Vanadium Complexes as Insulin Mimetic Agents: Coordination Chemistry and in Vivo Studies of Oxovanadium(IV) and Dioxovanadate(V) Complexes Formed from Naturally Occurring Chelating Oxazolinate, Thiazolinate, or Picolinate Units†

Marco Melchior, Katherine H. Thompson, Janet M. Jong, Steven J. Rettig, Ed Shuter, Violet G. Yuen, Ying Zhou, John H. McNeill, and Chris Orvig*

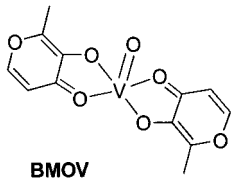
Medicinal Inorganic Chemistry Group, Department of Chemistry, University of British Columbia, Vancouver, British Columbia, V6T 1Z1, Canada and Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, V6T 1Z3, Canada

Received October 19, 1998

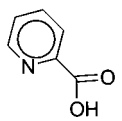
The synthesis and characterization of four complexes containing naturally occurring binding groups are reported: $VO(pic)_2 \cdot H_2O$ (Hpic = picolinic or pyridine-2-carboxylic acid), $NH_4[VO_2(pic)_2] \cdot 2H_2O$, $VO(oz)_2$ (Hoz = 2-(2′-hydroxyphenyl)-2-oxazoline), and $VO(thoz)_2$ (Hthoz = 2-(2′-hydroxyphenyl)-2-thiazoline). The X-ray structures of $NH_4[VO_2(pic)_2] \cdot 2H_2O$, $VO(oz)_2$, and $VO(thoz)_2$ have been determined. Crystals of $NH_4[VO_2(pic)_2] \cdot 2H_2O$ ($C_{12}H_{12}N_3O_6V \cdot 2H_2O$) are monoclinic, space group *Cc*, $a = 10.347(2)$ Å, $b = 26.318(2)$ Å, $c = 7.247(1)$ Å, $\beta = 128.37(1)°$, $V = 1547.3(5)$ Å^3, $Z = 4$; those of $VO(oz)_2$ ($C_{18}H_{16}N_2O_5V$) are triclinic, $P\bar{1}$, $a = 10.408(1)$ Å, $b = 11.282(1)$ Å, $c = 7.666(1)$ Å, $\alpha = 103.78(1)°$, $\beta = 109.64(1)°$, $\gamma = 84.75(1)°$, $V = 823.3(2)$ Å^3, $Z = 2$; and those of $VO(thoz)_2$ ($C_{18}H_{16}N_2O_3S_2V$) are orthorhombic, *Pbca*, $a = 12.331(2)$ Å, $b = 26.090(2)$ Å, $c = 11.125(2)$ Å, $V = 3579.3(9)$ Å^3, $Z = 4$. The structures of $NH_4[VO_2(pic)_2] \cdot 2H_2O$ and $VO(oz)_2$ were solved by direct methods and that of $VO(thoz)_2$ by the Patterson method. The structures were refined by full-matrix least-squares procedures to $R = 0.034$, 0.035, and 0.034 ($R_w = 0.031$, 0.030, and 0.035) for 2428, 1672, and 4595 reflections with $I \geq 3\sigma(I)$, respectively. In the case of $NH_4[VO_2(pic)_2] \cdot 2H_2O$, the $[VO_2(pic)_2]^-$ anion consists of a *cis*-VO_2^+ center with the two picolinate ligands bound with an interplanar angle of 97.25°. Both $VO(oz)_2$ and $VO(thoz)_2$ demonstrate the common distorted square pyramidal geometry, with bidentate oxazolinate or thiazolinate ligands bound to the vanadium center through trans phenolate oxygens and trans oxazoline or thiazoline ring nitrogens giving anti arrangements for the two ligands in each compound. The vanadium atoms in these complexes are elevated by approximately 0.6 Å from the basal square planes defined by the two pairs of ligand O and N donors. Of the four complexes, $VO(pic)_2 \cdot H_2O$, on the basis of solubility and stability, was selected as the most appropriate for in vivo testing. $VO(pic)_2 \cdot H_2O$ was administered in drinking water for 6 weeks to control−treated (CT) and streptozotocin-induced diabetic−treated (DT) male Wistar rats. Treatment in drinking water resulted in 38% of diabetic−treated animals responding with significant lowering of plasma glucose levels at an initial concentration of 1.52 mM; a subsequent increase to 2.28 mM resulted in 50% response. Lowering of plasma glucose in treated animals was not accompanied by any increase in plasma insulin levels. Intraperitoneal treatment with $VO(pic)_2 \cdot H_2O$ at an acute dose of 0.06 mmol kg^{-1} produced significant lowering of plasma glucose within 2 h of administration with a peak lowering effect at 8 h. Chronic intraperitoneal administration of $VO(pic)_2 \cdot H_2O$ at a dose of 0.03 mmol kg^{-1} d^{-1} for 3 weeks resulted in significant lowering of fasted plasma glucose without any increase in insulin output.

Introduction

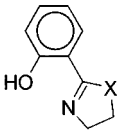
Although much effort has been expended studying the behavior and function of vanadium in naturally occurring systems, there is also considerable interest in the properties of synthetic vanadium compounds and their possible applications. The discovery of the *in vivo* insulin mimesis *per os* of oxovanadates(V)[1] and such oxovanadium(IV) complexes as vanadyl sulfate[2] and the much more potent bis(maltolato)-oxovanadium(IV) (BMOV)[3] stimulated the search for vanadium compounds which may have application in the treatment of type II diabetes.[4,5] Long-term treatment with BMOV effectively lowers glucose at significantly lower doses than vanadyl sulfate



BMOV
$VO(ma)_2$



Hpic



Hoz X=O
Hthoz X=S

† This paper is dedicated to the memory of our esteemed and beloved co-worker Ed Shuter, who passed away June 1998.

* To whom correspondence should be addressed, at the Department of Chemistry, UBC. Tel: (604)-822-4449. FAX: (604)-822-2847. E-mail: ORVIG@CHEM.UBC.CA.

(1) (a) Shechter, Y.; Karlish, S. J. D. *Nature* **1980**, *284*, 556. (b) Heyliger, C. E.; Tahiliani, A. G.; McNeill, J. H. *Science* **1985**, *227*, 1474.

(2) Ramanadham, S.; Mongold, J. J.; Brownsey, R. W.; Cros, G. H.; McNeill, J. H. *Am. J. Physiol.* **1989**, *257*, H904.

(3) McNeill, J. H.; Yuen, V. G.; Hoveyda, H. R.; Orvig, C. *J. Med. Chem.* **1992**, *35*, 1489.

10.1021/ic981231y CCC: $18.00
Published on Web 04/17/1999

without any overt toxic side effects.[6] Extending the search for more efficacious compounds, a study was undertaken of the coordination chemistry of vanadium with selected bidentate ligands containing mixed oxygen and nitrogen ligating moieties derived from naturally occurring motifs.

Found in humans as a metabolite of the amino acid tryptophan, Hpic (pyridine-2-carboxylic acid) forms a variety of coordination compounds with vanadium. Numerous solid-state structures have been elucidated, chiefly for the multiple oxoperoxovanadium(V)[7] and oxodiperoxovanadium(V) picolinates;[8] however, only one structure exemplifying the much less reactive dioxovanadium(V) picolinates, that of (hexamethylphosphodiamide)VO_2(pic),[7] has been determined to date. Since the first report of the oxovanadium(IV) complex $VO(pic)_2 \cdot H_2O$ in 1964,[9] there have been several studies of oxovanadium picolinate systems including the solution EPR of $VO(pic)_2 \cdot H_2O$,[10] potentiometric and spectrophotometric investigations of the oxovanadium(IV) ion with Hpic,[11] a combined ^{51}V and ^{1}H NMR study of the dioxovanadium(V) ion with Hpic,[12] as well as the autoxidation kinetics of hydrolyzed oxovanadium(IV) picolinates.[13] These have established the oxovanadium picolinate system as one possessing satisfactory hydrolytic stability as well as one in which interconversion between the oxovanadium(IV) and dioxovanadium(V) complexes is disfavored, a property desired in an insulin-mimetic drug. This is unlike the vanadium maltolate system which possesses superior hydrolytic stability[14] but also facile and spontaneous interconversions between the IV and V oxidation states with pH dependent autoxidations[15] and autoreductions[16] common. We therefore endeavored to examine $VO(pic)_2 \cdot H_2O$ as a potential orally active insulin mimetic agent. Although a study of the insulin mimetic activity of $VO(pic)_2 \cdot H_2O$ appeared while this work was in progress,[17] we present a study using significantly different dosing regimes from that of Sakurai and co-workers.

Hoz [2-(2′-hydroxyphenyl)-2-oxazoline] and Hthoz [2-(2′-hydroxyphenyl)-2-thiazoline] contain naturally occurring oxazoline and thiazoline binding motifs found in the microbial iron scavengers mycobactin and agrobactin (Hoz)[18] as well as in the iron chelator (*S*)-(−)-desferrithiocin (Thoz).[19] Hoz and Hthoz, as we have shown in studies of their group 13 complexes[20] as well as their oxotechnetium(V) and oxorhenium(V) complexes,[21] form hydrolytically resistant lipophilic complexes. Although the structure of a novel chiral oxovanadium(IV) oxazolinate has been very recently described,[22] the suitability of these and the related oxovanadium(IV) thiazolinates in the study of vanadium insulin mimesis has not been previously described.

Experimental Section

Materials and Methods. All chemicals were reagent grade and were used as received without further purification: $VOSO_4 \cdot 3H_2O$ (Aldrich), $NaOAc \cdot 3H_2O$ (Fisher), picolinic acid (Hpic) (Sigma). 2-(2′-Hydroxyphenyl)-2-oxazoline (Hoz)[23] and 2-(2′-hydroxyphenyl)-2-thiazoline (Hthoz)[24] were prepared according to literature methods. Water was deionized (Barnstead D8902 and D8904 cartridges) and distilled (Corning MP-1 Megapure still) before use. The yields are for analytically pure compounds and they are calculated based on vanadium.

Instrumentation. Infrared spectra were recorded as KBr disks in the range 4000−400 cm^{-1} on a Perkin-Elmer PE783 spectrophotometer and were referenced to polystyrene. Mass spectra were obtained with a Kratos Concept II H32Q (Cs^+, LSIMS) instrument or a Kratos MS50 (EIMS) instrument. Analyses for C, H, and N were performed in this department by Mr. Peter Borda. ^{51}V NMR spectra were recorded on a Varian XL-300 instrument and are referenced to external $VOCl_3$. The UV−vis spectra were recorded with a Shimadzu UV-2100 spectrophotometer. Room temperature (293.5 K) magnetic susceptibilities were measured on a Johnson Matthey magnetic susceptibility balance, using $Hg[Co(NCS)_4]$ as the susceptibility standard. Diamagnetic corrections were estimated by using Pascal's constants.

Syntheses of Vanadium Complexes. Bis(pyridine-2-carboxylato)-oxovanadium(IV) monohydrate, $VO(pic)_2 \cdot H_2O$. To 1.069 g (8.68 mmol) of picolinic acid in 20 mL of water was added 0.93 g (4.28 mmol) of $VOSO_4 \cdot 3H_2O$ in 20 mL of water. The pH was raised to 4.4 with dropwise additions of 2 M NaOH. The light blue material which precipitated was isolated by filtration, washed with methanol and ether, and dried in vacuo for a yield of 0.773 g (58% based on V). Anal. Calcd (found) for $C_{12}H_{10}N_2O_6V$: C, 43.79 (43.46); H, 3.06 (3.15); N, 8.51 (8.62). IR (cm^{-1}, KBr disk): 3400−3500 (ν_{OH}); 1640, 1630, 1600, 1570 ($\nu_{C=N}$ and $\nu_{C=C}$); 980, 970 ($\nu_{V=O}$). +LSIMS: $m/z = 157$ ($[VOL]^+$), 312 (M^+, $[HVOL_2]^+$). The solid-state magnetic moment was 1.75 μ_B. Solubility in aerobic water = 3 mM at 60 °C.

Ammonium Dioxobis(picolinato)vanadate(V) Dihydrate, $NH_4[VO_2(pic)_2] \cdot 2H_2O$. To a slurry of 0.80 g (6.84 mmol) of NH_4VO_3 in 5 mL of water was added 1.69 g (13.7 mmol) of picolinic acid in 3 mL of water to form a clear yellow solution that was stirred overnight and then stored at 4 °C for 2 days. Addition of 50 mL of acetone, with stirring, formed yellow crystals that were isolated by filtration and air-dried with a yield of 2.04 g (87% based on V). X-ray quality crystals were obtained by recrystallization of the product from water/acetone. Anal. Calcd (found) for $C_{12}H_{16}N_3O_8V$: C, 37.81 (38.44); H, 4.23 (4.16); N, 11.02 (11.13). IR (cm^{-1}, KBr disk): 3400−3500 (ν_{OH}); 1650, 1640, 1630, 1600 ($\nu_{C=N}$ and $\nu_{C=C}$); 920, 890 ($\nu_{V=O}$). −LSIMS: $m/z = 313$ ($[H_2VOL_2]^+$), 329 ($[H_2VO_2L_2]^-$), 346 ($M^- + 1$, ($H[NH_4][VO_2L_2]^-$). ^{51}V NMR (D_2O): −515, −554 ppm.

Bis[2-(2′-oxyphenyl)-2-oxazolinato]oxovanadium(IV), $VO(oz)_2$. To a solution of Hoz (0.31 g, 1.90 mmol) and $NaOAc \cdot 3H_2O$ (0.27 g, 1.95 mmol) in CH_3OH (10 mL) was added $VOSO_4 \cdot 3H_2O$ (0.20 g, 0.92 mmol) in 11 mL of CH_3OH:H_2O (10:1) solution. A gray-blue solid immediately precipitated and was collected by filtration. Recrystallization of this solid from CH_2Cl_2 yielded large blue crystals. The yield was 0.27 g (75% based on V). Anal. Calcd (found) for $C_{18}H_{16}N_2O_5V$:

(4) Schecter, Y.; Meyerovitch, J.; Farfel, Z.; Sack, J.; Bar-Meir, S.; Amir, S.; Degani, H.; Karlish, S. J. D. *Vanadium in Biological Systems*; Kluwer Academic Publishers: Norwell, 1990; p 129.
(5) Thompson, K. H.; McNeill, J. H.; Orvig, C. *Topics in Biological Chemistry Vol. 2: Metallopharmaceuticals*; Springer-Verlag: Heidelberg (in press).
(6) Yuen, V. G.; Orvig, C.; McNeill, J. H. *Can. J. Physiol. Pharmacol.* **1995**, 73, 55.
(7) Mimoun, H.; Suassine, L.; Daire, E.; Postel, M.; Fischer, J.; Weiss, R. *J. Am. Chem. Soc.* **1983**, *105*, 3101.
(8) Shaver, A.; Ng, J. B.; Hall, D. A.; Soo Lum, B.; Posner, B. I. *Inorg. Chem.* **1993**, *32*, 3109.
(9) Dutta, R. L.; Ghosh, S.; Lahiry, S. *Sci. Cult.* **1964**, *30*, 551.
(10) Wüthrich, K. *Helv. Chim. Acta* **1965**, *48*, 779.
(11) Paris, M.; Merlin, J.-C. *Bull. Soc. Chim. Fr.* **1962**, 800.
(12) Galeffi, B.; Tracey, A. *Inorg. Chem.* **1989**, *28*, 1726.
(13) Kaden, T. *Helv. Chim. Acta* **1966**, *227*, 1915.
(14) Caravan, P.; Gelmini, L.; Glover, N.; Herring, F. G.; Li, H,; McNeill, J. H.; Rettig, S. J.; Setyawati, I. A.; Shuter, E.; Sun, Y.; Tracey, A. S.; Yuen, V. G.; Orvig, C. *J. Am. Chem. Soc.* **1995**, *117*, 12759.
(15) Sun, Y.; James, B. R.; Rettig, S. J.; Orvig, C. *Inorg. Chem.* **1996**, *35*, 1667.
(16) Elvingson, K.; Baro, A. G.; Pettersson, L. *Inorg. Chem.* **1996**, *35*, 3388.
(17) Sakurai, H.; Fujii, K.; Watanabe, H.; Tamura, H. *Biochem. Biophys. Res. Commun.* **1995**, *214*, 1095.
(18) Eng-Wilmot, D. L.; van der Helm, D. *J. Am Chem. Soc.* **1980**, *102*, 7719.
(19) Anderegg, G.; Räber, M. *J. Chem. Soc. Chem. Commun.* **1990**, 1194.
(20) Hoveyda, H. R.; Karunaratne, V.; Rettig, S. J.; Orvig, C. *Inorg. Chem.* **1992**, *31*, 5408.
(21) Shuter, E.; Hoveyda, H. R.; Karuanaratne, V.; Rettig, S. J.; Orvig, C. *Inorg. Chem.* **1996**, *35*, 368.
(22) Bolm, C.; Luong, T. K. K.; Harms, K. *Chem. Ber. (Recueil)* **1997**, *130*, 887.
(23) Wilcox, B. E.; Heeg, M. J.; Deutsch, E. *Inorg. Chem.* **1984**, *23*, 2962.
(24) Wilcox, B. E.; Cooper, J. N.; Elder, R. C.; Deutsch, E. *Inorg. Chim. Acta* **1988**, *142*, 55.

Table 1. Selected Crystallographic Data

compound	$NH_4[VO_2(pic)_2]\cdot 2H_2O$	$VO(oz)_2$	$VO(thoz)_2$
formula	$C_{12}H_{16}N_3O_8V$	$C_{18}H_{16}N_2O_5V$	$C_{18}H_{16}N_2O_3S_2V$
fw	381.22	391.28	423.40
crystal system	monoclinic	triclinic	orthorhombic
space group	*Cc*	$P\bar{1}$	*Pbca*
a, Å	10.347(2)	10.408(1)	12.331(2)
b, Å	26.318(2)	11.282(1)	26.090(2)
c, Å	7.247(1)	7.666(1)	11.125(2)
α, deg	90	103.78(1)	90
β, deg	128.37(1)	109.64(1)	90
γ, deg	90	84.75(1)	90
V, Å^3	1547.3(5)	823.3(2)	3579.3(9)
Z	4	2	8
ρ_{calc}, g/cm^3	1.636	1.578	1.736
total reflections	4187	7576	4604
unique reflections	4025	7245	4604
R	0.034	0.034	0.035
R_w	0.031	0.035	0.030

C, 55.25 (55.12); H, 4.12 (4.17); N 7.16 (7.08). IR (cm^{-1}, KBr disk): 1620 ($\nu_{C=N}$); 1595 (w) ($\nu_{C=C}$); 990 ($\nu_{V=O}$). EIMS: m/z = 163 (L^+), 229 ([VOL]$^+$), 391 (M^+, [VOL_2]$^+$). The solid-state magnetic moment was 1.84 μ_B. UV/vis (CH_2Cl_2): λ_{max} (ϵ, M^{-1} cm^{-1}): 239 (63 700), 331 (12 000), 409sh (111), 543 (45), 597 (47).

Bis[2-(2′-oxyphenyl)-2-thiazolinato]oxovanadium(IV), $VO(thoz)_2\cdot 0.5H_2O$. The preparation was as for $VO(oz)_2$; $VOSO_4\cdot 3H_2O$ (0.20 g, 0.92 mmol), Hthoz (0.34 g, 1.90 mmol), and $NaOAc\cdot 3H_2O$ (0.27 g, 1.95 mmol) were employed. Recrystallization from CH_2Cl_2 yielded large yellow-green crystals. The yield was 0.32 g (82% based on V). Anal. Calcd (found) for $C_{18}H_{17}N_2O_{3.5}S_2V$: C, 50.00 (50.42); H, 3.98 (3.83); N 6.48 (6.47). IR (cm^{-1}, KBr disk): 1600 ($\nu_{C=N}$); 1570, 1540 ($\nu_{C=C}$); 980 ($\nu_{V=O}$). EIMS: m/z = 179 (L^+), 245 ([VOL]$^+$), 423 (M^+, [VOL_2]$^+$). The solid-state magnetic moment was 1.80 μ_B. UV/vis (CH_2Cl_2): λ_{max} (ϵ, M^{-1} cm^{-1}): 242 (56 500), 346 (11 200), 437sh (179), 537 (66), 595 (53).

X-ray Crystallographic Analyses of $NH_4[VO_2(pic)_2]\cdot 2H_2O$, $VO(oz)_2$ and $VO(thoz)_2$. Selected crystallographic data appear in Table 1. The final unit-cell parameters were obtained by least-squares on the setting angles for 25 reflections with 2θ = 25.0−32.1° for $NH_4[VO_2(pic)_2]\cdot 2H_2O$, 44.6−51.0° for $VO(oz)_2$, and 16.0−26.1° for $VO(thoz)_2$. The intensities of three standard reflections, measured every 200 reflections throughout the data collections, showed only small random fluctuations in each case. The data were processed[25] and corrected for Lorentz and polarization effects and absorption (empirical, based on azimuthal scans).

The structures of $NH_4[VO_2(pic)_2]\cdot 2H_2O$ and $VO(oz)_2$ were solved by direct methods and that of $VO(thoz)_2$ was solved by conventional heavy atom methods. The structure analysis of $VO(oz)_2$ was initiated in the centrosymmetric space group $P\bar{1}$, this choice being confirmed by subsequent calculations. All non-hydrogen atoms were refined with anisotropic thermal parameters. The O−H hydrogen atoms in $NH_4[VO_2(pic)_2]\cdot 2H_2O$ and all hydrogen atoms in $VO(oz)_2$ were refined with isotropic thermal parameters, and the ammonium N−H hydrogen atom positions in $NH_4[VO_2(pic)_2]\cdot 2H_2O$ were idealized from difference map positions but were not refined (N−H = 0.91 Å). The remaining hydrogen atoms in $NH_4[VO_2(pic)_2]\cdot 2H_2O$ and $VO(thoz)_2$ were fixed in calculated positions with C−H = 0.98 Å and $B_H = 1.2B_{bonded\ atom}$. Secondary extinction corrections were applied in each refinement, the final values of the extinction coefficients being 1.88(6) × 10^{-6} for $NH_4[VO_2(pic)_2]\cdot 2H_2O$, 5.0(14) × 10^{-8} for $VO(thoz)_2$, and 1.05(8) × 10^{-7} for $VO(oz)_2$. Neutral atom scattering factors and anomalous dispersion corrections were taken from the *International Tables for X-ray Crystallography*.[26] A parallel refinement of the structure of $NH_4[VO_2(pic)_2]\cdot 2H_2O$ having the opposite polarity gave significantly higher residuals, the *R* and R_w factor ratios being 1.074 and 1.071, respectively.

Selected bond lengths and bond angles for the three structures appear in Tables 2−4. Complete tables of crystallographic data, final atomic coordinates and equivalent isotropic thermal parameters, anisotropic thermal parameters, bond lengths, bond angles, hydrogen atom coordinates, torsion angles, intermolecular contacts, bond lengths and angles involving hydrogen bonding in $NH_4[VO_2(pic)_2]\cdot 2H_2O$ and least-squares planes are included as Supporting Information (see Supporting Information section at the end of this paper).

Table 2. Selected Bond Lengths (Å) and Angles (deg) in $NH_4[VO_2(pic)_2]\cdot 2H_2O$ with Estimated Standard Deviations in Parentheses

Bond Lengths			
V(1)−O(1)	1.989(2)	O(1)−C(6)	1.288(4)
V(1)−O(3)	2.125(3)	O(3)−C(12)	1.284(4)
V(1)−O(5)	1.637(2)	N(1)−C(1)	1.341(3)
V(1)−O(6)	1.638(2)	N(2)−C(7)	1.344(3)
V(1)−N(1)	2.314(2)	C(1)−C(6)	1.493(4)
V(1)−N(2)	2.126(2)	C(7)−C(12)	1.503(4)
Bond Angles			
O(1)−V(1)−O(3)	85.34(8)	O(5)−V(1)−N(2)	97.96(10)
O(1)−V(1)−O(5)	96.41(9)	O(6)−V(1)−N(1)	87.11(10)
O(1)−V(1)−O(6)	104.40(10)	O(6)−V(1)−N(2)	90.42(9)
O(1)−V(1)−N(1)	74.06(8)	N(1)−V(1)−N(2)	88.34(8)
O(1)−V(1)−N(2)	156.10(9)	V(1)−O(1)−C(6)	124.5(2)
O(3)−V(1)−O(5)	93.67(9)	V(1)−O(3)−C(12)	119.6(2)
O(3)−V(1)−O(6)	158.22(9)	V(1)−N(1)−C(1)	111.4(2)
O(3)−V(1)−N(1)	76.75(8)	V(1)−N(2)−C(7)	116.7(2)
O(3)−V(1)−N(2)	74.79(8)	O(1)−C(6)−C(1)	115.4(2)
O(5)−V(1)−O(6)	104.4(1)	N(2)−C(7)−C(12)	114.6(2)
O(5)−V(1)−N(1)	166.82(10)	O(3)−C(12)−C(7)	113.8(2)

Table 3. Selected Bond Lengths (Å) and Angles (deg) for $VO(oz)_2$ with Estimated Standard Deviations in Parentheses

Bond Lengths			
V(1)−O(2)	1.931(1)	O(3)−C(10)	1.346(2)
V(1)−O(4)	1.926(1)	O(3)−C(11)	1.458(2)
V(1)−O(5)	1.594(1)	O(4)−C(14)	1.319(2)
V(1)−N(1)	2.068(1)	N(1)−C(1)	1.284(2)
V(1)−N(2)	2.061(1)	N(1)−C(3)	1.472(2)
O(1)−C(1)	1.347(2)	N(2)−C(10)	1.287(2)
O(1)−C(2)	1.460(2)	N(2)−C(12)	1.472(2)
O(2)−C(5)	1.315(2)		
Bond Angles			
O(2)−V(1)−O(5)	108.41(5)	O(2)−V(1)−N(2)	85.25(5)
O(4)−V(1)−O(5)	108.81(5)	O(4)−V(1)−N(1)	85.23(5)
O(5)−V(1)−N(1)	104.73(6)	O(4)−V(1)−N(2)	86.21(5)
O(5)−V(1)−N(2)	103.79(5)	O(2)−V(1)−O(4)	142.79(5)
O(2)−V(1)−N(1)	85.28(5)	N(1)−V(1)−N(2)	151.47(5)

Table 4. Selected Bond Lengths (Å) and Angles (deg) for $VO(thoz)_2$ with Estimated Standard Deviations in Parentheses

Bond Lengths			
V(1)−O(2)	1.903(3)	S(2)−C(10)	1.761(4)
V(1)−O(4)	1.900(3)	S(2)−C(11)	1.788(5)
V(1)−O(5)	1.591(3)	O(4)−C(14)	1.328(4)
V(1)−N(1)	2.083(3)	N(1)−C(1)	1.295(5)
V(1)−N(2)	2.083(3)	N(1)−C(3)	1.479(5)
S(1)−C(1)	1.757(4)	N(2)−C(10)	1.291(4)
S(1)−C(2)	1.790(5)	N(2)−C(12)	1.474(5)
O(2)−C(5)	1.330(4)		
Bond Angles			
O(2)−V(1)−O(5)	115.2(1)	O(2)−V(1)−N(2)	86.4(1)
O(4)−V(1)−O(5)	114.9(1)	O(4)−V(1)−N(1)	85.5(1)
O(5)−V(1)−N(1)	99.1(1)	O(4)−V(1)−N(2)	85.7(1)
O(5)−V(1)−N(2)	99.4(1)	O(2)−V(1)−O(4)	129.9(1)
O(2)−V(1)−N(1)	86.8(1)	N(1)−V(1)−N(2)	161.4(1)

Biological Testing. Male Wistar rats (Animal Care Unit, University of British Columbia) weighing between 190 and 220 g were randomly

(25) *teXan*: Structure Analysis Package; Molecular Structure Corp.: The Woodlands, TX, 1985 & 1992.

(26) (a) *International Tables for X-ray Crystallography*; Kynoch Press: Birmingham, UK (present distributor Kluwer Academic Publishers: Boston, MA), 1974; Vol. IV, pp 99−102. (b) *International Tables for Crystallography*; Kluwer Academic Publishers: Boston, MA. 1992; Vol. C, pp 200−206.

divided into four groups: control (C), control−treated (CT), diabetic (D) and diabetic−treated (DT). Animals were lightly anesthetized with halothane and injected (via tail vein) with either NaCl, 9 g L^{-1}, (C and CT) or streptozotocin (STZ) 0.06 g kg^{-1} in NaCl, 9 g L^{-1} (D and DT). Animals were housed one or two per cage on a 12 h light:dark schedule. Food and fluid were freely available.[27] Only rats with blood glucose > 13 mM (Ames glucometer II, Miles Inc., Elkhart, IN, and Ames Glucostix, Miles Canada Inc., Etobicoke, Ontario, Canada) at 3 days after injection were accepted as diabetic. Treatments were initiated 7 days after injection and consisted of $VO(pic)_2 \cdot H_2O$ (freshly prepared on alternate days) at the concentrations given below. Tail vein blood samples were collected in heparinized capillary tubes and centrifuged (10000*g* × 15 min), red blood cells were discarded, and plasma was stored at −70 °C until analyzed. All metabolites were assayed in plasma. Glucose, triglyceride, and cholesterol were determined by kits (Boehringer Mannheim, Mannheim, West Germany); insulin was determined by RIA (Linco Research, Inc., St. Louis, MO). In all experiments, euglycemia was taken as glucose < 9 mM.

The testing protocol consisted of four methods, as follows.

Acute Oral. $VO(pic)_2 \cdot H_2O$ (0.55 mmol kg^{-1} the ED_{50} dose[28] for BMOV)[6] was given as a suspension in 3% gum arabic (Sigma) by mouth (oral gavage) to 11 DT rats. Blood was collected for glucose determination immediately prior to and at 1, 2, 4, 6, 8, 12, 24, 48, and 72 h following drug administration.

Chronic Oral. $VO(pic)_2 \cdot H_2O$ (0.50 g L^{-1} for 1 week, increased to 0.75 g L^{-1} for the next 5 weeks) was included in the drinking water of CT ($n = 8$) and DT ($n = 6$). Groups C ($n = 8$) and D ($n = 6$) received water only. Body weight and food and fluid consumption were measured daily. Blood was collected weekly for glucose, insulin, triglyceride, and cholesterol determinations.

Acute Intraperitoneal (ip) Injection. $VO(pic)_2 \cdot H_2O$ (0.06 mmol kg^{-1}, in a volume of 1 μL g^{-1} body weight) was administered ip to CT ($n = 8$) and DT ($n = 8$). C ($n = 8$) and D ($n = 8$) received an equivalent volume of saline. Blood was collected for glucose measurements immediately prior to and at 2, 4, 6, 8, 12, and 24 h following drug administration.

Chronic (ip) Injection. $VO(pic)_2 \cdot H_2O$ [0.03 mmol kg^{-1}, in a volume of 1 μL g^{-1} body weight (measured daily)] was administered ip to CT and DT ($n = 8$ for each) for 3 weeks. Food intake and fluid consumption were measured weekly. Blood was collected weekly for glucose, insulin, triglyceride, and cholesterol measurements.

Statistics. Values are presented as means (SEM). In the chronic studies, data were analyzed by two-way ANOVA. Data from the acute experiments were analyzed using GLM Repeated Measures ANOVA. The Student−Newman−Keuls test was applied following ANOVA analysis ($p < 0.05$), where appropriate.

Results and Discussion

Two oxovanadium complexes containing the picolinate anion were synthesized: $VO(pic)_2 \cdot H_2O$ and $NH_4[VO_2(pic)_2] \cdot 2H_2O$. Though single crystals proved elusive for $VO(pic)_2 \cdot H_2O$, the elemental analysis corresponds well to that previously reported for this compound by Sakurai and co-workers.[17] The solid-state structure of $NH_4[VO_2(pic)_2] \cdot 2H_2O$ was established by single crystal X-ray diffraction studies at 21 °C (Figure 1). The X-ray structure, along with the elemental analysis, unambiguously establishes the identity of $NH_4[VO_2(pic)_2] \cdot 2H_2O$. Single crystals of $NH_4[VO_2(pic)_2] \cdot 2H_2O$ were grown by slow evaporation from saturated acetone solutions, with relevant bond lengths and angles shown in Table 2. The $[VO_2(pic)_2]^-$ anion consists of the ubiquitous *cis*-VO_2^+ center [V(1)−O(5) = 1.637(2) Å and V(1)−O(6) = 1.632(2) Å, with a O(5)−V(1)−O(6) angle of 104.3(1)°] and two picolinate ligands bound at an interplanar angle of 97.25°. The two ligands possess distinctly different bond lengths, originating from the strong trans effect of the oxo

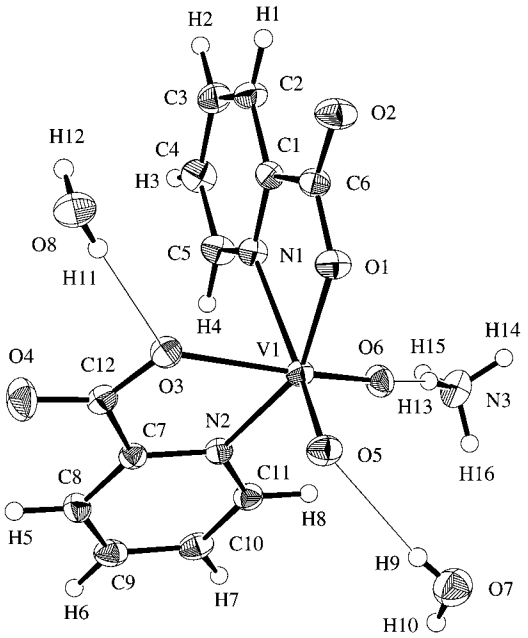


Figure 1. ORTEP diagram of $NH_4[VO_2(pic)_2] \cdot 2H_2O$ showing the crystallographic numbering. Thermal ellipsoids for the non-hydrogen atoms are drawn at the 33% probability level.

ligands, with the N bound [V(1)−N(1) = 2.314(2) Å and V(1)−O(1) = 1.989(2) Å] ligand trans to oxo O(5) possessing a significantly longer V−O distance and a significantly shorter V−N distance than the ligand O bound [V(1)−N(2) = 2.126(2) Å and V(1)−O(3) = 2.125(3) Å] trans to the remaining oxo O(6). Extensive intramolecular hydrogen bonding is seen in $NH_4[VO_2(pic)_2] \cdot 2H_2O$, with oxo O(6) hydrogen bonding with an ammonium ion and oxo O(5) hydrogen bonded to a water molecule. As well, one ligand hydrogen bonds through the electron deficient carboxylate trans to oxo O(6) to an additional water molecule. The ^{51}V NMR spectrum of $NH_4[VO_2(pic)_2] \cdot 2H_2O$ (0.1 M in H_2O) shows a peak at −545 ppm (attributable to free vanadate), as well as major resonances at −515 and −554 ppm attributed to $[VO_2(pic)_2]^-$ by Galeffi and Tracey,[29] which both confirms the identity of the complex and indicates that $[VO_2(pic)_2]^-$ predominates only when concentrated in solution at intermediate pH.

$VO(oz)_2$ and $VO(thoz)_2$ were synthesized simply from the addition of ligand, in a basic water/methanol solvent mixture to vanadyl sulfate. The solid-state structures of $VO(oz)_2$ and $VO(thoz)_2$ were established by single-crystal X-ray diffraction studies at 21 °C with crystals grown by slow evaporation from saturated CH_2Cl_2 solutions. These two complexes are isostructural, possessing the common [for oxovanadium(IV) complexes] distorted square pyramidal geometry with trans-coordinated bidentate ligands (Figures 2 and 3). The ligands are bound through the phenolate oxygen and oxazoline or thiazoline ring nitrogens with displacement of the vanadium from the plane defined by the ligand donor atoms. These displacements, 0.57 Å for $VO(oz)_2$ and 0.60 Å for $VO(thoz)_2$, as well as the V═O bond lengths in $VO(oz)_2$ and $VO(thoz)_2$, 1.594(1) Å and 1.591(3) Å, respectively, are typical for oxovanadium(IV) complexes. Within the six-membered chelate ring formed by the coordination of the phenolate oxygen and the ring nitrogen to the vanadium center, the average V−O bond lengths are 1.928(2) and 1.901(5) Å, and the average V−N bond lengths, 2.065(2) and 2.083(5) Å, respectively, for $VO(oz)_2$ and

(27) For determination of dose, animals were housed singly.

(28) That dose which alleviates symptomatology (in this case, diabetic) for 50% of rats tested.

(29) Galeffi, B.; Tracey, A. *Inorg. Chem.* **1989**, *28*, 1726.

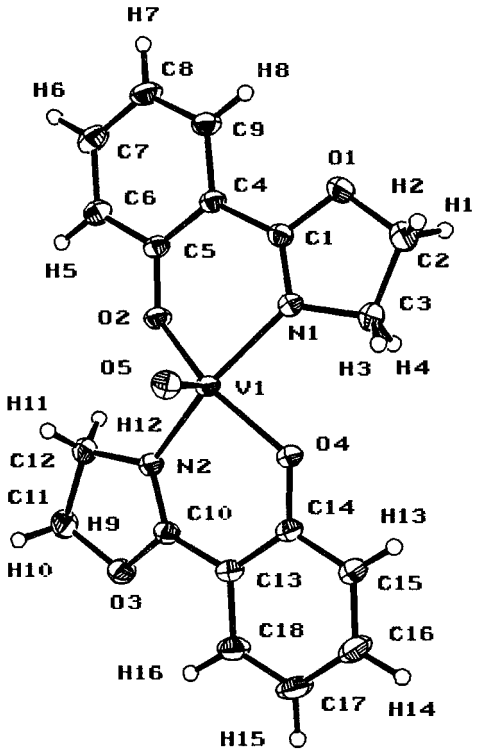


Figure 2. ORTEP diagram of $VO(oz)_2$ showing the crystallographic numbering. Thermal ellipsoids for the non-hydrogen atoms are drawn at the 33% probability level.

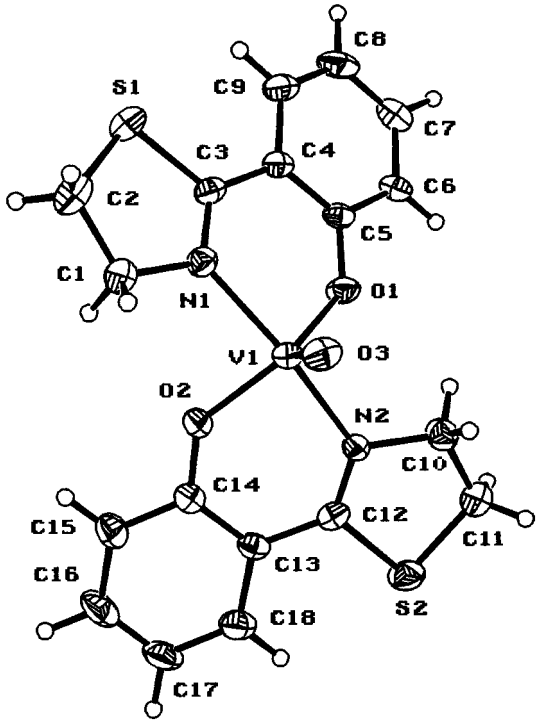


Figure 3. ORTEP diagram of $VO(thoz)_2$ showing the crystallographic numbering. Thermal ellipsoids for the non-hydrogen atoms are drawn at the 33% probability level.

$VO(thoz)_2$. The effect of sulfur substitution is seen as a significantly higher average V−O bond length and a significantly lower average V−N bond length. The V−O and V−N bond lengths of $VO(oz)_2$ and $VO(thoz)_2$ fall in the range of V−O and V−N bond lengths observed for many oxovanadium(IV) Schiff base complexes.[30] The V−O, V−N, and V═O bond lengths of $VO(oz)_2$ are identical to the (*S*,*S*)-isomer of a recently reported chiral bis(2-ethylthiazolinato)oxovanadium(IV) compound.[22] Unlike $VO(pic)_2 \cdot H_2O$, which possesses some water solubility, and $NH_4[VO_2(pic)_2] \cdot 2H_2O$, which is completely water soluble, $VO(oz)_2$ and $VO(thoz)_2$ are completely water insoluble; biological studies were precluded.

Biological Testing. All administration methods of $VO(pic)_2 \cdot H_2O$ lowered plasma glucose in diabetic rats. At the concentrations tested, none resulted in consistent declines to normal glucose ranges. Details follow, in the order listed under the experimental methods.

Acute Oral. Glucose in DT compared to D was significantly reduced by 4 h after oral gavage. At 12 h, 8 of 11 (73%) animals were euglycemic (plasma glucose: at time 0 h = 20.3 ± 0.6, at time 12 h = 7.3 ± 0.1 mM). Those that responded had diarrhea, which resolved within 48 h. At that time, seven animals remained euglycemic, declining to two (of the original 8) by 72 h.

Chronic Oral. Glucose in DT was 22% lower than in D at 6 weeks, but still significantly elevated compared to C and CT (C = 7.2 ± 0.1, CT = 6.7 ± 0.1, D = 18.3 ± 0.6, and DT = 14.2 ± 2.0 mM). Fluid and food intakes in DT were not different from C and CT. As is common in STZ-induced diabetes, D had much higher intakes than controls. The calculated oral dose of $VO(pic)_2 \cdot H_2O$ received by DT (0.48 ± 0.8 mmol kg^{-1} d^{-1}) was higher than that calculated for BMOV treatment (0.37 ± 0.3 mmol kg^{-1} d^{-1}).[3] Cholesterol and triglycerides remained within normal ranges for all animals. Insulin was 50% lower in CT compared to C (C = 4.5 ± 0.6, CT = 2.2 ± 0.2 μg L^{-1}); but was unchanged in DT compared to D (D = 2.6 ± 0.9, DT = 2.3 ± 0.4 μg L^{-1}).

Acute Intraperitoneal. $VO(pic)_2 \cdot H_2O$ lowered glucose in DT within 2 h (D = 17.8 ± 0.4; DT = 12.0 ± 0.8 mM). Two of eight DT became euglycemic by 8 h following ip administration; however, all DT reverted to hyperglycemia within 24 h of treatment.

Chronic Intraperitoneal. In the longer-term administration, $VO(pic)_2 \cdot H_2O$ lowered plasma glucose in DT compared to D (C = 7.1 ± 0.1, CT = 7.0 ± 0.1, D = 20.2 ± 0.2, and DT = 16.4 ± 0.6 mM), but only one of eight DT became euglycemic within the 3 week treatment period. Insulin, cholesterol, and triglycerides were not affected, and there was no reduction in food intake in CT or DT compared to their respective controls.

Thus, $VO(pic)_2 \cdot H_2O$, although shown to be an orally active insulin mimetic agent, did not lead to a majority of diabetic rats tested becoming euglycemic by any of the methods outlined. The calculated dose of $VO(pic)_2 \cdot H_2O$ self-administered by DT exceeded that of our "benchmark" compound, BMOV, by at least 50%, with less consistent glucose-lowering results. Rapid onset of glucose lowering in the short-term trials (at an ED_{50} dose for BMOV) resulted in gastrointestinal upset (which was seen only rarely with BMOV). $VO(pic)_2 \cdot H_2O$, as is common with other vanadium compounds, did not increase insulin levels nor depress food intake, compared to controls.

A recent study[17] provides additional evidence for the oral effectiveness of $VO(pic)_2 \cdot H_2O$ and also suggests that a lower dose (0.2 mmol kg^{-1} d^{-1} compared to 1.0 mmol kg^{-1} d^{-1}, in this study) for a longer testing period[31] may lessen the likelihood

(30) Carrano, C. J.; Nunn, C. M.; Quan, R.; Bonadies, J. A.; Pecoraro, V. L. *Inorg. Chem.* **1990**, *29*, 944.

(31) It is worth noting that these two studies are not strictly comparable, in that daily gavage dosing on an empty stomach[17] would preclude mitigating effects of food, which dosing in the drinking water over a longer period does not eliminate. Percent absorption of compound by these two methods may vary considerably.

of gastrointestinal distress and maximize the glucose-lowering potential of this compound.

An important feature of synthetic vanadium-containing insulin-mimetic compounds is their varying patterns of tissue uptake. The organ distribution of $VO(pic)_2 \cdot H_2O$ indicates a preferential accumulation in (total) bone vs kidney that is twice that seen with BMOV (9 times higher on a μg of V per g of rat basis).[32] The ratio of vanadium in kidney vs liver was also far greater for $VO(pic)_2 \cdot H_2O$ than for BMOV (7.4:1.0 compared to 1.4:1.0), which may be less suitable for an insulin-mimetic (in that much of insulin's effects take place in the liver). Overall levels of vanadium 110 days following cessation of oral $VO(pic)_2 \cdot H_2O$ administration[17] suggest that this compound may be cleared more slowly than BMOV and far more slowly than vanadyl sulfate.[32] Chromium picolinate also appears to result in long-term chromium retention, with uncertain biological ramifications.[33]

Another feature of relevance to vanadium's insulin mimesis is the lack of stimulation of endogenous insulin production. In our hands, no increase in insulin levels was observed with any of the test protocols for $VO(pic)_2 \cdot H_2O$. Fujimoto observed 3-fold-increased insulin levels in rats treated by oral gavage with $VO(pic)_2 \cdot H_2O$.[34] This apparent discrepancy suggests a difference in uptake resulting from gavage to fasted rats, as opposed to administration in the drinking water accompanied by ad libitum food intake. A lower concentration of the vanadium complex in the gastrointestinal tract, combined with rapid and progressive pH change accompanying normal food digestion, clearly affects the overall absorption and eventual disposition of the ingested vanadium complex. Studies that specifically address these concerns are indicated.

Insofar as the mechanism of vanadium's insulin mimesis may involve formation of a phosphotyrosine phosphatase (PTPase) transition state analogue in a reversible fashion,[35,36] the $VO(O_4)$ complex could be expected to perform better than the $VO(N_2O_2)$ coordination mode proposed here. Nonetheless, the $VO(N_2O_2)$ complex with fewer ligand O atoms to hydrogen bond with water would be expected *a priori* to have the potential advantage of greater lipophilicity, with associated increased cell membrane permeability. Assessing the relative in vivo importance of lipophilic/hydrophilic balance and the capacity to serve as a phosphate analogue can only be assessed experimentally, preferably in a standardized *in vivo* model.

Conclusions

Vanadium complexes based on naturally occurring binding motifs, $VO(pic)_2 \cdot H_2O$, $NH_4[VO_2(pic)_2] \cdot 2H_2O$, $VO(oz)_2$, and $VO(thoz)_2$, were synthesized. Of these, $VO(pic)_2 \cdot H_2O$ was chosen for in vivo testing and was found to have modest glucose-lowering activity, without accompanying plasma insulin elevation or food intake suppression, in experimentally diabetic rodents. Rapid (acute) glucose lowering was accompanied by gastrointestinal upset, a side-effect which may be avoidable with more appropriate ligand choices. It is worth noting that the differences in tissue distribution and retention of $VO(pic)_2 \cdot H_2O$ compared to BMOV may result in substantially different availability in insulin target organs (e.g. liver) for a similar calculated dose of vanadium and that this may influence the range of responsiveness to this compound.

Acknowledgment. The authors gratefully acknowledge the Medical Research Council of Canada for operating funds and the Natural Sciences and Engineering Research Council for a graduate fellowship (M.M.).

Supporting Information Available: Complete tables of crystallographic data, final atomic coordinates and equivalent isotropic thermal parameters, anisotropic thermal parameters, bond lengths, bond angles, hydrogen atom coordinates, torsion angles, intermolecular contacts, bond lengths and angles involving hydrogen bonding in $NH_4[VO_2(pic)_2] \cdot 2H_2O$, and least-squares planes. This material is available free of charge via the Internet at http://pubs.acs.org.

IC981231Y

(32) Setyawati, I. A.; Thompson, K. H.; Yuen, V. G.; Sun, Y.; Battell, M.; Lyster, D. M.; Vo, C.; Ruth, T. R.; Ziesler, S., McNeill, J. H., Orvig, C. *J. Appl. Physiol.* **1998**, *84*, 569.

(33) Stearns, D. M.; Belbruno, J. J.; Wetterkahn, K. E. *FASEB J.* **1995**, *9*, 1650.

(34) Fujimoto, S.; Fujii, K.; Yasui, H.; Matsushita, R.; Takada, J.; Sakurai, H. *J. Clin. Biochem. Nutr.* **1997**, *23*, 113.

(35) Li, J.; Elberg, G.; Crans, D. C.; Shechter, Y. *Biochemistry* **1996**, *35*, 8314.

(36) Zhang, M.; Zhou, M.; Van Etten, R. L., Stauffacher, C. V. *Biochemistry* **1997**, *36*, 15.