.0 g, 23.5 mmol) was suspended in 10 mL of water and allowed to oxidize under ambient air and moisture for 1 month, with the resulting gray precipitate collected by filtration and air-dried (0.359 g, 1.13 mmol, ($VO(ma)_2$) 97% yield). The filtrate was strongly acidic (pH ~1). Similarly, intensely red $V(ema)_3$ (1.34 g, 2.86 mmol) was suspended in 10 mL of water and allowed to oxidize under ambient air and moisture for 1 month, resulting in a mixture of yellow and blue-green solids. Allowing the mixture to sit for a further 3 months gave only a blue-green solid ($VO(ema)_2$) which was collected by filtration and air-dried (0.920 g, 2.66 mmol, 93% yield based on V). The filtrate was strongly acidic (pH ~1).

Table 2. Bond Lengths (Å) and Angles (deg) in $V(dpp)_3 \cdot 12H_2O$ with Estimated Standard Deviations in Parentheses

Bond Lengths (Å)			
V(1)−O(1)	2.007(1)	V(1)−O(2)	2.035(1)
O(1)−C(2)	1.355(2)	O(2)−C(3)	1.294(2)
N(1)−C(1)	1.363(2)	N(1)−C(5)	1.370(3)
N(1)−C(6)	1.470(3)	C(1)−C(2)	1.390(3)
C(1)−C(7)	1.482(3)	C(2)−C(3)	1.405(3)
C(3)−C(4)	1.412(3)	C(4)−C(5)	1.359(3)

Bond Angles[a] (deg)			
O(1)−V(1)−O(1′)	91.30(6)	O(1)−V(1)−O(2)	80.57(6)
O(1)−V(1)−O(2′)	167.94(6)	O(1)−V(1)−O(2″)	97.74(6)
O(2)−V(1)−O(2′)	91.64(6)	V(1)−O(1)−C(2)	111.8(1)
V(1)−O(2)−C(3)	113.0(1)	C(1)−N(1)−C(5)	120.8(2)
C(1)−N(1)−C(6)	121.8(9)	C(5)−N(1)−C(6)	117.3(2)
N(1)−C(1)−C(2)	118.8(2)	N(1)−C(1)−C(7)	118.7(2)
C(2)−C(1)−C(7)	122.5(2)	O(1)−C(2)−C(1)	121.7(2)
O(1)−C(2)−C(3)	116.9(2)	C(1)−C(2)−C(3)	121.4(2)
O(2)−C(3)−C(2)	117.4(2)	O(2)−C(3)−C(4)	125.1(2)
C(2)−C(3)−C(4)	117.5(2)	C(3)−C(4)−C(5)	119.6(2)
N(1)−C(5)−C(4)	121.8(2)		

[a] Symmetry operations: (′) $-x + y$, $1 - x$, z; (″) $1 - y$, $1 + x - y$, z.

For solution studies, maltol and either NaCl or KCl were weighed and placed into a two-neck 25 mL flask. Both necks were sealed with septa. The flask was thoroughly flushed with Ar. $V(ma)_3$ was added into the flask while under constant Ar flux to minimize any solid-state oxidation of the complex. An 0.05 M buffer solution (either (1) pH 4.0−potassium biphthalate or (2) pH 7.4−*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid, Hepes) was prepared and vigorously degassed with Ar. An aliquot (25.0 mL) of the buffer solution was injected into the flask by syringe, and the resulting red-orange solution was stirred magnetically until all reactants had dissolved. A quartz cuvette was fitted with a septum and flushed thoroughly with Ar. To the cuvette was added an aliquot of the reaction solution by syringe. The cuvette was kept under Ar until the experiment was initiated. The cuvette was inserted into a thermostated cell holder, and at experiment initiation, the atmosphere in the cuvette was changed to 1 atm of O_2. The reaction was followed by monitoring the absorbance of the solution at 400 nm.

Animal Studies. Male Wistar rats (Animal Care Unit, University of British Columbia), weighing between 190 and 220 g, were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care. Experimental animals were randomly divided into four groups: control (C), control-treated (CT), diabetic (D), and diabetic-treated (DT) ($n = 5$ for each group). Housing and feeding of the animals, the induction of a hyperglycemic state through streptozotocin (STZ) injection, and the monitoring of glucose levels were all conducted as described previously.[10,11,13] In experiments involving administration of the appropriate complex by intraperitoneal (i.p.) injection, D rats were given saline (0.9% w/w NaCl), whereas DT animals were given a single i.p. dose of 0.1 mmol kg^{-1} of the appropriate compound in saline. In the oral gavage experiments, D rats were given 3% w/w gum arabic; DT were given a single dose of 0.6 mmol kg^{-1} of the appropriate compound suspended in 3% w/w gum arabic. Glucose levels were determined immediately prior to administration of the compound and at 2, 4, 6, 8, 12, 16, 20, 24, 48, and 72 h following compound administration. A positive response to testing was taken as blood glucose lowering to <9 mM within the first 24 h following compound administration. Values are presented as means ± SEM. Data from the acute studies were analyzed using GLM repeated measures ANOVA, with a significance level taken as $p < 0.05$.

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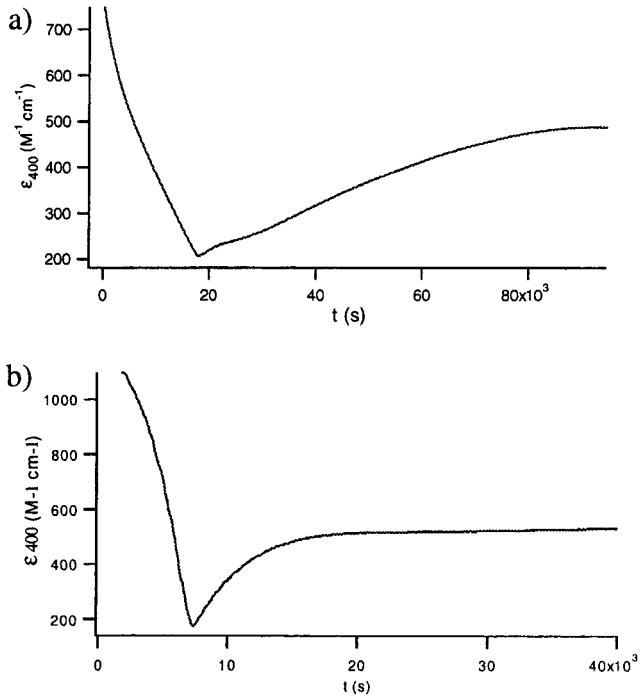

Figure 1. Oxidation of $V(ma)_3$ in the presence of excess Hma: (a) $[V(ma)_3]_{total} = 1.77$ mM, $[Hma]_{total} = 35.5$ mM, $p(O_2) = 1$ atm, [KCl] = 1 M, [KPTH] = 0.05 M, pH 4.0, 298 K, $\lambda = 400$ nm; (b) $[V(ma)_3]_{total} = 1.87$ mM, $[Hma]_{total} = 36.9$ mM, $p(O_2) = 1$ atm, [NaCl] = 1 M, [Hepes] = 0.05 M, pH 7.35, 298 K, $\lambda = 400$ nm.

Results and Discussion

New V(III) complexes—$V(ma)_3$, $V(ema)_3$, $V(koj)_3 \cdot H_2O$, and $V(dpp)_3 \cdot 12H_2O$—were synthesized on a large scale by dithionite reduction of an aqueous vanadyl complex: a convenient method to access V(III) complexes in aqueous solutions that has been employed previously in the facile synthesis of vanadium(III) diketonates.[43] The "one-pot" synthesis of the 3-hydroxy-4-pyridinone complex $V(dpp)_3 \cdot 12H_2O$ from maltol, methylamine, dithionite, and vanadyl was adapted from the previously reported syntheses of $Al(dpp)_3 \cdot 12H_2O$ and $Ga(dpp)_3 \cdot 12H_2O$.[44] The synthesis of $V(ema)_3$ was accomplished through the dithionite reduction of an insoluble V(IV) precursor formulated, based on the available data, as $[VO(ema)_2(Hema)] \cdot H_2O$. Unlike $V(pic)_3 \cdot H_2O$ but similar to $V(acac)_3$,[43,45] $V(ma)_3$, $V(ema)_3$, $V(koj)_3 \cdot H_2O$, and $V(dpp)_3 \cdot 12H_2O$ are all air-sensitive; however, the degree of air sensitivity varies considerably from that of $V(ema)_3$ which is readily decomposed in air, to $V(ma)_3$, the most air-stable, which only slowly generates $VO(ma)_2$ in solution over extended periods.

In aqueous solution, the oxidations of two of the vanadium(III) complexes were probed spectrophotometrically and proved to be complicated and nonlinear. Although the oxidation could not be modeled using any simple mathematical treatment, the lifetimes of the V(III) complexes could be estimated from inflection points in the absorbance vs time graphs, as the oxidations are typically biphasic (Figure 1). The first phase corresponds to the oxidation of V(III) to V(IV); the second phase corresponds to the oxidation of V(IV) to V(V). V(III), a strong reductant, and V(V), a moderate oxidant, react together rapidly. It is likely that the major oxidant of V(III) is in fact V(V) and not O_2, with V(V) the major oxidant accounting for the nonlinear nature of the reaction kinetics.

Oxidations of $V(ma)_3$ and $V(ema)_3$ are slow under acidic conditions, each having a lifetime of approximately 16 h at pH 4.0 under 1 atm of $p(O_2)$. These lifetimes decrease with increasing basicity; at pH 7.4, $V(ma)_3$ was shown to have a lifetime of 2 h. Lifetimes are also greatly reduced in organic solvents such as methanol, in which $V(ma)_3$ and $V(ema)_3$ are least stable, possessing lifetimes on the order of only 10 s at $p(O_2) = 1$ atm. Intermediate lifetimes are seen in increasingly basic conditions. The V(III) complexes $V(ma)_3$, $V(ema)_3$, $V(koj)_3 \cdot H_2O$, and $V(dpp)_3 \cdot 12H_2O$ are all ultimately sensitive to oxidation in the solid state. Because the oxidation of $V(ma)_3$, the most oxidatively stable of the vanadium(III) complexes, is relatively slow (particularly at the acidic pH range of the stomach) and is characterized by an induction period in which no appreciable oxidation occurs, it was deemed most suitable for biological testing (vide infra).

Significant lowering of the pyrone ($\sim$40 cm^{-1}) and pyridinone ($\sim$20 cm^{-1}) ring stretching frequencies, inseparable combinations of the ligand carbonyl and high energy ring vibrations,[46] were observed when the infrared spectra of the uncomplexed heterocycles, and their analogous V(III) complexes were compared. The V(IV) precursor of $V(ema)_3$, formulated as $[VO(ema)_2(Hema)] \cdot H_2O$, had pyrone ring vibrations attributable to deprotonated ema^- (with a low energy shift of 40 cm^{-1} from Hema) as well as pyrone ring vibrations intermediate in energy between those of Hema and $V(ema)_3$, suggestive of neutral pyrone coordination (with a 10 cm^{-1} reduction from values for Hema). The infrared spectra of the V(III) complexes, as expected, were devoid of any peaks attributable to vanadyl $\nu_{V=O}$ stretching frequencies.

The positive ion detection mode LSIMS spectra of all the V(III) complexes were dominated by a 100% relative intensity ion with *m*/*z* characteristic of $[VL_2]^+$, with the parent molecular mass, the $[VL_3]^+$ ion, appearing as a much less intense peak. Higher molecular-weight ions, including $[V_2L_5]^+$, were particularly intense and diagnostic; the $[V_2L_5]^+$ ion has been shown in previous work to be characteristic of trivalent metal ion tris-(oxypyridinonato) and -(oxypyronato) complexes.[30,31,47−49] Subtle distinctions were found between the mass spectra of the V−pyrone complexes in different oxidation states. The mass spectra of $V(ema)_3$ and its V(IV) precursor differ only in the intensity of key ions: for example, the $[HVO(ema)_2]^+$ ion is significantly less intense in the mass spectrum of $V(ema)_3$ than in the spectrum of $[VO(ema)_2(Hema)] \cdot H_2O$.

$V(dpp)_3 \cdot 12H_2O$ crystallizes from saturated aqueous solutions at 4 °C as large brown crystals which are robust, but not indefinitely stable to solvent loss and oxidation. Key bond lengths and angles are summarized in Table 2. $V(dpp)_3 \cdot 12H_2O$ is isomorphous with the previously reported exoclathrate dodecahydrates of other metal ions: $Al(dpp)_3 \cdot 12H_2O$,[48] $Ga(dpp)_3 \cdot 12H_2O$,[48] $In(dpp)_3 \cdot 12H_2O$,[49] and $Fe(dpp)_3 \cdot 12H_2O$.[50,51] The structure consists of $V(dpp)_3$, a distorted octahedron of three planar 1,2-dimethyl-3-oxy-4-pyridinonato ligands (Figure 2),

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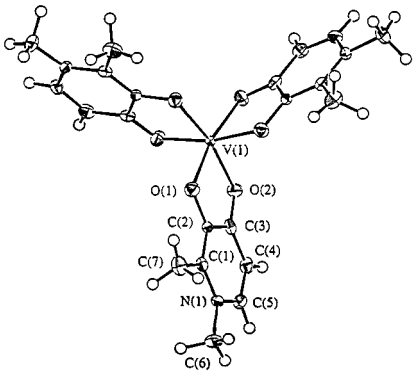

Figure 2. Structure of the metal complex in $V(dpp)_3 \cdot 12H_2O$ showing the crystallographic numbering; thermal ellipsoids for non-hydrogen atoms are drawn at 33% probability.

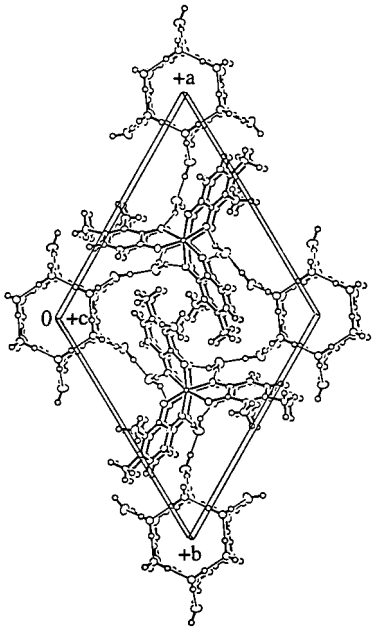

Figure 3. Packing diagram for $V(dpp)_3 \cdot 12H_2O$ showing the hexagonal channels of water molecules.

enclosed in an exoclathrate hydrate structure,[47,49] in which the complex is excluded from a hydrogen-bonded network provided by 12 water molecules forming hexagonal channels (Figure 3). In $V(dpp)_3 \cdot 12H_2O$, there is a compression of the octahedron along the trigonal axis; the compression is seen in a large exocyclic angle O(1)−V(1)−O(2) of 97.74(6)° as well as in a relatively small bite angle O(1)−V(1)−O(2) of 80.57(6)°, characteristics which are shared by homologues such as $Fe(dpp)_3 \cdot 12H_2O$.[50,51] The bond lengths, 2.007(1) Å for V(1)−O(1) and 2.035(1) Å for V(1)−O(2), and the bite angle, 80.57(6)°, in this complex are well within the range of those in the V(III) catecholates, including $[V(cat)_3]^{3-}$ [23] and $[V(trencam)]^{3-}$, which contains a macrocyclic tricatecholate.[52] $[V(cat)_3]^{3-}$ and $[V(trencam)]^{3-}$ possess, respectively, mean V−O bond lengths of 2.013(9) Å and 1.996(7) Å as well as mean bite angles of 81.3(9)° and 80.75(8)°.[23,52]

The glucose lowering characteristics of $V(ma)_3$, $V(ema)_3$, $V(koj)_3 \cdot H_2O$, and $V(dpp)_3 \cdot 12H_2O$ as well as $VO(ma)_2$ (BMOV, for purposes of comparison) were determined following intraperitoneal (i.p.) administration to STZ-diabetic rats. The glucose levels at 24 h after i.p. injection were for $V(ma)_3$, 12.3 ± 3.7 mM; for $V(ema)_3$,10.5 ± 1.8 mM; and for $VO(ma)_2$, 8.2 ± 3.0 mM, which were all significantly lower than untreated diabetic rat (D) plasma glucose levels (>20 mM). Positive response (defined as [glucose] < 9 mM within 24 h of injection) was seen in 3 of 5 rats treated with $V(ma)_3$, none of the rats treated with $V(ema)_3$, and 4 of 5 rats treated with $VO(ma)_2$. Neither $V(koj)_3 \cdot H_2O$ ($[glucose]_{24h}$ = 19.7 ± 0.4 mM) nor $V(dpp)_3 \cdot 12H_2O$ ($[glucose]_{24h}$ = 19.9 ± 0.9 mM) proved active as insulin-enhancing agents; animals treated with these compounds had no appreciable glucose-lowering compared to D ($[glucose]_{24h}$ = 20.9 ± 0.7 mM). There were no fatalities, and the only observable toxicity manifested as mild gastrointestinal distress; however $V(dpp)_3 \cdot 12H_2O$ produced severe diarrhea.

The glucose-lowering abilities of $V(ma)_3$, $V(ema)_3$, and $VO(ma)_2$, when administered by oral gavage, were also examined. $V(koj)_3 \cdot H_2O$ and $V(dpp)_3 \cdot 12H_2O$ were excluded from the oral gavage studies because of their poor performance in the i.p. experiments. Both $V(ma)_3$ ([glucose] = 14.5 ± 3.2 mM) and $VO(ma)_2$ ([glucose] = 15.7 ± 2.2 mM) resulted in comparable glucose-lowering 24 h after administration by oral gavage; the glucose levels were significantly different from D ([glucose] = 19.0 ± 0.3). $V(ema)_3$ (n = 5, 19.0 ± 3.2 mM) proved inactive. There were also no fatalities in these groups.

Previous comparisons of biodistribution[53] and pharmacology[54] of V(III), V(IV), and V(V) compounds in vivo suggest that all are handled similarly in terms of tissue uptake and anticipated toxicity. Nonetheless, because V(III) complexes are known to be notoriously unstable to oxidation at pH >3, further pursuit of these compounds as pharmaceutical agents was generally considered likely to be futile. This did not turn out to be the case. Treatment with either $V(ma)_3$ and $VO(ma)_2$ resulted in significant glucose lowering in STZ-diabetic rats of comparable magnitudes, both when administered by i.p. injection or by oral gavage, with no overt toxicity other than mild gastrointestinal distress and no fatalities.

Concluding Remarks

$V(ma)_3$, $V(koj)_3$, $V(ema)_3$, and $V(dpp)_3 \cdot 12H_2O$ have been synthesized in high-yield large-scale preparations. All were screened for insulin-enhancing potential by i.p. injection in STZ-diabetic rats, and biologically effective compounds were then also tested for oral availability. Despite the fact that prior art teaches against it, V(III) compounds proved as equally effective as many V(IV) complexes in terms of insulin-enhancing behavior. Speciation and biodistribution studies are currently underway for these novel insulin-enhancing V(III) complexes.

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Supporting Information Available: An X-ray crystallographic file in CIF format for the structure determination of $V(dpp)_3 \cdot 12H_2O$. This material is available free of charge via the Internet at http://pubs.acs.org.

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