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Coordination Chemistry and Insulin-Enhancing Behavior of Vanadium Complexes with Maltol $C_6H_6O_3$ Structural Isomers

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Syntheses of vanadium complexes using the naturally occurring ligands isomaltol (Hima) and allomaltol (Hama), as well as a newly synthesized, potentially tetradentate diaminodipyrone [H_2(en(ama)$_2$], are reported. Complete characterization of the resulting compounds [*trans*-VO(ima)$_2$(H_2O), VO(ama)$_2$, V(ima)$_3$, V(ama)$_3$ and VO(en(ama)$_2$)], including X-ray crystallography analyses for *trans*-VO(ima)$_2$(H_2O) and V(ima)$_3$, are presented herein. Potentiometric titrations (25°C, I = 0.16 M NaCl) were used to measure stability constants in the V(IV)–Hima system; these data were compared to previous data collected on the V(IV)–L (L = Hma, Hama) systems. The in vivo efficacy of these compounds to lower the blood glucose levels of STZ-diabetic rats was tested; all but VO(en(ama)$_2$) produced significant decreases in plasma glucose levels. The results were compared to those of the benchmark compound BMOV [VO(ma)$_2$, bis(maltolato)oxovanadium(IV)], a known insulin-enhancing agent.

Introduction

Vanadium involvement in the treatment of diabetes mellitus predates the discovery of insulin; in 1899, Lyonnet and Martin reported that two of three diabetic individuals treated with sodium vanadate excreted less glucose in their urine.[1] However, the insulin-enhancing activity of vanadium compounds has received growing interest only over the past two decades.[2] Physiological effects of vanadium may result from the structural resemblance between phosphate and vanadate, as well as from the fact that vanadium can form cationic and anionic compounds.[3] The ease of conversion between vanadyl {[VO]$^{2+}$, V(IV)} and vanadate {[VO_4]$^{3-}$, V(V)}, the two forms of vanadium present in equilibrium in the human body, both permits and complicates various interactions with biological molecules.[4−6] Vanadium is essential to the functioning of some enzymes in simple organisms; however, the essentiality of vanadium in higher animals and humans has a long and controversial history, and to date, the words of Schroeder still hold true: "No other trace metal has so long had so many supposed biological activities, without having been proven to be essential."[7]

Diabetes mellitus, a heterogeneous metabolic disorder, is becoming a major health concern in industrialized countries; currently, more than 6% of the populations of developed countries is estimated to have diabetes. In underdeveloped countries, the many undiagnosed diabetic individuals make estimation difficult.[8] Diabetes is distinguished as either type 1 (IDDM, insulin-dependent diabetes mellitus), in which the minimum sufficient insulin is not produced, or type 2 (NIDDM, non-insulin-dependent diabetes mellitus), in which there is insensitivity to insulin.

Insulin, a pancreatic signaling hormone, is the principal treatment for type 1 diabetes and is often required for type 2 diabetes as well. Insulin is not orally active and must be administered via intramuscular injection. Vanadium com-

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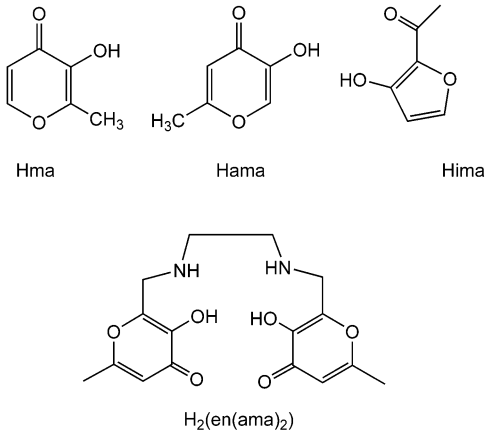
Chart 1. Structures of $C_6H_6O_3$ Structural Isomers and $H_2(en(ama)_2)$.



pounds stimulate glucose metabolism without affecting the concentration of insulin. This makes them promising candidates for the treatment of type 2 diabetic individuals (which includes the majority of people diagnosed with diabetes) where hyperinsulinemia is of concern because of secondary complications resulting from excess insulin.[9]

Coordination complexes of vanadium (mostly vanadyl) are current candidate insulin-enhancing compounds; they can be tailored to optimize the desired properties for a drug. A considerable number of these compounds have been synthesized and tested for their insulin-enhancing actions.[2] In 1992, we reported the oral insulin-enhancing behavior of the vanadium complex BMOV [bis(maltolato)oxovanadium(IV)].[10] The insulin-enhancing (i.e., augmenting the effects of insulin) ability of BMOV has been investigated thoroughly.[10−14] BEOV [bis(ethylmaltolato)oxovanadium(IV)],[14] a BMOV analogue, completed phase I clinical trials in humans in late 2000. Both of these complexes have the desired properties, namely, water solubility, balanced lipophilicity and/or hydrophilicity, neutral charge and thermodynamic stability, for a candidate oral drug.[13,14] However, the latest studies have shown that, shortly after administration, vanadium complexes dissociate[14] and the ligand may act only as a shuttle.[15]

Maltol, the proligand in BMOV, has two structural isomers, allomaltol (Hama) and isomaltol (Hima), which are also nontoxic natural products and potential ligands for chelation to vanadium. Herein, we report the chemical and biological properties of vanadium complexes with these ligands. Chart 1 shows the structures of the three $C_6H_6O_3$ structural isomers along with the structure of $H_2(en(ama)_2)$.

Allomaltol (3-hydroxy-6-methyl-4-pyrone, Hama) has a structure very close to that of maltol (3-hydroxy-2-methyl-4-pyrone, Hma) wherein the methyl group is shifted from the 2 to the 6 position on the pyrone ring.[16] Isomaltol [1-(3-hydroxy-2-furanyl) ethanone, Hima] is a β-hydroxyeneone with a metal binding site analogous to that in acetylacetone.[17,18] Investigating these systems was deemed worthwhile because allomaltol is the closest in structure to the maltol motif and vanadium complexes of acetylacetone have been reported for their insulin-enhancing activity.[19,20] *N*,*N*′-Bis(3-hydroxy-6-methyl-2-methylene-4-pyrone)ethylenediamine [H_2en(ama)$_2$] is a potentially tetradentate N_2O_2 donor, incorporating two allomaltol moieties bridged by an ethylenediamine backbone.[21]

The syntheses and complete characterizations of various vanadium(III, IV) complexes with the two bidentate ligands and the V(IV) complex of the aminopyrone are reported herein, along with comparison studies between the acidity and stability constants for these compounds. Biological testing of the insulin-enhancing behavior of these new vanadium compounds is also presented.

Experimental Section

Materials. All solvents were reagent-grade and were used without further purification. All chemicals were obtained from commercial sources (Aldrich, Sigma, Fisher) and were used without further purification. Reactions were carried out in air unless otherwise specified. Allomaltol,[16] *O*-galactosylisomaltol,[18] and H_2en(ama)$_2$[21] were prepared according to the previously published methods.

Instrumentation. Infrared spectra were recorded as KBr disks with a Mattson Galaxy Series 5000 FTIR spectrophotometer in the 4000−400 cm^{-1} range. Mass spectra were obtained with a Kratos MS 50 (electron-impact ionization mass spectrometry, EIMS), a Micromass LCT (electrospray ionization mass spectrometry, ESIMS), or a Kratos Concept II H32Q [Cs^+, liquid secondary ion mass spectrometry (LSIMS) with positive ion detection] instrument. Elemental analyses were performed by Mr. Peter Borda or Mr. Minaz Lakha in the Department of Chemistry, University of British Columbia, or by Delta Microanalytical Services. ^{1}H NMR spectra were recorded on a Bruker AC-200E or a Bruker AM-300 NMR spectrometer at 200 or 300 MHz, respectively. Room-temperature magnetic susceptibilities were measured using a Johnson Matthey balance. Diamagnetic corrections were estimated using Pascal's constants.[22] EPR spectra were recorded in CH_2Cl_2 solvent on a Bruker ESC-106 EPR spectrometer in 20-μL quartz capillaries. Simulation of the isotropic EPR spectrum was performed using Bruker's WINEPR/SIMFONIA package. Water was deionized (Barnstead D8902 and D8904 cartridges), distilled (Corning MP-1 Megapure Still), and degassed by being boiled under Ar for 30 min.

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Potentiometric measurements were carried out with an automatic titration system consisting of a Metrohm 713 pH meter with a Metrohm 6.0233.100 electrode, a model 665 Metrohm Dosimat autoburet, water-jacketed titration vessels and a Julabo UC circulating bath. Both the pH meter and the autoburet were controlled with an IBM-compatible PC. Titrations were controlled by a locally written Qbasic program. The concentration of NaOH (1 M; Fisher) used for potentiometric titrations was confirmed by titration with potassium biphthalate (Anachemia Canada Inc.). NaCl was used to control the ionic strength ($I = 0.16$ M). The electrode was calibrated before each titration using a known amount of HCl(aq) solution titrated with a known amount of NaOH. Plotting potential (mV, calculated) vs pH gave a working slope and an intercept; therefore the pH could be read as $-\log [H^+]$ directly. The value of pK_w used at $I = 0.16$ and $T = 25$ °C was 13.76.[23]

Syntheses of Compounds. (i) **Isomaltol, $C_6H_6O_3$ (Hima).** *O*-Galactosyl isomaltol (10 g, 35 mmol) was placed in a Schlenk tube equipped with a coldfinger under vacuum. The system was immersed in a preheated Wood's metal bath at 110 °C, and the bath was heated to 210 °C. Sublimation of isomaltol started with caramelization of the galactoside. After the sublimation was finished, the light beige product was collected from the coldfinger, recrystallized from acetone/water, and dried in a desiccator under vacuum over P_2O_5 to yield 3.22 g, 73%, mp 98−100 °C. Anal. Calcd (found) for $C_6H_6O_3$: C, 57.14 (57.42); H, 4.80 (4.72). IR (cm^{-1}, KBr disk): $\nu_{C=O/C=C}$ 1577, 1615, 1636. ^1H NMR (200 MHz, $CDCl_3$): δ 2.40 (s, 3H, CH_3), 6.27 (d, 1H, $J = 2$ Hz), 7.27 (d, 1H, $J = 2$ Hz), 8.50 (s, b, 1H, OH). LSIMS (+): $m/z = 127$ [$(M + 1)^+$].

(ii) *trans*-bis(isomaltolato)aquaoxovanadium(IV), BIMOV, *trans*-($VO(ima)_2(H_2O)$). Method A. Isomaltol (6.15 g, 35 mmol) was dissolved in water (100 mL). The temperature was increased to 50 °C and the pH adjusted to 6 with 1 M NaOH solution. Vanadyl sulfate tetrahydrate (5.75 g, 25 mmol) was added to the isomaltol solution. The color changed to green immediately and the pH was then adjusted to 7 with 1 M NaOH. After 1 h the reaction mixture was filtered and the green precipitate dried under vacuum over P_2O_5 in a desiccator to yield 7.28 g, 89% yield. Anal. Calcd (found) for $C_{12}H_{12}O_8V$: C, 43.00 (43.29), H, 3.60 (3.56). IR (cm^{-1}, KBr disk): $\nu_{V=O}$ 967, $\nu_{C=C/C=O}$ 1511, 1537, 1584. LSIMS (+): $m/z = 318$ [$(M + H)^+$]. EPR: 8 line pattern, $g_{iso} = 1.966 \pm 0.001$, $A_{iso} = 100.0 \pm 0.1$ (10^{-4}) cm^{-1}. Solid-state magnetic moment: $\mu = 1.76\ \mu_B$.

Method B. Isomaltol (78 mg, 0.62 mmol) and vanadyl sulfate (67 mg, 0.32 mmol) were dissolved in ice cold water (15 mL). Urea (19 mg, 0.32 mmol) was added, and the solution was warmed to room temperature and left overnight. The resulting green X-ray-quality crystals (35 mg, 35% yield) were filtered out and found to be genuine BIMOV.

Method C. $V(ima)_3$ (10 mg, 0.023 mmol) was dissolved in 5 mL of acetone in a 25-mL vial; the vial containing the red solution was closed and kept still. The red solution eventually became green after a few days. Crystals suitable for X-ray crystal structure analysis were grown by slow diffusion of *n*-pentane into this solution. The isolated product was characterized as *trans*-$VO(ima)_2(H_2O)$.

(iii) Tris(isomaltolato)vanadium(III), V(ima)$_3$. Under Ar, Hima (188 mg, 1.49 mmol) was dissolved in 50 mL of hot (55 °C) degassed water. Vanadyl sulfate (108 mg, 0.50 mmol) was added to this solution. Sodium dithionite (250 mg, 1.44 mmol) was dissolved in Ar saturated water (10 mL) and added to the reaction solution very slowly. The green solution changed to red upon addition of the dithionite. The system was kept at 55 °C with stirring overnight. The next morning, the reaction mixture was filtered, and the red precipitate was washed with cold water. A red solid (170 mg, 80% yield) was isolated. Crystals suitable for X-ray structure analysis were grown by diffusion of *n*-pentane into an acetone solution of this complex under dry Ar. Anal. Calcd (found) for $C_{18}H_{15}O_9V$: C, 50.72 (50.61); H, 3.54 (3.48). IR (cm^{-1}, KBr disk): $\nu_{C=C/C=O}$ 1534, 1561, 1629 cm^{-1}. LSIMS (+): $m/z = 427$ [$(M + 1)^+$]. Solid-state magnetic moment: $\mu = 2.85\ \mu_B$.

(iv) Bis(allomaltolato)oxovanadium(IV) monohydrate, $VO(ama)_2 \cdot H_2O$. A solution of $VO(acac)_2$ (50 mg, 0.19 mmol) in 2 mL of CH_2Cl_2 was slowly added to a solution of Hama (54 mg, 0.42 mmol) in 3 mL of CH_2Cl_2 under Ar. The color changed from light yellow to dark burgundy after a few minutes. The solution was stirred overnight; a burgundy solid was isolated by filtration (47 mg, 78% yield). Anal. Calcd (found) for $C_{12}H_{12}O_8V$: C, 43.00 (42.80), H, 3.60 (3.44). IR (cm^{-1}, KBr disk): $\nu_{V=O}$ 988; $\nu_{C=C/C=O}$ 1551, 1609. EIMS: $m/z = 317$ $[M]^+$. Solid-state magnetic moment: $\mu = 1.78\ \mu_B$.

(v) Tris(allomaltolato)vanadium(III) monohydrate, $V(ama)_3 \cdot H_2O$. The synthesis of this compound was done by a method similar to that used for $V(ima)_3$, except that allomaltol was substituted for isomaltol, 75% yield. Anal. Calcd (found) for $C_{18}H_{17}O_{10}V$: C, 48.65 (48.45); H, 3.82 (3.68). IR (cm^{-1}, KBr disk): $\nu_{C=C/C=O}$ 1563, 1610 cm^{-1}. LSIMS (+): $m/z = 427$ $[M + 1]^+$. Solid-state magnetic moment: $\mu = 2.84\ \mu_B$.

(vi) Bis(isomaltolato)methoxyoxovanadium(V), $VO(ima)_2(OCH_3)$. *trans*-$VO(ima)_2(H_2O)$ (27.2 mg, 0.08 mmol) was dissolved in 1.5 mL of MeOH· H_2O_2 (10 μL) was added to this bright green solution and the color changed to blood red. The reaction flask was kept at −45 °C for 4 h and then placed in the freezer overnight. The dark red precipitate was filtered and dried under vacuum (13.1 mg, 47% yield). Anal. Calcd (found) for $C_{13}H_{13}O_8V$: C, 44.85 (45.19); H, 3.76 (3.98). IR (cm^{-1}, KBr disk): $\nu_{V=O}$ 965. ^1H NMR (CD_3OD): δ 2.30 (s, 3H, CH_3), 3.39(s, 3H, OCH_3), 6.28 (d, 1H), 7.53 (d, 1H).

(vii) (*N*,*N*′-Bis(3-hydroxo-6-methyl-2-methylene-4-pyrone)-ethylenediamine) oxovanadium(IV) Dihydrate, $VO(en(ama)_2) \cdot 2H_2O$. Vanadyl sulfate (130 mg, 0.51 mmol) and $H_2(en(ama)_2)$, (172 mg, 0.52 mmol) were added to 20 mL of water with stirring; the pH of the mixture was increased to 8.5 by the addition of 1 M NaOH. A clear solution of dark brown color was obtained. The temperature was then increased to 55 °C with stirring for 2 h. The reaction flask was cooled to room temperature prior to filtration. A dirty pink (light burgundy) solid was obtained (105 mg, 51% yield). Anal. Calcd (found) for $C_{16}H_{22}N_2O_9V$: C, 43.95 (44.24); H, 5.07 (5.00); N, 6.41 (6.53). IR (cm^{-1}, KBr disk): $\nu_{V=O}$ 951, $\nu_{C=C/C=O}$ 1586, 1627. (+) ESIMS $m/z = 402$ [$(M + 1)^+$]. Solid-state magnetic moment, $\mu = 1.79\ \mu_B$.

Potentiometric pH Titrations. The acidity constants for Hama and Hima were redetermined by titrating 50 mL of aqueous 0.6 mM HCl solution ($I = 0.16$ M NaCl, $T = 25$ °C) in the presence of 0.92−2.0 mM Hama or Hima under Ar with 1.5 mL of 0.11 M NaOH. The calculations were done using a curve fit procedure (a Newton−Gauss nonlinear least-squares program). The considered pH range was $4 \le pH \le 7.3$ for isomaltol. This corresponds to the range of about 2−98% neutralization for the equilibrium between Hima and ima^-. The final results were averages of 12 independent titrations.

The stability constants of V(IV) with isomaltol were determined under the same conditions as used for the acidity constant, except that the acid (HCl) was partly replaced by the respective metal ion

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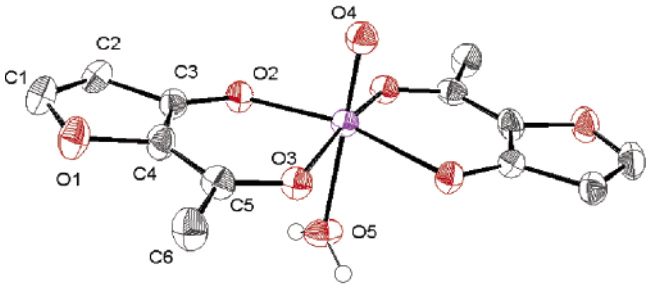

Figure 1. ORTEP drawing of *trans*-VO(ima)$_2$(H$_2$O), showing 50% thermal probability ellipsoids.

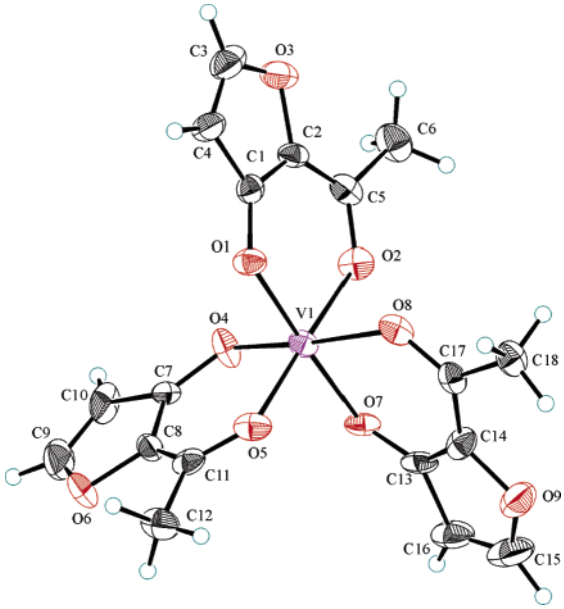

Figure 2. ORTEP illustration of V(ima)$_3$ (two of the ligands are each shown in one of two disordered orientations), showing 50% thermal probability ellipsoids.

(I = 0.16 M, 25 °C). The ligand-to-metal ratio used was ≥4:1 to prevent the hydrolysis of V(IV). The calculations were carried out using a similar curve-fitting procedure by the same Newton−Gauss nonlinear least-squares program. Each titration was repeated at least eight times; the final results are the averages of the eight trials.

X-ray Crystallographic Analyses. The crystal structure of VO(ima)$_2$(H$_2$O) was determined at the University of Minnesota X-ray Crystallographic Laboratory, and that of V(ima)$_3$ was determined at UBC. Selected crystallographic data and selected bond angles and bond lengths are presented in Tables 1−3 below. The ORTEP diagrams of the two complexes are shown in Figures 1 and 2.

***trans*-VO(ima)$_2$(H$_2$O).** Teal crystals of excellent quality were mounted on the tip of a glass capillary on a Bruker smart platform CCD system for data collection at 173(2) K using Mo Kα radiation (graphite monochromator) with a frame time of 10 s. The cell constants were based on 7846 reflections with 1577 being unique (R_{int} = 0.0552). The data set was collected 4.42 < 2θ < 55.12; Data were processed and corrected for absorption as well as for Lorentz and polarization effects.

The structure was solved and refined.[24,25] The space group $C2/c$ was determined according to systematic absences and intensity statistics. Full-matrix least-squares/difference Fourier cycles were performed to locate the remaining non-hydrogen atoms and fractional sites of O1 and C1. All non-hydrogen atoms were refined with anisotropic displacement parameters. H5 was found in the difference map, and all other hydrogen atoms were placed in ideal positions. The full-matrix least-squares refinement converged to R1 = 0.0392 and wR2 = 0.1098. The goodness of fit was 1.080. In addition to the two ligands and the oxo group, a water molecule was also coordinated to the metal center. This water molecule interacts with other oxygens of the neighboring molecules via hydrogen bonds. One ligand is disordered over two sites that results in a structural trans/cis ratio of 88:12.

V(ima)$_3$. A red chip crystal was mounted on a glass fiber and measurements were done on a Rigaku/ADSC CCD area detector with graphite-monochromated Mo Kα radiation. The cell constants were based on 5750 reflections with 5.5° < 2θ < 50.0°; they correspond to a C-centered monoclinic cell. Statistical analysis of the intensity distribution and the successful solution and refinement of the structure determined the space group to be $C2/c$. Data were collected at 173(1) K in oscillation with 47.00-s exposures. Of the 14139 reflections collected, 3369 were unique (R_{int} = 0.073) after equivalent reflections were merged. Data were collected,[26] processed, and corrected for Lorentz and polarization effects. The structure was solved by direct methods and expanded using Fourier transform techniques.[27,28] Two of the ligands about vanadium were disordered, each adopting two orientations. The first disordered ligand had a relative major/minor fragment population ratio of 68:32, whereas the proportion in the second ligand was 86:14. Restraints were used to ensure that each disordered ligand fragment had roughly the same geometry as the nondisordered ligand. All non-hydrogen atoms in the major fragment were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinements was based on 3130 observed reflections [I > 0.00$\sigma(I)$], and 330 variable parameters converged with unweighted and weighted agreement factors of R1 = 0.086, wR2 = 0.134. Goodness of fit was 0.96.

in Vivo Animal Studies. Male Wistar rats (Animal Care Unit, UBC), weighing 190−210 g, were cared for according to the guidelines of the Canadian Council for Animal Care. The housing and feeding of the animals, the induction of experimentally induced diabetes with streptozotocin (STZ), and the monitoring of glucose levels were conducted as described previously.[11,13,29] Screening for anti-diabetic activity of VO(ima)$_2$, V(ima)$_3$, V(ama)$_3$, and VO(en-(ama)$_2$) was carried out in a different trial for each complex. For most bioactivity tests, rats were divided into five groups: control (nondiabetic, C), diabetic (D), diabetic treated with BMOV (DT), and diabetic treated with a new V(III) or V(IV) complex. Doses for testing were those established as the ED$_{50}$ doses for BMOV in previous testing.[11,30] For oral gavage, compound suspensions were 0.6 mmol kg^{-1}; for intraperitoneal (ip) injection, they were 0.01 mmol kg^{-1}, in a 1-mL volume of 1% CMC. Control and diabetic

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(29) In one case, no nondiabetic group was included; in another, a BMOV-nondiabetic group was included. As these screening tests have been repeated many times by our group, we can safely say that these additional groups are not germane to the screening process. BMOV served as a yardstick for comparison of glucose-lowering effectiveness.

groups (untreated) received an equivalent volume of 1% CMC alone, whether by oral gavage or ip injection. Data [mean ± standard error of the mean (SEM)] were analyzed by ANOVA followed by a Newman−Keuls test where applicable ($p < 0.05$), with an NCSS (Number Cruncher Statistical System) package.

Results and Discussion

Proligands. Isomaltol (Hima) was made in high yield by vacuum sublimation of *O*-galactosylisomaltol at 190 °C. The two-step synthesis of isomaltol has been reported previously by Nelson and Hodge.[18] One glucose moiety from lactose is dehydrated to form *O*-galactosylisomaltol, and hydrolysis of this product by steam distillation forms isomaltol. The first part of the synthesis used here followed that reported previously in the literature,[18] but the second part, the synthesis of isomaltol from this precursor, was modified. Herein is reported a simple, less hazardous and less time-consuming method with comparable yield. The tetradentate aminopyrone chelator H_2(en(ama)$_2$) was synthesized by the procedure recently reported by our group[21] with minor modifications. The second step of the reaction was carried out at 0 °C (as opposed to room temperature) to slow the process and avoid decomposition. The product was isolated from the reaction mixture in one step in comparable yield.

V(IV) Complexes, VOL_2 (L = ima$^-$, ama$^-$), VOL (L = [en(ama)$_2$]$^{2-}$). All of the proligands, when deprotonated, are bidentate Lewis bases[31] and are expected to bind strongly to vanadyl, a hard Lewis acid. Combining $VOSO_4$ and the proligand Hima in water produces VO(ima)$_2$(H_2O) with the maximum yield obtained at pH = 6. The same compound was also prepared by air oxidation of the V(III) complex [V(ima)$_3$] in solution.

The same synthetic procedure as described above was used for allomaltol, but isolation of pure VO(ama)$_2$ was not possible because of its high solubility in water. Also, if left in aqueous solution or exposed to air, the complex would oxidize over time. Therefore, VO(ama)$_2$ was synthesized from VO(acac)$_2$ via ligand substitution in methylene chloride.

Vanadium complexation to [en(ama)$_2$]$^{2-}$ was carried out by dissolution of vanadyl sulfate and the proligand [H_2(en(ama)$_2$] in water and adjusting the pH to 8.5 with 1 M NaOH. Isolation of the product was not possible at pH < 8. VO(en(ama)$_2$) precipitates from the solution as a dirty pink solid. It was characterized using spectroscopic techniques (IR, ESIMS), magnetic measurements, and elemental analysis.

Infrared spectra of the complexes show the vanadyl stretch peaks at 967, 988, and 951 cm^{-1} for *trans*-VO(ima)$_2$(H_2O), VO(ama)$_2$·H_2O, and VO(en(ama)$_2$)·2H_2O, respectively. These are within the previously reported range of 930−1030 cm^{-1}.[32] For all complexes examined here, the peak shifted to lower energy compared to that in BMOV or VO(acac)$_2$, at 995 or 998 cm^{-1}, respectively. The shift is particularly noticeable in the case of *trans*-VO(ima)$_2$(H_2O) and VO(en(ama)$_2$)·2H_2O; the bound water trans to V=O reduces its bond order, resulting in a bathochromic shift. It is not possible to distinguish the C=O stretch from that of C=C. Despite the observed bathochromic shifts in the IR spectra of *trans*-VO(ima)$_2$(H_2O) and VO(ama)$_2$·H_2O, the pyrone stretching frequencies (C=O and C=C) did not shift noticeably upon complexation for VO(en(ama)$_2$)·2H_2O. This suggests that the carbonyl is not coordinated to the metal center and that the pyrone ring is coordinated to the metal ion only through the hydroxo oxygen atoms.

Magnetic measurements showed room-temperature magnetic moments of $\mu = 1.76\ \mu_B$ for *trans*-VO(ima)$_2$(H_2O), $\mu = 1.78\ \mu_B$ for VO(ama)$_2$, and $\mu = 1.79\ \mu_B$ for VO(en(ama)$_2$) in the solid state, values very close to the spin-only value of $\mu = 1.73\ \mu_B$ for a d^1 V(IV) system and within the accepted range of 1.7−1.8 μ_B for such systems.[22] The typical eight-line pattern EPR spectrum for a V(IV) system was also as expected for *trans*-VO(ima)$_2$(H_2O) at room temperature. The isotropic g and A values are $g_{iso} = 1.966 \pm 0.001$ and $A_{iso} = 100 \pm 0.1$ (10^{-4}) cm^{-1} in CH_2Cl_2. These are similar to other reported values for vanadyl complexes.[13,14]

VOL_2 (L = ima$^-$, ama$^-$) complexes were also studied by electron impact ionization mass spectrometry; for both bis complexes, the parent peak [VOL_2]$^+$ and the fragment ion peaks [VOL]$^+$ and L$^+$ were observed at m/z = 317, 192, and 126, respectively (the coordinated water molecule was not observed by this method). The parent peak for *trans*-VO(ima)$_2$(H_2O) was observed in the electrospray ionization mass spectrum at m/z = 335; the molecular ion peak [VOL + 1]$^+$ for VO(en(ama)$_2$) was detected using the same technique at m/z = 402.

Vanadyl complexes are oxidized in water or alcoholic solutions upon prolonged exposure to air, resulting in V(V) complexes. This oxidation process was thoroughly studied for the maltol complexes.[13,33] A methanol solution of VOL_2 (L = ima$^-$, ama$^-$), when exposed to air for a few hours, turns red in color; this is indicative of oxidation, which can be accelerated if excess H_2O_2 is used (3−6 equiv). The resulting complex has a six-coordinate V(V) metal center with an alkoxy group filling the sixth coordination site. Only VO(ima)$_2$(OCH_3) was actually isolated and characterized. The ^{1}H NMR spectrum of this compound is diagnostic of a V(V) complex; the ring hydrogens show up as doublets at 6.28 and 7.53 ppm (4H), and the methyl group has a chemical shift of 2.3 ppm (6H). The methoxy hydrogens are observed at 3.39 ppm (3H). The room-temperature NMR spectrum of this complex shows that the two ligands are in similar environments; however, as a V(V) d^0 system, VO(ima)$_2$(OCH_3) is very labile, meaning that the cis and trans isomers cannot be resolved at room temperature on the NMR time scale. Previous variable-temperature NMR studies of the maltol analogue showed a cis structure at lower temperatures.[13] The mass spectrum of the ima$^-$ complex did not show

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the parent peak but instead indicated the bis complex {*m*/*z* = 317, $VO(ima)_2^+$; $[M - OCH_3]^+$}.

Elemental analyses for the two bis ligand complexes indicated one water of crystallization; for VO(en(ama)$_2$), two waters of crystallization were present. Whether this water molecule was actually coordinated was not clear until the X-ray crystal structure of *trans*-VO(ima)$_2$(H_2O) was solved (Figure 1). The two ligands are bound in a trans arrangement, similar to that seen for BMOV.[13] The geometry around the vanadium center is pseudo-octahedral. The asymmetric unit cell consists of one-half of a formula unit − the V═O and the aqua ligand define a 2-fold axis of symmetry. VO bond lengths are consistent among this compound, BMOV, and VO(acac)$_2$. The V═O bond distance is [1.596(2) Å], exactly as long as that in BMOV [1.596(7) Å][13] and slightly longer than that in VO(acac)$_2$ [1.584(2) Å].[34] Upon coordination of isomaltol to vanadyl, a six-membered chelate ring is formed. The average V−O bond length in this ring is 2.075 Å as compared to 1.988 Å for BMOV and 1.968 Å for VO(acac)$_2$.[13,34] The bond between the coordinated water and the vanadium ion is significantly longer (2.187 Å) than the other V−O bonds. This could be due to steric strain, as well as the trans influence imparted by the oxo group; the vanadyl moiety is sitting above the plane of the other four equatorial oxygen atoms. The isomaltolato bond lengths are also consistent with those reported for other metal complexes of this ligand.[35] The average CO bond length is 1.286 Å, close to the average length in VO(acac)$_2$, in which a typical carbonyl bond is about 1.25 Å whereas a single C−O bond is about 1.45 Å. This is indicative of charge delocalization in the isomaltolato moiety.

There is also intermolecular hydrogen bonding between the hydrogens of the coordinated water molecule and the hydroxo oxygens of the isomaltolato ligands of the neighboring complexes. This provides a network of hydrogen bonding throughout the extended structure. The structure of *trans*-VO(ima)$_2$(H_2O) is closely related to that of VO(acac)$_2$ (originally reported in 1961 and redetermined in 1995);[34,36] the isomaltolato ligand has a binding site similar to that of the acetylacetonato ligand. Bond lengths are comparable between the two structures; however, comparison of bond angles is difficult because of the disorder (the syn/anti orientation of the ligand) in *trans*-VO(ima)$_2$(H_2O), in that the ligand is disordered over two sites in a ratio of 88:12. The geometry around the vanadium is octahedral as opposed to square-pyramidal in VO(acac)$_2$. The vanadium(IV) ion is sitting above the equatorial plane in both complexes. The two isomaltolato ligands are trans to one another, similar to the structure of BMOV.[13] Crystallographic data are presented in Table 1, and selected bond lengths and angles are presented in Table 2.

V(III) Complexes, VL_3 (L = ima$^-$ and ama$^-$). Vanadium(III) tris complexes of isomaltol and allomaltol were synthesized using dithionite reduction of aqueous vanadyl solution. This method has been reported before in the synthesis of V(III) diketonates[37] and has been used in our laboratories to make V(III) complexes of maltol, ethylmaltol, and kojic acid.[38] These V(III) compounds are air-sensitive to various degrees, V(ima)$_3$ being more stable in the solid form than is V(ama)$_3$, and both complexes being more stable than V(ma)$_3$. Either complex oxidizes in solution when exposed to air over several days. Both complexes were completely characterized in the solid state by elemental analysis, IR spectroscopy, MS, and magnetic measurements, as well as by X-ray crystallography for V(ima)$_3$. Elemental analyses for these complexes were consistent with the proposed VL_3 structure. Both compounds are hygroscopic, and V(ama)$_3$ is isolated with one water of crystallization.

Infrared spectroscopy shows that the OH stretch of the proligand disappears, indicative of chelation. Also, the IR spectra of both tris complexes showed vibrations related to the bidentate pyrone or furan ligands. The absence of the characteristic V═O stretch peak was a good indication of the reduction of V(IV) vanadyl starting material to V(III). Similarly to the bis complexes, the C═O band has resolved and shifted to lower energy. The bathochromic shift relative to isomaltol is indicative of metal binding, because electron donation from the oxygen atom to the metal center weakens

Table 1. Selected Crystallographic Data for *trans*-VO(ima)$_2$(H_2O) and V(ima)$_3$

compd	*trans*-VO(ima)$_2$(H_2O)	V(ima)$_3$
empirical formula	$C_{12}H_{12}O_8V$	$C_{18}H_{15}O_9V$
cryst color, habit	teal, block	red, chip
cryst size, mm	0.25 × 0.21 × 0.19	0.20 × 0.20 × 0.05
cryst system	monoclinic	monoclinic
space group	*C*2/*c*	*C*2/*c*
a, Å	12.376(4)	29.187(6)
b, Å	13.879(5)	8.239(1)
c, Å	8.011(3)	15.126(3)
β, deg	96.513(6)	100.734(8)
V, Å^3	1367.2(8)	3547(1)
Z	4	8
FW	335.16	426.25
ρ (g/cm^3)	1.628	1.584
temperature	173(2) K	173(2) K
θ (deg)	2.21−27.56	2.75−25
F(000)	684	1744
total reflns	7846	14139
unique reflns	1577 (R_{int} = 0.0552)	3369 (R_{int} = 0.073)
R1	0.0392	0.086
wR2	0.1059	0.134
GOF	1.080	0.96

Table 2. Selected Bond Lengths (Å) and Angles (deg) in *trans*-V(ima)$_2$(H_2O)

V(1)−O(2)	2.001(4)	C(3)−C(4)	1.401(4)
V(1)−O(3)	2.038(3)	C(5)−C(4)	1.384(4)
V(1)−O(5)	2.187(2)	C(5)−C(6)	1.495(4)
C(3)−O(2)	1.297(3)	C(4)−O(1)	1.408(3)
C(5)−O(3)	1.279(3)		
O(4)−V(1)−O(3)	95.99(9)	O(3)−V(1)−O(5)	84.01(9)
O(4)−V(1)−O(2)	97.5(3)	C(4)−C(3)−C(2)	105.7(2)
O(2)−V(1)−O(3)	93.02(8)	C(4)−C(5)−C(6)	121.1(2)
O(2)−V(1)−O(5)	82.5(3)		

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Table 3. Selected Bond Lengths (Å) and Angles (°) in $V(ima)_3$

V(1)−O(1)	1.951(2)	C(5)−O(2)	1.274(4)
V(1)−O(4)	1.953(6)	C(3)−O(3)	1.367(4)
V(1)−O(7)	1.960(5)	C(2)−O(3)	1.398(3)
V(1)−O(5)	1.991(7)	C(1)−C(2)	1.380(4)
V(1)−O(2)	2.033(3)	C(1)−C(4)	1.442(4)
V(1)−O(8)	2.082(6)	C(2)−C(5)	1.382(5)
C(1)−O(1)	1.287(4)	C(3)−C(4)	1.336(5)
C(3)−O(3)−C(2)	104.9(3)	O(2)−V(1)−O(4)	83.7(2)
O(2)−V(1)−O(1)	91.23(10)	O(2)−V(1)−O(7)	87.96(19)
O(4)−V(1)−O(1)	94.9(2)	O(2)−V(1)−O(5)	176.6(2)
O(7)−V(1)−O(1)	177.20(18)	O(2)−V(1)−O(8)	88.0(2)
O(4)−V(1)−O(7)	87.6(3)	O(5)−V(1)−O(8)	93.6(3)
O(5)−V(1)−O(1)	91.9(2)	O(8)−V(1)−O(1)	86.86(19)
O(4)−V(1)−O(5)	94.6(3)	C(2)−C(1)−C(4)	106.6(3)
O(7)−V(1)−O(5)	89.0(3)	C(2)−C(5)−C(6)	121.7(3)

the carbonyl bond. Here as well, C═C stretches cannot be distinguished from C═O stretches, and the bands are assigned as combination bands.

Liquid secondary ion mass spectra of both of these complexes show the parent peak $m/z = 427$ $[ML_3 + 1]^+$, with a correct isotope distribution; high-resolution LSIMS confirms the molecular mass and formula. The 100% intensity peak is the VL_2^+ ion for both complexes and not the actual parent peak. The $V_2L_5^+$ peak is also observed at $m/z = 727$ for both compounds; this peak is characteristic of the V(III)−tris-ligand complexes.[38,39] Magnetic moments were measured for $V(ima)_3$ and $V(ama)_3$ at room temperature, and the data were in agreement with previously reported two-electron d^2 vanadium systems.

Selected bond lengths and angles are presented in Table 3. Selected crystallographic data for $V(ima)_3$ are shown in Table 1. The ORTEP diagram of this complex is shown in Figure 2; the vanadium(III) ion is situated in a distorted octahedral O_6 coordination sphere. The disorder (the syn/anti orientation of two of the ligands) in the structure makes detailed comparisons of bond angles difficult; however, bond lengths are quite comparable. In the ordered ligand, the C−C bonds for the binding site are identical [C1−C2 = 1.380(4) Å, C2−C5 = 1.382(5) Å] within experimental error. The average C−C bond length in $V(acac)_3$ is 1.376(4) Å. The C−O bond lengths are 1.274(4) Å for the carbonyl and 1.287(4) Å for the hydroxyo, both slightly longer than those observed in the acac complex. Overall, the bond lengths in $V(ima)_3$, compared to those in $V(acac)_3$,[40] show delocalization of the double bonds in isomaltol when it is coordinated to the V(III) center. Mer and fac isomers cannot be assigned to the structure, because of the structural disorder. The V−O distance varies from 1.951 to 2.082 Å (all within the previously reported bond length range for VO single bond).

Solution Studies. Acidity constants (eq 1) of Hma, Hama, and Hima have been reported previously[13,21,35] and were redetermined here for the latter proligand; herein, the stability constants for the V(IV)−ima^- system are reported for the first time. Table 4 shows the acidity and formation constants

Table 4. Acidity Constants of the Three $C_6H_6O_3$ Structural Isomers, Stability Constants of the Complexes Formed between V(IV) and These Ligands ($I = 0.16$ M NaCl, 25 °C), and Corresponding Values for Hacac and $VO(acac)_2$ ($I = 0.1$ M $NaClO_4$, 25 °C)

ligand	pK_a	$\log K_{110}$	$\log \beta_{120}$	pK_{12-1}	ref
Hima	5.64(2)	5.97(1)	11.37(4)	7.1(1)	this work
Hama	8.04(2)	7.90(12)	14.83(8)	8.8(2)	21
Hma	8.44(2)	8.80(2)	16.29(2)	7.5(1)	39
Hacac	8.83	8.59	16.10	−	41

defined by eqs 1−5, with the corresponding values for the V(IV)−L systems of each of the $C_6H_6O_3$ structural isomers (L = Hima, Hama, and Hma).[13,21]

$$HL \leftrightarrows H^+ + L^- \qquad K_a \tag{1}$$

$$VO^{2+} + L^- \leftrightarrows [VOL]^+ \qquad K_{110} \tag{2}$$

$$[VOL]^+ + L^- \leftrightarrows VOL_2 \qquad K_{120} \tag{3}$$

$$VO^{2+} + 2L^- \leftrightarrows VOL_2 \qquad \beta_{120} \tag{4}$$

$$VOL_2 + OH^- \leftrightarrows [VOL_2(OH)]^- \qquad K_{12-1} \tag{5}$$

Throughout the titrations, no precipitation was observed at any point; all experimental curves were smooth and fitted properly, indicating that hydrolysis was not a major factor. By comparing the acidity constants of the three structural isomers, one can easily conclude that isomaltol should be a stronger acid than allomaltol or maltol; therefore, it can be predicted that the former will not be as strong a binding group for vanadium.

As seen in the V(IV)−ama^- system,[21] L/V(IV) = 2:1 complexes form in solution for the bidentate ima^- ligand. A deprotonation from $VO(ima)_2$ was observed at higher pH values (about 7) to form hydroxo species with $pK_a = 7.1 \pm 0.1$. Our previous investigations of the V(IV)−ama^- or V(IV)−$[en(ama)_2]^{2-}$ systems showed that, in each case, in aqueous solution, there is a coordinated water molecule.[13] This water molecule is deprotonated at pH > 7, an observation that was also reported by Buglyó et al. recently for BMOV.[41]

In determinations of the stability constants, the hydrolysis of vanadyl was included in the model using constants from the literature.[42] Using stability constants and hydrolysis constants, species distribution diagrams can be calculated for the V(IV)−ima^- system (Figure 3). For $VO(ima)_2$, the formation constants are less than those for the other two isomers [$VO(ama)_2$, $VO(ma)_2$] or for $VO(acac)_2$ ($\log K_1 = 8.59$, $\log \beta_2 = 16.10$).[13,21,43] $VO(ima)_2$ is the predominant species in the range $3 \le pH \le 7$. It is obvious from the diagram that, when L/V(IV) > 4, the hydrolysis of vanadyl can be ignored for all of these bidentate ligands (in the case of isomaltol, the hydrolysis is negligible up to pH = 6 and <10% below pH = 7). As the pH elevates, the concentration

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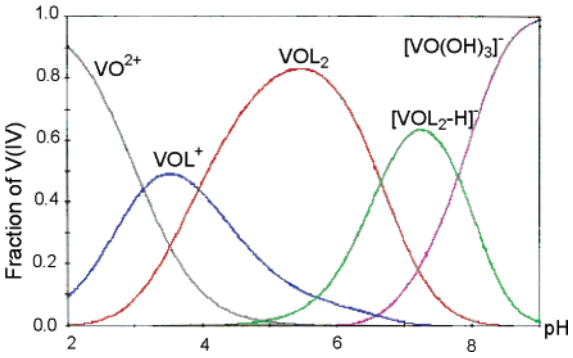

Figure 3. Species distribution diagrams for the complexation of V(IV) with Hima {L/V(IV) = 4:1, [V(IV)] = 0.1 mM, I = 0.16 mM NaCl, 25 °C}.

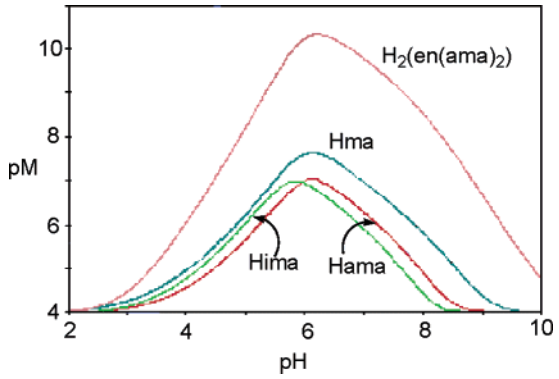

Figure 4. Plot of pM vs pH for the V(IV)−Hma, Hama, Hima and H_2-(en(ama)$_2$) systems (L:V(IV) = 4:1; [V(IV) = 0.1 mM; I = 0.16 M NaCl; 25 °C).

of hydrolysis species increases; above pH 7 for isomaltol, hydrolysis dominates the solution.

A pM vs pH plot (Figure 4), where pM is defined as the sum of all noncomplexed "free" vanadium species (eq 6), can be used to compare the stabilities of different structurally isomeric V(IV) species. It is apparent that ma$^-$ forms the most stable vanadyl complex among the three structural isomers, followed by ama$^-$ and finally ima$^-$ (Figure 4); however, the VO(en(ama)$_2$) system is the most stable vanadyl complex.

$$\mathrm{pM} = -\log([\mathrm{VO}^{2+}] + [\mathrm{VO(OH)}^{+}] + [\,(\mathrm{VO})_2(\mathrm{OH})_2]^{2+} + [\,\mathrm{VO(OH)_3}]^{-}) \quad (6)$$

Biological Results. Three of the four compounds screened showed evidence of significant glucose lowering, the one exception being VO(en(ama)$_2$). The average glucose levels at 24 h after treatment for VO(ima)$_2$, V(ima)$_3$, and V(ama)$_3$ were 16.9 ± 3.0, 16.9 ± 2.8, and 16.1 ± 3.4 mM, as compared to the BMOV-treated group for each of these trials (13.7 ± 2.3, 11.0 ± 4.0, and 14.3 ± 4.3 mM, respectively). No serious side effects were noted in these test groups, although 2 of 10 of the VO(ima)$_2$-treated rats had a mild diarrhea 24 h after gavage.

Figure 5 compares the percent glucose lowering for the new vanadium complexes. VO(ima)$_2$ was as effective as BMOV for the first 24 h; however, the activity dropped to about one-half that of BMOV by 48 h after gavage. The

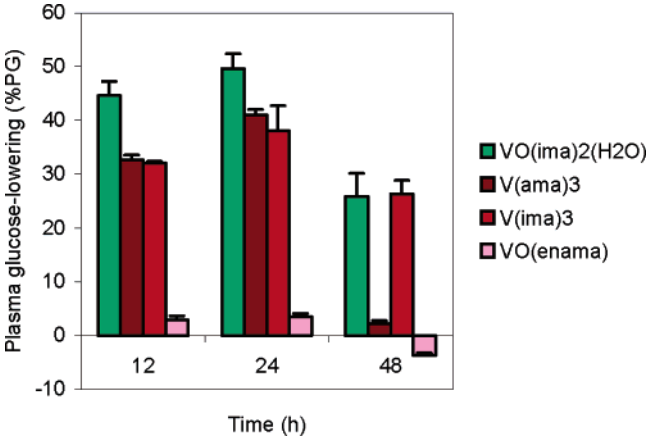

Figure 5. Plasma glucose lowering (%) of VO(ima)$_2$, V(ima)$_3$, V(ama)$_3$, and VO(en(ama)$_2$). % PG = (PG_D − PG_{test})/PG_D × 100%, where PG is plasma glucose, PG_D is the mean plasma glucose for the untreated diabetic group at the same time point in that assay, and PG_{test} is the mean plasma glucose for the test compound group. All compounds were given as suspensions in 1% carboxymethylcellulose (CMC). VO(ima)$_2$ and VO-(en(ama)$_2$) were administered by oral gavage, 0.6 mmol kg^{-1}; V(ima)$_3$ and V(ama)$_3$ were administered by intraperitoneal (ip) injection, 0.1 mmol kg^{-1}.

two tris complexes showed significant glucose-lowering activity at 24 h after injection [38.1% for V(ima)$_3$ and 41.9% for V(ama)$_3$]; the mean percentages plasma glucose (%PG) for the comparison BMOV groups in each of these trials were 59.7% and 47.6%, respectively. Of the 28 diabetic rats treated with BMOV in these four trials, four animals died prior to sacrifice. The vanadyl tetradentate diaminodipyrone complex, VO(en(ama)$_2$), resulted in no antidiabetic activity at all (but also no toxicity), on a par with the bis and tris thiopyrone complexes tested previously.[44] Solution studies of the V(IV)−en(ama)$_2$ system[21] indicated a higher stability compared to either bis[13,14] or tris[37,44] ligand vanadium complexes. Vanadyl compounds that are effective insulin-enhancing agents appear to dissociate rapidly.[14,45] High complex stability might, in fact, be counterproductive to relief of diabetic symptomatology.[46,47]

Compared to previous trials of VOL$_2$ complexes,[13,14,29,37,48] VO(ima)$_2$ was as good as, but no better than, others that resulted in roughly 50% glucose lowering in acute screening. The two new VL$_3$ complexes [V(ima)$_3$ and V(ama)$_3$] were, once again, not as potent as the VOL$_2$ compounds,[2] but they appeared to be somewhat more effective than V(ma)$_3$ and V(ema)$_3$, tested previously.[38] In the case of V(ama)$_3$ and, to a lesser extent, V(ima)$_3$, the effect was short-lived, with rebound to hyperglycemia by 48 h after injection.

Conclusions

Vanadium(III, IV, V) complexes of the structural isomers of maltol, allomaltol, and isomaltol were synthesized and

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completely characterized. The acidity constant of Hima and the stability constants of its V(IV) complexes were determined using potentiometric titrations; these results were compared to our previously reported data. The V(IV)−isomaltol system was the least stable among the three ligands (Hima, Hama, Hma) in the physiological pH range. The X-ray crystal structures for the isomaltolato complexes *trans*-$VO(ima)_2(H_2O)$ and $V(ima)_3$ show a coordinated water molecule in the vacant site for the former and disorder in both complexes. The reaction of $H_2(en(ama)_2)$ with vanadyl sulfate afforded $VO(en(ama)_2)$. All characterization agrees with 1:1 complex formation. The new vanadyl complex, $VO(ima)_2$, and both tris complexes significantly lowered plasma glucose in STZ-diabetic rats; none were considered as effective as BMOV in terms of sustained effect. The tetradentate complex did not correct diabetic hyperglycemia, possibly because of the high stability of this complex.

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Supporting Information Available: CIF files for $VO(ima)_2(H_2O)$ and $V(ima)_3$ and a table reporting glucose-lowering data for the proposed insulin mimetic agents are available free of charge via the Internet at http://pubs.acs.org.

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