Chemistry and Insulin-Mimetic Properties of Bis(acetylacetonate)oxovanadium(IV) and Derivatives[1]

Sean S. Amin,[†] Kirk Cryer,[†] Boyan Zhang,[†] Subodh K. Dutta,[†] Sandra S. Eaton,[‡] Oren P. Anderson,[†] Susie M. Miller,[†] Benedicte A. Reul,[§] Sonia M. Brichard,[§] and Debbie C. Crans*[,†]

Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523-1872, Department of Chemistry and Biochemistry, University of Denver, Denver, Colorado 80208, and Department of Pharmacology, University of Louvain, Belgium

Received May 21, 1999

The syntheses and the solid state structural and spectroscopic solution characterizations of VO(Me-acac)$_2$ and VO(Et-acac)$_2$ (where Me-acac is 3-methyl-2,4-pentanedionato and Et-acac is 3-ethyl-2,4-pentanedionato) have been conducted since both VO(acac)$_2$ and VO(Et-acac)$_2$ have long-term *in vivo* insulin-mimetic effects in streptozotocin-induced diabetic Wistar rats. X-ray structural characterizations of VO(Me-acac)$_2$ and VO(Et-acac)$_2$ show that both contain five-coordinate vanadium similar to the parent VO(acac)$_2$. The unit cells for VO(Et-acac)$_2$ and VO(Me-acac)$_2$ are both triclinic, $P\bar{1}$, with $a = 9.29970(10)$ Å, $b = 13.6117(2)$ Å, $c = 13.6642(2)$ Å, $\alpha = 94.1770(10)°$, $\beta = 106.4770(10)°$, $\gamma = 106.6350(10)°$ for VO(Et-acac)$_2$ and $a = 7.72969(4)$ Å, $b = 8.1856(5)$ Å, $c = 11.9029(6)$ Å, $\alpha = 79.927(2)°$, $\beta = 73.988(2)°$, $\gamma = 65.1790(10)°$ for VO(Me-acac)$_2$. The total concentration of EPR-observable vanadium(IV) species for VO(acac)$_2$ and derivatives in water solution at 20 °C was determined by double integration of the EPR spectra and apportioned between individual species on the basis of computer simulations of the spectra. Three species were observed, and the concentrations were found to be time, pH, temperature, and salt dependent. The three complexes are assigned as the *trans*-VO(acac)$_2$·H$_2$O adduct, *cis*-VO(acac)$_2$·H$_2$O adduct, and a hydrolysis product containing one vanadium atom and one R-acac$^-$ group. The reaction rate for conversion of species was slower for VO(acac)$_2$ than for VO(malto)$_2$, VO(Et-acac)$_2$, and VO(Me-acac)$_2$; however, in aqueous solution the rates for all of these species are slow compared to those of other vanadium species. The concentration of vanadium(V) species was determined by ^{51}V NMR. The visible spectra were time dependent, consistent with the changes in species concentrations that were observed in the EPR and NMR spectra. EPR and visible spectroscopic studies of solutions prepared as for administration to diabetic rats documented both a salt effect on speciation and formation of a new halogen-containing complex. Compound efficacy with respect to long-term lowering of plasma glucose levels in diabetic rats traces the concentration of the hydrolysis product in the administration solution.

Introduction

Vanadium salts have potent insulin-mimetic effects[2−4] and recently several studies have been conducted with these simple salts in humans.[5−7] Peroxovanadium complexes[8−12] and bis(maltolato)oxovanadium(IV)[13,14] (VO(malto)$_2$) have been found to be more effective than the simple vanadium(IV) and -(V) salts, both in cell cultures and in animal studies. Furthermore, a bis(maltolato)oxovanadium(IV) derivative (KP-102) has now entered phase I clinical trials in humans.[15] A related compound, bis(2,4-pentanedionato-*O*,*O*′)oxovanadium(IV) (VO(acac)$_2$) also was found to have insulin-mimetic properties superior to those of $VOSO_4$ in cell culture studies.[16] Recently, a comparative study was carried out with $VOSO_4$ and three vanadium coordination complexes ((VO(acac)$_2$), vanadyl 3-ethylacetylacetonate (VO(Et-acac)$_2$), and bis(maltolato)oxovanadium(IV)

[†] Colorado State University.
[‡] University of Denver.
[§] University of Louvain.

(1) Abbreviations: acac = 2,4-pentanedionato; Me-acac = 3-methyl-2,4-pentanedionato; Et-acac = 3-ethyl-2,4-pentanedionato; pb = 1-phenyl-1,3-butanedionate; acen = *N*,*N*′-ethylenebis(acetylacetoneiminato); R-acac = 3-alkyl-2,4-pentanedionato.

(2) Shechter, Y.; Karlish, S. J. D. *Nature* **1980**, *284*, 556−558.
(3) Heyliger, C. E.; Tahiliani, A. G.; McNeill, J. H. *Science* **1985**, *227*, 1474−1477.
(4) Srivastava, A. K.; Chiasson, J.-L. *Mol. Cell. Biochem.* **1995**, *153*.
(5) Cohen, N.; Halberstam, M.; Shlimovich, P.; Chang, C. J.; Shamoon, H.; Rossetti, L. *J. Clin. Invest.* **1995**, *95*, 2501−2509.
(6) Goldfine, A. B.; Simonson, D. C.; Folli, F.; Patti, M.-E.; Kahn, C. R. *Mol. Cell. Biochem.* **1995**, *153*, 217−231.
(7) Goldfine, A. B.; Simonson, D. C.; Folli, F.; Patti, M.-E.; Kahn, C. R. *J. Clin. Endocrinol. Metab.* **1995**, *80*, 3311−3320.
(8) Posner, B. I.; Shaver, A.; Fantus, I. G. In *New Antidiabetic Drugs*; Bailey, C. J., Flatt, P. R., Eds.; Smith-Gordon: London, 1990; Chapter 8, pp 107−118.
(9) Posner, B. I.; Faure, R.; Burgess, J. W.; Bevan, A. P.; Lachance, D.; Zhang-Sun, G.; Fantus, I. G.; Ng, J. B.; Hall, D. A.; Lum, B. S.; Shaver, A. *J. Biol. Chem.* **1994**, *269*, 4596−4604.
(10) Shaver, A.; Hall, D. A.; Ng, J. B.; Lebuis, A.-M.; Hynes, R. C.; Posner, B. I. *Inorg. Chim. Acta* **1995**, *229*, 253−260.
(11) Shaver, A.; Ng, J. B.; Hall, D. A.; Soo Lum, B.; Posner, B. I. *Inorg. Chem.* **1993**, *32*, 3109−3113.
(12) Kadota, S.; Fantus, I. G.; Deragon, G.; Guyda, H. J.; Hersh, B.; Posner, B. I. *Biochem. Biophys. Res. Commun.* **1987**, *147*, 259−266.
(13) McNeill, J. H.; Yuen, V. G.; Hoveyda, H. R.; Orvig, C. *J. Med. Chem.* **1992**, *35*, 1489−1491.
(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*, 12759−12770.
(15) Kinetek Pharmaceutical, Inc., Press Release Dec 18, 1998, via NewsEdge Corporation.
(16) Li, J.; Elberg, G.; Crans, D. C.; Shechter, Y. *Biochemistry* **1996**, *35*, 8314−8318.

10.1021/ic9905897 CCC: $19.00
Published on Web 01/08/2000

($VO(malto)_2$)) in nonketotic, insulin-deficient diabetic rats.[17] Oral administration of the three vanadium complexes induced a faster and greater lowering in glycemia than $VOSO_4$, and the effects persisted for up to 3 months without significant toxicity. Since $VO(acac)_2$ and $VO(Et\text{-}acac)_2$ both were more effective than $VO(malto)_2$ in these respects, Hacac-derived vanadium complexes represent a new and interesting group of insulin-mimetic compounds.[17] Importantly, this study provided a direct comparison of the performance of four vanadium compounds. Here we report the synthesis of one of the $VO(R\text{-}acac)_2$ compounds, the X-ray structures and solid state characterization of two derivatives, the aqueous solution chemistry of $VO(acac)_2$ and derivatives, and the speciation of the four vanadium compounds that were investigated *in vivo*: $VO(acac)_2$, $VO(Et\text{-}acac)_2$, $VO(malto)_2$, and $VOSO_4$, including solutions similar to the administration liquid.

Since the first published account of the synthesis of $VO(acac)_2$ in 1914,[18] the complex has been used extensively as a reagent in organic synthesis.[19−21] The physical properties of $VO(acac)_2$ have been examined by numerous workers,[22−24] and the crystal structure was published in 1962.[25] A variety of derivatives in which the terminal methyl groups in the $acac^-$ ligand were substituted have been prepared and studied.[26,27] Such substitutions include both symmetric and asymmetric modifications of the parent $acac^-$ ligand and formation of the corresponding $VO(acac)_2$-type complexes.[18,28] In the solid state, the complexes are five-coordinate; however, upon dissolution in organic solvents, the vanadium coordinates a donor ligand in the vacant site, generating products expressed as $[VO(acac)_2L]$ (where L = coordinated ligand and acac is 2,4-pentanedionato or corresponding $acac^-$ derivatives). Previously, many spectroscopic methods were employed to determine the coordination number,[22,23,29−32] the geometry of the complex, and whether the incoming ligand was trans or cis to the oxo group.[33−36] Although the literature is not in complete agreement,[37] the consensus strongly favors[35,36,38,39] that the incoming ligand coordinates *trans* to the oxo group in the case of neutral oxygen donors (see Figure 1 for illustration of *trans*-$VO(acac)_2{\cdot}H_2O$ and *cis*-$VO(acac)_2{\cdot}H_2O$ adducts). Despite many studies exploring the geometry of the $VO(acac)_2L$ adducts, no information currently is available on the chemistry of the adduct complex or its five-coordinate parent in aqueous solution.

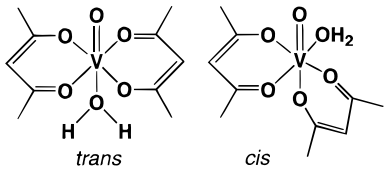


Figure 1. Structural representation of the *trans*- and *cis*-$VO(acac)_2{\cdot}H_2O$ adducts.

The need for understanding of the biologically relevant aqueous chemistry of $VO(R\text{-}acac)_2$ derivatives is underscored by the ongoing phase I clinical trial of KP-102[15] and the recent demonstration that $VO(R\text{-}acac)_2$ derivatives may be superior to $VO(malto)_2$ as insulin-mimetic agents.[17] This paper describes the synthesis and the solid state and solution characterization for water-soluble $VO(R\text{-}acac)_2$ complexes in which the R group on the center methylene carbon atom is H, Me, or Et, in distilled water and in solutions similar to the administration solutions for the diabetic rat studies.

Experimental Section

A. Materials and Methods. All chemicals obtained from Aldrich were of reagent grade and were used without further purification. $VO(malto)_2$ was prepared as previously described.[14] IR spectra were recorded on a Perkin-Elmer 1600 FT-IR spectrometer in KBr pellets. Microanalyses were performed by Desert Analytics, Tucson, AZ.

B. Syntheses. [VO(Et-acac)$_2$]. Vanadyl sulfate trihydrate (3.80 g, 17.5 mmol) was dissolved in 10 mL of 10% H_2SO_4. Then, 3-ethyl-2,4-pentanedione (5.00 g, 38.2 mmol) was added, and the resulting blue solution was pH adjusted with 10% sodium bicarbonate solution to pH 4. The solution was kept at 4 °C overnight, and the blue-green precipitate was collected by filtration. The isolated solid contained green product, brown precipitates of vanadyl hydroxides, and residual amounts of sodium bicarbonate. This material was purified by dissolving the $VO(Et\text{-}acac)_2$ in chloroform, as neither the vanadyl hydroxides nor sodium bicarbonate is soluble in this solvent. The particulate matter was removed by filtration through a sintered glass funnel. The solid compound was obtained by evaporation of the solvent to dryness. Yield: 3.25 g, 58% (based on $VOSO_4$). Anal. Found: C, 52.01; H, 6.81; V, 16.27. $C_{14}H_{22}O_5V$ requires C, 52.29; H, 6.85; V, 15.86. λ_{max}/nm (50 mM aqueous HEt-acac, species A): 820, 575 (ϵ/dm^3 mol^{-1} cm^{-1}: 200, 90). IR (KBr, cm^{-1}): 2963, 2884, 1560, 1467, 1368, 1332, 1295, 1260, 1174, 1066, 1000, 958, 916, 790, 723, 686, 618.

[VO(Me-acac)$_2$]. Vanadyl sulfate trihydrate (7.60 g, 35.0 mmol) was dissolved in 20 mL of 10% H_2SO_4. 3-Methyl-2,4-pentanedione (9.98 g, 74.3 mmol) then was added, and the resulting blue solution was pH adjusted with 10% sodium bicarbonate solution to pH 4. A blue-green precipitate began to form immediately and was collected by filtration. The precipitate was washed with distilled water and dried. Yields before dissolution in $CHCl_3$ were 75−85%. This material was purified as described above for the $VO(Et\text{-}acac)_2$ compound. Yield: 6.66 g, 65% (based on $VOSO_4$). Anal. Found: C, 48.45; H, 6.14; V, 16.94. $C_{12}H_{18}O_5V$ requires C, 48.77; H, 6.10; V, 17.25. λ_{max}/nm (50 mM aqueous HMe-acac, species A): 830, 565 (ϵ/dm^3 mol^{-1} cm^{-1}: 200, 60). IR (KBr, cm^{-1}): 3015, 2924, 1558, 1481, 1423, 1334, 1299, 1176, 998, 976, 898, 732, 660, 618.

(17) Reul, B. A.; Amin, S. S.; Buchet, J. P.; Ongemba, L. N.; Crans, D. C.; Brichard, S. M. *Br. J. Pharmacol.* **1999**, *126*, 467−477.
(18) Morgan, G. T.; Moss, H. W. *J. Chem. Soc.* **1914**, *103*, 78−90.
(19) Kaneda, K.; Jitsukawa, K.; Itoh, T.; Teranishi, S. *J. Org. Chem.* **1980**, *45*, 3004−3009.
(20) Hirao, T. *Chem. Rev.* **1997**, *97*, 2707−2724.
(21) Wender, P. A.; Rice, K. D.; Schnute, M. E. *J. Am. Chem. Soc.* **1997**, *119*, 7897−7898.
(22) Taguchi, H.; Isobe, K.; Nakamura, Y.; Kawaguchi, S. *Chem. Lett.* **1975**, 757−760.
(23) Selbin, J.; Manning, H. R.; Cessac, G. *J. Inorg. Nucl. Chem.* **1963**, *25*, 1253−1258.
(24) Atherton, N. M.; Gibbon, P. J.; Shohoji, M. C. B. *J. Chem. Soc., Dalton Trans.* **1982**, 2289−2290.
(25) Dodge, R. P.; Templeton, D. H.; Zalkin, A. *J. Chem. Phys.* **1961**, *35*, 55−67.
(26) Al-Niaimi, N. S.; Al-Karaghouli, A. R.; Aliwi, S. M.; Jalhoom, M. G. *J. Inorg. Nucl. Chem.* **1974**, *36*, 283−288.
(27) Pandya, B. J.; Bhattacharya, P. K. *Indian J. Chem.* **1986**, *25A*, 776−778.
(28) Hon, P.-K.; Belford, R. L.; Pfluger, C. E. *J. Chem. Phys.* **1965**, *43*, 1323−1333.
(29) Caira, M. R.; Haigh, J. M.; Nassimbeni, L. R. *Inorg. Nucl. Chem. Lett.* **1972**, *8*, 109−112.
(30) Isobe, K.; Nakamura, Y.; Kawaguchi, S. *J. Inorg. Nucl. Chem.* **1978**, *40*, 607−611.
(31) Caira, M. R.; Haigh, J. M.; Nassimbeni, L. R. *J. Inorg. Nucl. Chem.* **1972**, *34*, 3171−3176.
(32) Carlin, R. L.; Walker, F. A. *J. Am. Chem. Soc.* **1965**, *87*, 2128−2133.
(33) Nath, P. K.; Dash, K. C. *Indian J. Chem.* **1986**, *25A*, 968−969.
(34) Frausto da Silva, J. J. R.; Wooton, R. *J. Chem. Soc., Dalton Trans.* **1969**, 421−422.
(35) Van Willigen, H. *Chem. Phys. Lett.* **1979**, *65*, 490−492.
(36) Kirste, B.; van Willigen, H. *J. Phys. Chem.* **1982**, *86*, 2743−2749.
(37) Hanson, G. R.; Sun, Y.; Orvig, C. *Inorg. Chem.* **1996**, *35*, 6507−6512.
(38) Linert, W.; Herlinger, E.; Margl, P.; Boca, R. *J. Coord. Chem.* **1993**, *28*, 1−16.
(39) Caira, M. R.; Nassimbeni, L. R.; Scott, J. L.; Wildervanck, A. F. *J. Chem. Crystallogr.* **1996**, *26*, 117−122.

Table 1. Details of the Crystallographic Experiments and Computations for $[VO(Et\text{-}acac)_2]$ and $[VO(Me\text{-}acac)_2]$

	$[VO(Et\text{-}acac)_2]$	$[VO(Me\text{-}acac)_2]$
empirical formula	$C_{14}H_{22}O_5V$	$C_{12}H_{18}O_5V$
fw	321.26	293.20
cryst syst	triclinic	triclinic
space group	$P\bar{1}$	$P\bar{1}$
lattice consts		
a, Å	9.29970(10)	7.7296(4)
b, Å	13.6117(2)	8.1856(5)
c, Å	13.6642(2)	11.9029(6)
α, deg	94.1770(10)	79.927(2)
β, deg	106.4770(10)	73.988(2)
γ, deg	106.6350(10)	65.1790(10)
V, Å^3	1566.74(4)	655.52(6)
Z	4	2
ρ(calcd), Mg m^{-3}	1.362	1.485
radiation (λ, Å)	Mo Kα (0.71073)	Mo Kα (0.71073)
abs coeff μ, mm^{-1}	0.648	0.766
θ range for data collection (deg)	1.58−28.26	1.78−23.30
reflns collected	10 418	3052
indep reflns	7177	1878
R ($[I > 2\sigma(I)]$)	0.0457	0.0328
R_w ($[I > 2\sigma(I)]$)	0.1202	0.0901
R (all data)	0.0580	0.0371
R_w (all data)	0.1286	0.0930
abs corr	SADABS	SADABS

C. Spectroscopic Methods. Crystallographic Studies of $VO(Et\text{-}acac)_2$ and $VO(Me\text{-}acac)_2$. Rectangular blue-green crystals of $VO(Et\text{-}acac)_2$ were obtained from a saturated solution of CH_2Cl_2 on standing at −15 °C for 24 h. Rodlike blue-green crystals of $VO(Me\text{-}acac)_2$ were obtained from a saturated benzene solution on standing for 12 h at ambient temperature and pressure. X-ray diffraction data from crystals of dimensions 0.45 × 0.35 × 0.35 mm^3 ($VO(Et\text{-}acac)_2$) and 0.48 × 0.18 × 0.18 mm^3 (for $VO(Me\text{-}acac)_2$) were recorded on a Bruker AXS SMART CCD diffractometer employing Mo Kα radiation (graphite monochromator). Crystallographic results and other details are listed in Table 1. The cell parameters were obtained from a least-squares fit to the angular coordinates of all reflections for both compounds. Intensities were integrated from a series of frames (0.3° rotation) covering more than a hemisphere of reciprocal space. Absorption and other corrections were applied by using SADABS.[40] A total of 10 418 reflections for $VO(Et\text{-}acac)_2$ and 3052 reflections for $VO(Me\text{-}acac)_2$ were merged to provide data sets containing 7177 unique reflections (R_{int} = 0.014) for $VO(Et\text{-}acac)_2$ and 1878 unique reflections (R_{int} = 0.017) for $VO(Me\text{-}acac)_2$. The structures were solved by direct methods and refined (on F^2 using all data) by a full-matrix, weighted least-squares process (R = 0.0457, R_w = 0.1286 for $VO(Et\text{-}acac)_2$; R = 0.033, R_w = 0.0901 for $VO(Me\text{-}acac)_2$). All non-hydrogen atoms were refined by using anisotropic displacement parameters. Hydrogen atoms were placed in idealized positions and refined by using a riding model. The final electron density map showed features in the range from −0.39 to + 0.45 e Å^{-3} for $VO(Et\text{-}acac)_2$ and from −0.26 to +0.30 e Å^{-3} for $VO(Me\text{-}acac)_2$. Standard Bruker control (SMART) and integration (SAINT) software was employed, and Bruker SHELXTL[41] software was used for structure solution, refinement, and graphics.

Sample and Stock Solution Preparation. Sufficient compound was weighed to prepare stock solutions of millimolar concentration and was dissolved in double-distilled deionized water (pH about 7). It was necessary to sonicate solutions for up to 1 h (no global temperature increases were observed during this time) to obtain complete dissolution of the $VO(Me\text{-}acac)_2$ and $VO(Et\text{-}acac)_2$ complexes. The colors of the solutions were as follows: $VOSO_4$, blue; $VO(acac)_2$, light blue; $VO(malto)_2$, green-yellow; and $VO(Me\text{-}acac)_2$ or $VO(Et\text{-}acac)_2$, light green. Because of the low solubility of $VO(Me\text{-}acac)_2$ and $VO(Et\text{-}acac)_2$, only concentrations of up to 1−1.5 mM in aqueous solution could be prepared. Sonication was always used to dissolve $VO(Et\text{-}acac)_2$ and $VO(Me\text{-}acac)_2$ and frequently was used to dissolve $VO(acac)_2$ and $VO(malto)_2$ in a timely manner (≤5 min). Time zero was defined as the time when dissolution was complete. Unless otherwise stated, the pH reported was that observed upon dissolution of the compound. To characterize the species present in solution, measurements also were made on samples prepared by sonication of the compound in the presence of excess ligand. A noticeable increase in solubilities and stabilities of particularly $VO(Me\text{-}acac)_2$ and $VO(Et\text{-}acac)_2$ was observed upon sonication of compounds in the presence of excess ligand. Studies also were carried out on solutions to which 85 mM NaCl (and other salts) had been added; the compounds were administered to the diabetic rats in 85 mM NaCl solutions thus warranting an interest in speciation under these conditions.[17]

Visible Spectroscopy. Visible spectra were recorded on a Perkin-Elmer Lambda 4B spectrometer from 400 to 900 nm with temperature regulated at 21 °C using a Haake A81 circulating water bath. Solutions of 1.0−10 mM $VO(acac)_2$ and $VO(malto)_2$ and near-saturated solutions of $VO(Me\text{-}acac)_2$ and $VO(Et\text{-}acac)_2$ were studied. Spectra were recorded immediately after solution preparation and as a function of time. Preparations of the solutions were as described above.

EPR Spectroscopy: Data Acquisition. The EPR spectra were recorded on Bruker ESP 300 and Varian E-9 spectrometers. The first-derivative X-band EPR spectra were recorded in 1 mm quartz capillary tubes that were placed in 4 or 5 mm quartz tubes at ambient temperature; alternatively spectra were recorded at 20 ± 2 °C in disposable 0.4 mm path length glass flat cells.[42] Samples of the vanadium(IV) compounds were varied in concentration from 1.0−5.0 mM and were recorded in aqueous solution, aqueous sodium chloride, aqueous sodium bromide, and aqueous sodium fluoride solutions. Spectra were routinely corrected for background signals by subtraction of a spectrum obtained for a water-containing EPR flat cell.

To permit quantitative analysis of the concentrations of the vanadyl species, it is necessary to obtain spectra at microwave powers that do not saturate the signals.[43] Although the literature suggests that VO^{2+} complexes do not saturate at power levels as high as 200 mW,[44] no data were reported. Due to the fact that several species were discovered in our studies, and because there was little precedent for quantitation of such species in aqueous solution, particular care was exercised in these first quantitative measurements. The nonresonant absorption of microwaves by aqueous samples can cause sample heating which can affect the signal line widths. For most vanadyl complexes in fluid solution, EPR line widths are due to incomplete motional averaging of g and A anisotropy.[45] Heating of the sample causes faster tumbling, a narrowing of the lines, and an increase in signal amplitude. This effect can complicate determination of the appropriate power level for signal quantitation.

A plot of EPR signal amplitude as a function of the square root of the microwave power (a power saturation curve) is expected to be linear if the electron spin relaxation rates are fast enough that the signal does not saturate. Slower electron spin relaxation rates cause the signal intensity to increase more slowly at high power than predicted by linear extrapolation from the low-power data. Power saturation leads to broadening of the EPR signal and a decrease in integrated signal intensity. A power saturation curve for an aqueous solution of $VO(acac)_2$ in a flat cell with a 0.4 mm path length was anomalous in that signal amplitude increased more rapidly at high power than predicted by linear extrapolation from the low-power data (trace a in Figure 2). Expanded plots of the center lines of the spectra indicated that the line widths were narrower in the high-power spectra than in the low-power spectra. A smaller deviation from linearity was observed for data obtained in a 0.2 mm path length flat cell (trace b in Figure 2), and linear behavior was observed for a 0.2 mm ID capillary tube (trace c

(40) Sheldrick, G. M. *SADBS (a Program for Siemens Area Detection Absorption Correction)*, to be published.
(41) Sheldrick, G. M. *SHELXTL*; Siemens: Madison, WI, 1996; Vol. 5.
(42) Eaton, S. S.; Eaton, G. R. *Anal. Chem.* **1977**, *49*, 1277−1278.
(43) Swartz, H. M.; Bolton, J. R.; Borg, D. C. *Biological Applications of Electron Spin Resonance*; Wiley-Interscience: New York, 1972.
(44) Chasteen, N. D. In *Biological Magnetic Resonance*; Berliner, L., Reuben, J., Eds.; Plenum Press: New York, 1981; Vol. 3, pp 53−119.
(45) Wilson, R.; Kivelson, D. *J. Chem. Phys.* **1966**, *44*, 154−168.

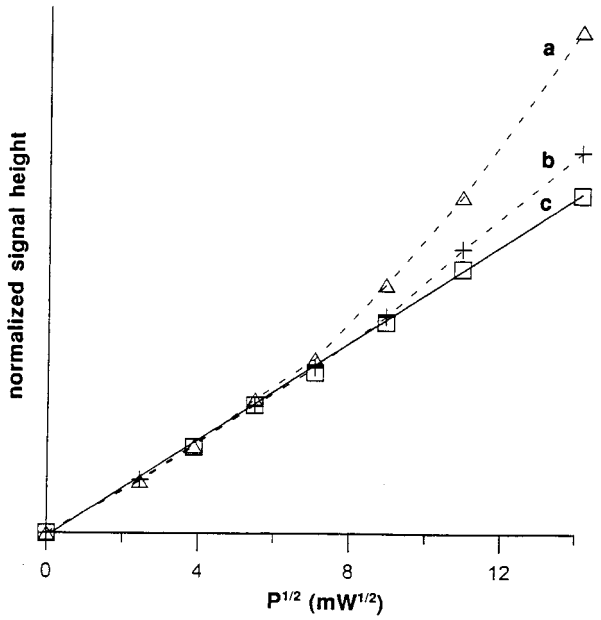


Figure 2. Power saturation curve for 5.0 mM $VO(acac)_2$ in aqueous solution with 5.0 mM Hacac ligand: (a) 0.4 mm flat cell; (b) 0.2 mm flat cell; (c) 0.2 mm capillary tube.

in Figure 2). The greater deviation from linearity for larger sample volumes is consistent with the expected dependence of sample heating on sample volume. Furthermore, the detector current tended to drift at high power for the samples in 0.4 or 0.2 mm flat cells, which is an additional indication that the sample temperature was changing. These observations of heating of larger sample volumes by the microwaves are an important reminder that caution must be exercised in working with samples containing water at higher microwave powers. Although powers up to 200 mW could have been used without power saturation occurring, data were recorded at 50 mW to avoid problems with detector current drift or line shape changes due to sample heating.

The spectra shown in this paper were recorded at 20 °C at 9.17 GHz (X-band) and 50 mW microwave power. The 20 ± 2 °C temperatures were recorded by a thermocouple located in the resonator close to the EPR sample tube. A modulation frequency of 100 kHz, a modulation amplitude of 5.00 G, a time constant of 128 ms, a sweep width of 2000 G, and a sweep time of 240 s were used. Typically four scans with 2048 points per scan were recorded with a center field of 3238.0 G. The spectrometer center field was calibrated using a powder sample of 2,2-bis(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH, $g = 2.0037 \pm 0.0002$[46]), and sweep width was calibrated with Mn^{2+} in CaO ($A = 86.38$ G, $g = 2.0015$). The composition of the vanadyl samples changed as a function of time, thus reported times are the halfway point for data acquisition. Quantitation of EPR signals was performed by double integration of the first-derivative spectra to obtain the area under the absorption curve. Intensities were compared with a calibration curve obtained for 1.0−10 mM $VOSO_4$ in 0.1 M H_2SO_4. The signals observed for freshly prepared 1.0 mM $VO(acac)_2$ (pH 5.7) solutions accounted for 90−100% of the vanadium in the sample.[47] The concentrations of spins for $VO(Et\text{-}acac)_2$ and $VO(Me\text{-}acac)_2$ in the presence of excess ligands were about 60% and 75%, respectively. The observation of integrated intensities less than 100% is attributed to the presence of EPR-silent vanadium(IV) species and to oxidation to vanadium(V). The quantitation of the vanadium(V) by NMR is described below.

EPR Spectroscopy: Analysis of Spectra. Several components were observed in the ambient temperature EPR spectra. As described in Results and Discussion these are assigned as follows: species A, *trans*-$VO(R\text{-}acac)_2 \cdot H_2O$ adduct; species B, *cis*-$VO(acac)_2 \cdot H_2O$ adduct (species B was not actually observed for $VO(R\text{-}acac)_2$ when R = Me and Et, although its presence cannot be ruled out); and species C, the hydrolysis complex that contains one vanadium atom and one R-acac$^-$ ligand. For simplicity, species C is referred to as the 1:1 hydrolysis product. Spectra were recorded at different pH values, but no changes in the values of g_0 or A_0 were observed between pH 3 and pH 7. The signal recorded for vanadyl cation is in agreement with those observed previously for $VO(H_2O)_5^{2+}$ and for $VO(H_2O)_4(OH)]^+$.[44,48] In this paper both of these species will be referred to as hydrated VO^{2+} since the pH of the samples under investigation is both above and below 5.3, the pK_a value for $VO(H_2O)_5^{2+}$.

Computer simulations of the fluid solution EPR spectra were performed with the program ASYM (Table 4[51]), which includes second-order corrections to the nuclear hyperfine interaction[49] and the dependence of line width on vanadium nuclear spin.[45,50] On the basis of repeated data collection on the same sample and repeated simulation of the spectra, the estimated uncertainties for the hyperfine coupling, g values, and populations of species are $\pm 1 \times 10^{-4}$ cm^{-1}, ±0.002, and ±2%, respectively. Percentages for species given in this paper are based on the integrated vanadium(IV) signal intensity apportioned between individual species based upon the relative intensities obtained by the simulations.

^{51}V NMR Spectroscopy. The ^{51}V NMR spectra were recorded on a Varian INOVA-300 spectrometer at 78.9 MHz (7.0 T). The parameters used for the quantitative spectra were as follows: sweep width, 83 600 Hz; pulse width, 40°; and acquisition time, 0.096 s. No observable differences in integrations (and resulting quantitations) were found when these parameters were modified. The quantitative measurements were carried out in a precision tube apparatus. A sodium metavanadate standard solution (pH 11−12) with a known concentration (ranging from 0.1 to 1.0 mM) was placed in a capillary. A calibration curve was generated for the concentration of vanadate solution in the 5 mm NMR tube, relative to the concentration in the capillary. The solutions of $VO(acac)_2$, $VO(Et\text{-}acac)_2$, and $VO(malto)_2$, prepared as described above, were placed in the NMR tube outside the capillary. The spectral integrations allowed calculation of the vanadium(V) concentration in the solutions of the vanadium(IV) complexes with an estimated 10% accuracy. Typically spectra with reasonable signal-to-noise ratios required 20 000 transients or more. In cases of low concentrations (0.05−0.1 mM) the number of transients increased to as many as 100 000. The composition of the samples changed as a function of time; thus reported times are the halfway point for data acquisition. When necessary, a calibration plot of vanadate in the capillary tube and known vanadate solutions in the outer tube could be used to improve the accuracy below 10%.

Experiments Relating to the Administration of Vanadium Compounds to Streptozotocin (STZ) Diabetic Rats. Male Wistar rats (∼220 g) were made insulin-deficient and diabetic by iv injection of streptozotocin (STZ; 38 mg/kg of body weight dissolved in 0.1 M citrate buffer (pH 4.5)). Seven days after the injection of STZ, all diabetic rats had glycemia consistently above 22 mmol/L. They were divided into several experimental groups: untreated diabetic rats ($n = 8$) and diabetic rats treated with one of the following vanadium compounds: $VO(acac)_2$, $n = 11$; $VO(Et\text{-}acac)_2$, $n = 11$; $VO(malto)_2$, $n = 11$; and $VOSO_4$, $n = 10$.[17] The four treated groups received the appropriate vanadium compounds dissolved in drinking solutions containing NaCl

(46) Drago, R. S. *Physical Methods for Chemists*, 2nd ed.; Saunders College Publishing: Orlando, 1992.

(47) A variety of standard solutions were used ($VOSO_4$ in H_2SO_4 and $VOCl_2$ in HCl), and it was found that high pH (>2) was usually the cause when less than 100% spins were observed for solutions of hydrated VO^{2+}.

(48) Francavilla, J.; Chasteen, N. D. *Inorg. Chem.* **1975**, *14*, 2860−2862.

(49) Atherton, N. M. In *Principles of Electron Spin Resonance*; Atherton, N. M., Ed.; Ellis Horwood/PTR Prentice Hall: New York, 1993; pp 114−122.

(50) Chasteen, N. D.; Hanna, M. W. *J. Phys. Chem.* **1972**, *76*, 3951−3958.

(51) The visible spectroscopy data in Table 4 reflect quantitated solution speciation and indicate values for the hypothetical case where a solution of 100% of each separate species could be prepared. The observed absorbances (for mixtures of species) for freshly prepared 1 mM $VO(R\text{-}acac)_2$ in the presence of excess ligand (1 mM for Hacac, 50 mM for HMe-acac, and 50 mM for HEt-acac) are 0.043, 0.050, and 0.043 at ca. 820 nm for acac, Me-acac, and Et-acac, respectively, and 0.015, 0.016, and 0.018 at ca. 570 nm.

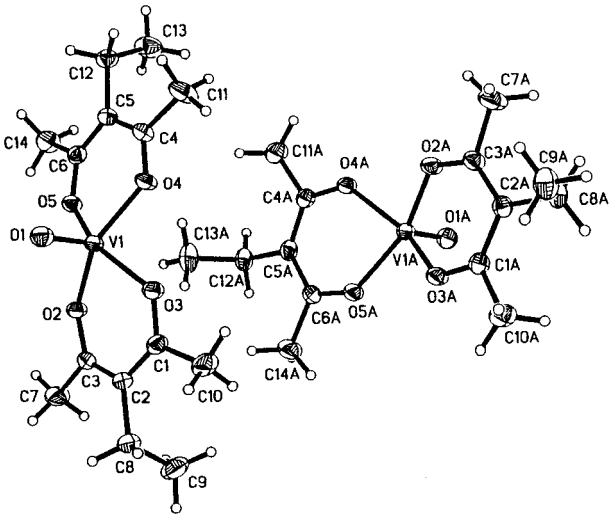


Figure 3. Structure and atomic labeling scheme for VO(Et-acac)$_2$.

to disguise the taste,[52] which eliminated differences between the four vanadium compounds relating to palatability.

The administration solutions were freshly prepared every 2 days by first dissolving the vanadium compound in distilled water (at the desired concentration ranging from 40 to 125 mg/L in elemental vanadium) and then adding solid NaCl to a concentration of 85 mM.[51] Sonication was always used to dissolve VO(Et-acac)$_2$ and VO(Me-acac)$_2$ and frequently was used to dissolve VO(acac)$_2$ and VO(malto)$_2$ in a timely manner. The quantity of elemental vanadium in the administration solutions was progressively increased from 40 to 125 mg/L during the first 4 weeks and then remained unchanged until the end of the study (12 weeks).

Results and Discussion

Synthesis of Complexes. Initially VO(acac)$_2$ and VO(Me-acac)$_2$ were prepared from vanadyl hydroxide and Hacac or HMe-acac in alcohol.[18] Purification of VO(acac)$_2$ typically is done by recrystallization from a chloroform solution.[53] However, the high solubility of VO(Me-acac)$_2$ and VO(Et-acac)$_2$ in chloroform makes recrystallization from this solvent inconvenient and difficult. Since the principal impurities in the preparation of the vanadium(IV) complexes were sodium bicarbonate and vanadyl hydroxides, which are insoluble in chloroform, an alternative purification method was developed. Vanadium(IV) compounds were extracted into $CHCl_3$, and solid impurities were removed by filtration. Evaporation of the solution to dryness yielded analytically pure compounds. The resulting yield of 65% for VO(Me-acac)$_2$ is higher than previously reported. VO(Et-acac)$_2$ was isolated in 58% yield after similar workup. If a purification step is not performed, yields of crude product as high as 85% can be obtained.

Infrared spectroscopy. The solid state properties of VO(acac)$_2$, VO(Me-acac)$_2$, and VO(Et-acac)$_2$ were examined by infrared spectroscopy. For each of these compounds, the characteristic ν(V═O) stretch at ca. 1000 cm^{-1} was observed, as reported for other oxovanadium(IV) derivatives.[54]

Crystallographic Characterization of VO(Et-acac)$_2$ and VO(Me-acac)$_2$. The structures and atomic labeling schemes for VO(Et-acac)$_2$ and VO(Me-acac)$_2$ are presented in Figures 3 and 4, respectively. Selected interatomic distances and angles are given in Tables 2 and 3. Both VO(Et-acac)$_2$ and VO(Me-acac)$_2$ exist in the solid state as discrete mononuclear complexes, with the vanadium atoms in distorted square pyramidal coordination environments. The asymmetric unit of the unit cell of VO(Et-acac)$_2$ contains two independent molecules of VO(Et-acac)$_2$, but there are only minor structural differences between them. The oxygen atoms of the Et-acac$^-$ or Me-acac$^-$ ligand coordinate in the equatorial plane, while apical coordination by the oxo group completes the square pyramidal geometry in each case. Both structures and those of related complexes, including VO(acac)$_2$,[25] show the vanadium atom lying above the basal equatorial plane. For VO(Et-acac)$_2$ and VO(Me-acac)$_2$, the vanadium atoms lie 0.56 and 0.55 Å, respectively, above the best least-squares planes through O2, O3, O4, and O5. These distances are very similar to those observed for VO(acac)$_2$ (0.55 Å),[25] VO(pb)$_2$ (bis(1-phenyl-1,3-butanedionate)oxovanadium(IV)) (0.54 Å),[28] and [VO(acen)] (acen = *N*,*N*-ethylenebis(acetylacetoneiminato)) (0.58 Å).[55] One can conclude that changing the substituents on the Hacac functionality and replacing the oxygen atoms in the equatorial plane with nitrogen atoms has little effect on the out-of-plane displacement of the vanadium atom. Bond distances and angles also are very similar among VO(Et-acac)$_2$, VO(Me-acac)$_2$, VO(acac)$_2$,[25] VO(pb),[28] and [VO(acen)].[55]

The longer VO1 bond lengths (1.561(10) Å for VO(acac)$_2$, 1.5922(2) Å for VO(Me-acac)$_2$, and 1.603(2) Å for VO(Et-acac)$_2$) suggest that Et-acac$^-$ and Me-acac$^-$ compared with the acac$^-$ ligand have increased electron-donating abilities. These

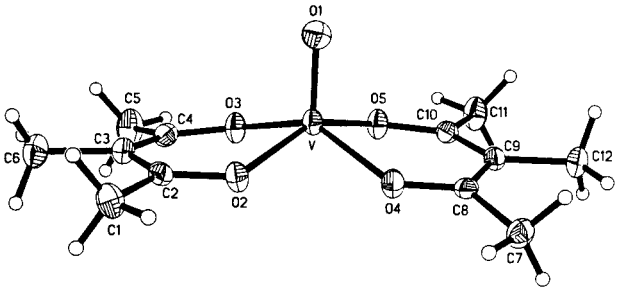


Figure 4. Structure and atomic labeling scheme for VO(Me-acac)$_2$.

Table 2. Selected Bond Lengths (Å) for [VO(Et-acac)$_2$] and [VO(Me-acac)$_2$]

	[VO(Et-acac)$_2$]		
V1−O1	1.605(2)	V1−O4	1.962(2)
V1−O2	1.962(2)	V1−O5	1.965(2)
V1−O3	1.966(2)	O2−C2	1.289(3)
O3−C4	1.291(3)	O4−C8	1.291(3)
O5−C10	1.289(3)	C1−C2	1.499(3)
C2−C3	1.407(3)	C3−C4	1.409(3)
C3−C6	1.532(3)	C4−C5	1.502(3)
V1A−O1A	1.603(2)	V1A−O2A	1.963(2)
V1A−O4A	1.964(2)	V1A−O5A	1.963(2)
V1A−O3A	1.966(2)	O2A−C2A	1.290(3)
O3A−C4A	1.290(3)	O4A−C8A	1.292(3)
O5A−C10A	1.289(3)		
	[VO(Me-acac)$_2$]		
V−O1	1.592(2)	V−O5	1.956(2)
V−O4	1.958(2)	V−O2	1.968(2)
V−O3	1.971(2)	O2−C2	1.289(3)
O3−C4	1.286(3)	O4−C8	1.292(3)
O5−C10	1.295(3)	C1−C2	1.503(4)
C2−C3	1.399(4)	C3−C4	1.406(4)
C3−C6	1.520(4)	C4−C5	1.506(4)
C7−C8	1.502(4)	C8−C9	1.405(4)
C9−C10	1.394(4)	C9−C12	1.519(3)
C10−C11	1.502(4)		

(52) Becker, D. J.; Ongemba, L.-N.; Henquin, J.-C. *Eur. J. Pharmacol.* **1994**, *260*, 169−175.
(53) Rowe, R. A.; Jones, M. M. *Inorg. Synth.* **1957**, *5*, 114−115.
(54) Selbin, J. *Coord. Chem. Rev.* **1966**, *1*, 293−314.
(55) Bruins, D.; Weaver, D. L. *Inorg. Chem.* **1970**, *9*, 130−135.

Table 3. Selected Bond Angles (deg) for [VO(Et-acac)$_2$] and VO(Me-acac)$_2$]

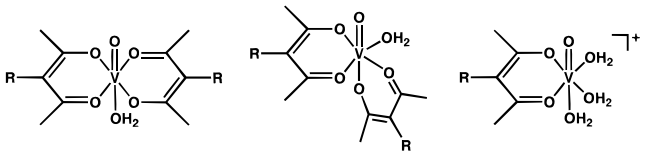
	[VO(Et-acac)$_2$]		
O1−V1−O4	106.10(8)	O1−V1−O2	106.83(8)
O4−V1−O2	84.75(7)	O1−V1−O5	106.61(8)
O4−V1−O5	85.74(7)	O2−V1−O5	146.56(8)
O1−V1−O3	106.04(8)	O4−V1−O3	147.86(8)
O2−V1−O3	85.59(7)	O5−V1−O3	85.65(7)
C2−O2−V1	130.0(2)	C4−O3−V1	129.2(2)
C8−O4−V1	129.54(14)	C10−O5−V1	129.76(14)
O2−C2−C3	124.5(2)	O2−C2−C1	113.2(2)
C3−C2−C1	122.3(2)	C2−C3−C4	120.2(2)
C2−C3−C6	120.0(2)	C4−C3−C6	119.7(2)
O3−C4−C3	125.0(2)	O3−C4−C5	113.2(2)
C3−C4−C5	121.8(2)	O4−C8−C9	124.7(2)
O4−C8−C7	113.5(2)	C9−C8−C7	121.8(2)
C10−C9−C8	120.2(2)	C10−C9−C12	120.3(2)
C8−C9−C12	119.4(2)	O5−C10−C9	124.8(2)
O5−C10−C11	113.5(2)	C9−C10−C11	121.7(2)
O1A−V1A−O2A	106.82(8)	O1A−V1A−O4A	105.88(8)
O2A−V1A−O4A	84.90(7)	O1A−V1A−O5A	107.02(8)
O2A−V1A−O5A	146.16(8)	O4A−V1A−O5A	85.83(7)
O1A−V1A−O3A	105.99(8)	O2A−V1A−O3A	85.56(7)
O4A−V1A −O3A	148.13(8)	O5A−V1A−O3A	85.38(7)
C2A−O2A−V1A	129.0(2)	C4A−O3A−V1A	128.5(2)
C8A−O4A−V1A	129.1(2)	C10A−O5A−V1A	129.4(2)
	[VO(Me-acac)$_2$]		
O1−V−O5	105.32(9)	O1−V−O4	108.11(8)
O5−V−O4	86.25(7)	O1−V−O2	105.06(9)
O5−V−O2	149.61(8)	O4−V−O2	85.07(7)
O1−V−O3	108.44(9)	O5−V−O3	84.25(7)
O4−V−O3	143.45(8)	O2−V−O3	85.58(7)
C2−O2−V	127.8(2)	C4−O3−V	129.1(2)
C8−O4−V	129.2(2)	C10−O5−V	128.2(2)
O2−C2−C3	124.7(2)	O2−C2−C1	114.9(2)
C3−C2−C1	120.4(2)	C2−C3−C4	120.7(2)
C2−C3−C6	118.8(2)	C4−C3−C6	120.3(2)
O3−C4−C3	124.6(2)	O3−C4−C5	114.1(2)
C3−C4−C5	121.3(2)	O4−C8−C9	124.1(2)
O4−C8−C7	114.6(2)	C9−C8−C7	121.2(2)
C10−C9−C8	121.0(2)	C10−C9−C12	118.5(2)
C8−C9−C12	120.3(2)	O5−C10−C9	125.1(2)
O5−C10−C11	114.1(2)	C9−C10−C11	120.8(2)

bond lengths are in the range reported for related vanadyl complexes, including VO(bzac)$_2$ (1.612(10) Å)[28] and [VO(acen)] (1.585(7) Å).[55] Studies of related vanadium complexes with acac$^-$ ligands alkyl- and aryl-substituted at the terminal methyl carbon document minor structural differences as a result of substitution. The present results demonstrate that only minor structural changes in the complexes are observable when the central methylene carbon of the acac$^-$ ligand is substituted by an alkyl group. Given the potent insulin-mimetic effects of VO(acac)$_2$ and VO(Et-acac)$_2$, the solution properties of these and related complexes were investigated next.

Species in Aqueous Solutions of VO(acac)$_2$ as Monitored by EPR and Visible Spectroscopy. Although many studies designed to characterize vanadyl speciation have been performed on frozen samples,[35,37] the present studies were performed in fluid solution to accurately reflect the conditions of the animal studies. The EPR spectra of 1.0 mM VO(acac)$_2$ in aqueous solution at 20 °C indicate a mixture of three species (See Table 4). These will be referred to as species A, B, and C as indicated in Figure 5 (the bases for these assignments are discussed below). In a freshly prepared solution of VO(acac)$_2$, species A ($A_0 = 92 \times 10^{-4}$ cm^{-1}; $g_0 = 1.970$) was the major species (85%) and B ($A_0 = 97 \times 10^{-4}$ cm^{-1}; $g_0 = 1.970$) was the minor species (5%) (Figure 5a). The concentration of species B was very low at all times, and because of the similarity of its parameters to those for species A, it was observed as a shoulder (most readily detected at high power settings where heating of the sample caused narrowing of the lines). Species C ($A_0 = 101 \times 10^{-4}$ cm^{-1}; $g_0 = 1.970$) was not observed in freshly prepared VO(acac)$_2$ solution (Figure 5a), but its concentration increased over time (Figure 5b,c). Furthermore, spectra recorded of solutions of crystalline compound that had been aged or solutions refluxed for several hours contained higher fractions of complex C (data not shown). Spectra of some aged solutions of VO(acac)$_2$ (data not shown) were found to contain hydrated VO^{2+} (in several cases the pH of the solution is near the pK_a value for $VO(H_2O)_5^{2+}$ (5.36)[44] and both this species and the deprotonated species $[VO(H_2O)_4(OH)]^+$ will exist).

The EPR spectrum of freshly prepared VO(acac)$_2$ in a solution containing 1 equiv of Hacac was indistinguishable from the spectrum in Figure 5a (data not shown). In the presence of 1

Table 4. The Ambient Temperature EPR Parameters for VO(R-acac)$_2$ (R = H, CH_3, CH_2CH_3) and Hydrated VO^{2+}



	g_0 and A_0 (10^4 cm^{-1}) λ_{max}/nm (ϵ/dm^3 mol^{-1} cm^{-1})		
species	acac	Me-acac	Et-acac
A	1.970, 92 820 (50); 570 (20)	1.970, 91 830 (200); 565 (60)	1.970, 91 820 (200); 575 (90)
B	1.970, 97		
C	1.970, 101	1.970, 100	1.970, 100
hydrated VO^{2+}	1.964, 106.3 750 (18); 625 (7)[a]		

[a] References 44 and 57.

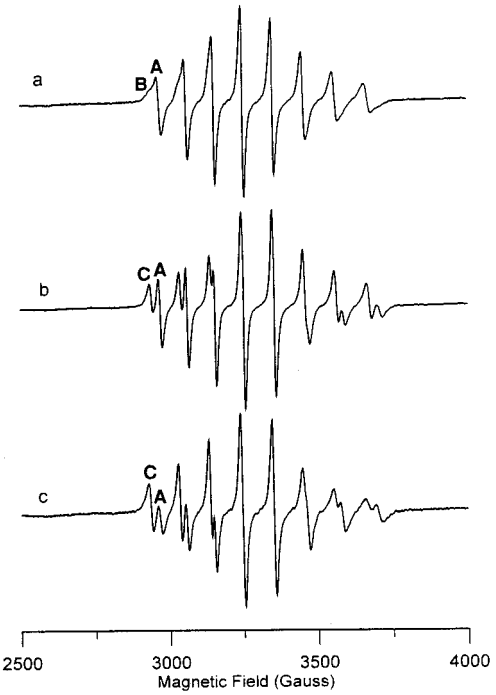


Figure 5. EPR spectra recorded at 20 ± 2 °C: (a) 1.0 mM VO(acac)$_2$ in aqueous solution (t = 10 min, pH = 5.8); (b) 1.0 mM VO(acac)$_2$ in aqueous solution (t = 2 days, pH = 5.3); (c) 1.0 mM VO(acac)$_2$ in aqueous solution (t = 24 days, pH = 4.5). t = 0 is defined as the time at which complete dissolution was obtained.

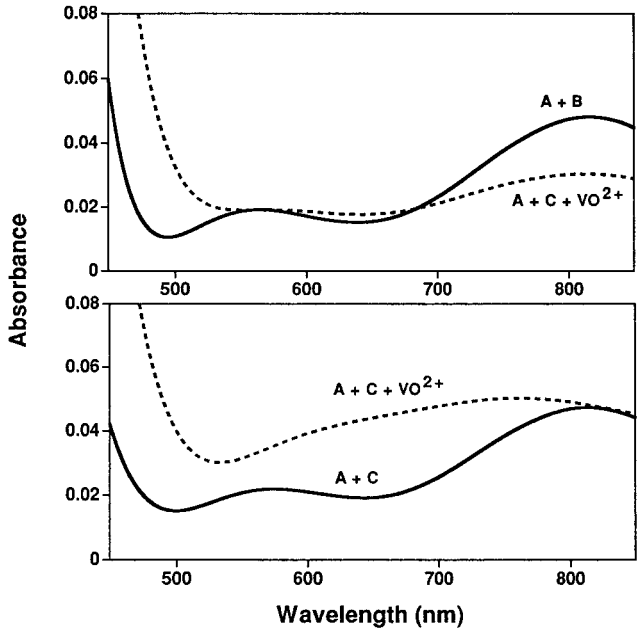


Figure 6. Visible spectra of the major species in solutions of 1.0 mM VO(R-acac)$_2$. Top: VO(acac)$_2$ with 1.0 mM Hacac (pH 5.8) (—) and VO(acac)$_2$ after 38 days with no added ligand (pH 4.2) (– – –). Bottom: VO(Et-acac)$_2$ with 50 mM HEt-acac (pH 4.8) (—) and VO(Et-acac)$_2$ after dissolution with no added ligand (pH 4.4) (– – –).

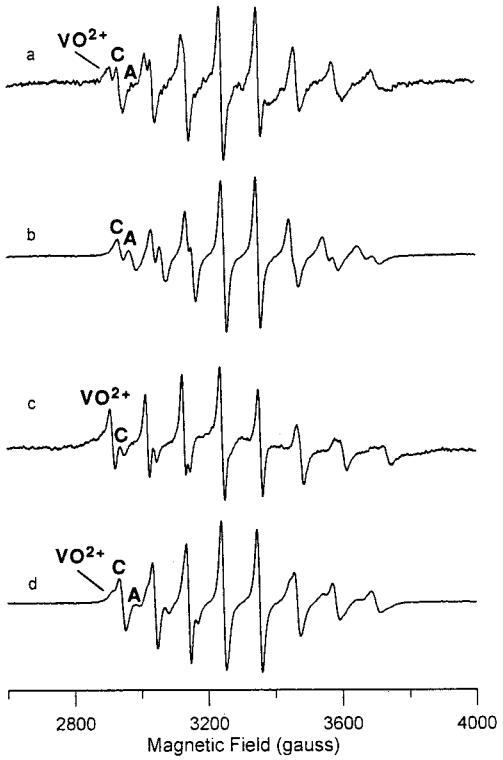


Figure 7. EPR spectra recorded at 20 ± 2 °C: (a) 1.0 mM VO(Me-acac)$_2$ in H_2O (t = 10 min, pH = 5.2); (b) 1.0 mM VO(Me-acac)$_2$ with 50 mM HMe-acac ligand (t = 10 min, pH = 5.8); (c) 1.0 mM VO(Et-acac)$_2$ in H_2O (t = 10 min, pH = 4.4); (d) 1.0 mM VO(Et-acac)$_2$ and 50 mM HEt-acac ligand (t = 10 min, pH = 4.8).

equiv of Hacac there was no observable change in the EPR spectra after 7 days at 20 ± 2 °C (data not shown). In contrast, the spectra in Figure 5b and Figure 5c of dissolved crystalline VO(acac)$_2$ recorded after 2 days and 24 days, respectively, showed significantly higher concentrations of species C.

To further characterize the major species A and C in solution, visible spectroscopic studies were carried out. A spectrum was recorded of a solution containing 1.0 mM VO(acac)$_2$ (pH 5.7) immediately after dissolution. Solutions prepared in this manner for EPR spectroscopy contained 85% species A (5% species B, no observable vanadium(V), and 10% EPR-silent vanadium(IV)) (see Table 4). Initially two distinct bands with λ_{max}/nm (H_2O) 820 and 570 (ϵ/dm^3 mol^{-1} cm^{-1}: 50 and 20) were observed which are attributed to species A. A visible spectrum of a sample solution containing 1.0 mM VO(acac)$_2$ and 1.0 mM Hacac (Figure 6, top) was indistinguishable from that of a freshly prepared VO(acac)$_2$ solution. Over a period of several weeks at ambient temperature, the absorbances for the 1.0 mM VO(acac)$_2$ solution changed in tandem with the EPR and NMR spectra. After 24 days at ambient temperature, EPR spectroscopic analysis of an analogous solution indicated about 25% A and 65% C. The 10% of spins that are observed by neither EPR nor NMR spectroscopy are assigned to vanadium(IV)−acac complexes with strongly antiferromagnetically coupled spins (such as the dimeric VO(acac) complexes). The visible spectra for all of these species are distinguishable from hydrated VO^{2+} (see Table 4).[44,57]

Species in Solutions of VO(Et-acac)$_2$ and VO(Me-acac)$_2$ as Monitored by EPR and Visible Spectroscopy. Analogous EPR and visible spectroscopic studies were carried out at 20 °C for VO(Et-acac)$_2$ and VO(Me-acac)$_2$. For the same molar concentration of vanadium, EPR spectra of solutions of crystalline VO(Et-acac)$_2$ showed a lower signal-to-noise ratio and lower integrated signal intensity than the parent VO(acac)$_2$. Even in solutions containing 50 mM HEt-acac, integrals accounted for about 60% of the total vanadium. The lower signal intensity indicates that these compounds had increased tendencies to form EPR-silent vanadium(IV) species and/or to be oxidized to vanadium(V). Quantitative NMR studies indicated that both vanadium(V) and EPR-silent vanadium(IV) species had formed. The decreased signal-to-noise ratio resulted in unambiguous identification of only species A and C. These observations, however, do not rule out the presence of low concentrations of species B.

The EPR spectra for 1.0 mM VO(Et-acac)$_2$ and VO(Me-acac)$_2$ are shown in Figure 7a−d in the absence (Figure 7a,c) and presence (Figure 7b,d) of 50 mM excess free ligand. The notation used above for VO(acac)$_2$ has been maintained, and the parameters for species A and C of VO(Et-acac)$_2$ and VO(Me-acac)$_2$ are shown in Table 4. In the absence of excess ligand, hydrated VO^{2+} is the major species present in solutions of crystalline VO(Et-acac)$_2$ and VO(Me-acac)$_2$. The two minor species observed are A and C. In the presence of 50 mM free ligand, species C is the major complex observable by EPR spectroscopy in solutions of crystalline VO(Et-acac)$_2$ and VO(Me-acac)$_2$, which demonstrates that excess ligand shifts the equilibrium from VO^{2+} to species C. The parameters for species

(56) Preparation of solutions by sonication of VO(R-acac)$_2$ before the NaCl and other salts were added was found to be critical. In contrast to the studies described in the text, sonication of VO(Et-acac)$_2$ in the presence of NaCl generated aqueous solutions with a dark purplish color. This color changed within 1 h of preparation to a yellow-green similar to that observed by dissolution in the absence of NaCl. Recording the EPR spectra of purple solutions showed that most of the compound in solution was EPR silent, and that only very low concentrations of presumably hydrated VO^{2+} (or a chloride derivative thereof) were left in solution. The solutions for administration to the rats were obtained by addition of NaCl after sonication. On the basis of the studies describing the interaction of salts with vanadium compounds, addition of NaCl before or during sonication would have resulted in administration of a different species mixture and may yield very different results.

(57) Ballhausen, C. J.; Gray, H. B. *Inorg. Chem.* **1962**, *1*, 111−122.

C are indistinguishable for R = Et and R = Me, with $A_0 = 100 \times 10^{-4}$ cm^{-1} and $g_0 = 1.970$. For both VO(Et-acac)$_2$ and VO(Me-acac)$_2$, species A was observed as a minor signal, even in the presence of 25−50 mM excess ligand. The parameters for species A also are indistinguishable for R = Et and R = Me with $A_0 = 91 \times 10^{-4}$ cm^{-1} and $g_0 = 1.970$. On the basis of the observed high concentrations of species C for the alkylated acac frameworks, ligand alkylation generates a complex that is significantly less stable in H_2O than the parent. However, since most of the spins are not observable and only a fraction of these were accounted for by ^{51}V NMR spectroscopy, the major type of vanadium in these solution is the EPR-silent vanadium(IV). Since the solid state data show that alkylation makes HEt-acac and HMe-acac better donors and the complex stability in aqueous solution follows the reverse pattern, the solution stability must also be affected by factors other than ligand donor properties.

EPR spectra (at 20 °C) of VO(acac)$_2$ demonstrated that species A converted to C over the course of days, whereas the conversion from species A to C for VO(Et-acac)$_2$ and VO(Me-acac)$_2$ was nearly complete within the time required for dissolution (the ≤1 h sonication period). Visible spectra of solutions of VO(acac)$_2$ compared to those of VO(Me-acac)$_2$ and VO(Et-acac)$_2$ also showed that the latter two complexes were more labile and less stable than VO(acac)$_2$. In the case of VO(acac)$_2$, it was necessary to use only 1 equiv of excess ligand to keep the [VO(acac)$_2$] unit intact in solution for weeks, whereas for both VO(Et-acac)$_2$ and VO(Me-acac)$_2$, excesses of greater than 10 equiv were needed. Concentrations of EPR-silent vanadium(IV)−R-acac complexes were also greater for the VO(R-acac)$_2$ systems (R = Me or Et) than for the parent VO(acac)$_2$. However, once formed, species C is stable in aqueous solutions for several days, a point that may be important for the abilities of these complexes to induce insulin-mimetic properties (see below).

The complexes formed in solutions of VO(Et-acac)$_2$ and VO(Me-acac)$_2$ absorb at wavelengths similar to those of complexes formed in solutions of VO(acac)$_2$ (see Table 4 and Figure 6). Aqueous solutions of VO(Me-acac)$_2$ and VO(Et-acac)$_2$ with 50 mM excess ligand generated visible spectra similar to that of VO(acac)$_2$ with 1 mM Hacac ligand. Some of the differences in the visible spectra of solutions of crystalline VO(acac)$_2$ and VO(Et-acac)$_2$ are related to the lower stability of species A and B of the Et-acac system and are most clearly seen for the VO(Et-acac)$_2$ complex (Figure 6, bottom). In addition, large differences in the extinction coefficients for species A were observed when the quantitation of species was taken into account (Table 4).[56] The wavelengths and extinction coefficients for species A in the parent VO(acac)$_2$ system are 820 (50 dm^3 mol^{-1} cm^{-1}) and 570 (20 dm^3 mol^{-1} cm^{-1}), and for VO(Et-acac)$_2$ they are 820 (200 dm^3 mol^{-1} cm^{-1}) and 575 (90 dm^3 mol^{-1} cm^{-1}). In solutions containing species C and VO^{2+} derived vanadium species, only one broad absorption signal from 545 to 900 nm was observed for all three acac-type complexes. The absorbance spectra for the VO(Et-acac)$_2$ system are shown in Figure 6 (bottom). The maxima for the mixture of species for both VO(Et-acac)$_2$ and VO(Me-acac)$_2$ are in the region of 745 nm. In summary, these studies have shown that VO(Et-acac)$_2$ and VO(Me-acac)$_2$ are more labile than VO(acac)$_2$ but are still more inert than many of the vanadium compounds used in insulino-mimetic studies.[58]

Structural Assignment of Species A, B, and C. Limited data are available in the literature on complexes that provide precedents for the assignment of the species present in ambient temperature aqueous solutions of VO(R-acac)$_2$.[44] Reports examining adducts of VO(acac)$_2$ with alcohols in organic solvents all have concluded that the major isomer has the *trans* geometry.[35,38] Given the similarity of H_2O to an alcohol, the major isomer of VO(R-acac)$_2$·H_2O is most likely the *trans* isomer. We therefore propose that species A is the *trans* isomer and species B is the *cis* isomer. On the basis of these assignments for the VO(acac)$_2$·H_2O complexes, the value of A_0 for species A, *trans* isomer ($A_0 = 92 \times 10^{-4}$ cm^{-1}), is lower than that for species B, *cis* isomer ($A_0 = 97 \times 10^{-4}$ cm^{-1}). The relative magnitudes of the coupling constants for the isomers of VO(R-acac)$_2$ are consistent with a study of the related vanadium(IV) complex, VO(malto)$_2$·H_2O, in which the species with the smaller hyperfine coupling ($A_0 = 85.0 \times 10^{-4}$ cm^{-1}) was assigned as the *trans* isomer, and the species with the larger hyperfine coupling ($A_0 = 95.5 \times 10^{-4}$ cm^{-1}) was assigned as the *cis* isomer.[37] For VO(R-acac)$_2$·H_2O the *trans* isomer is the dominant isomer, but for VO(malto)$_2$·H_2O the *cis* isomer is dominant.[37] The structure of the major isomer for VO(malto)$_2$·H_2O is analogous to vanadium(V) VO(malto)$_2$OR (R = Me, Et, *i*-Pr) complex[14] in which the alkoxide group is coordinated *cis* to the oxo group. The facts that the chelate rings are of different sizes for R-acac and malto and that ligand electronic properties vary could explain the observed differences in isomer population between the VO(R-acac)$_2$ and VO(malto)$_2$ systems. A recent study of vanadium(V)−acac derivatives[59] described isolation and structural characterization of a neutral VO(acac)$_2$-(O-*i*-Pr) complex. Similar to VO(malto)$_2$OR, this vanadium(V) isopropoxide complex also has the alkoxide group *cis* to the oxo group and thus it differs from the major solution species of VO(acac)$_2$·H_2O adducts.

The population of species C was observed to increase with time and to decrease in the presence of excess ligand. These observations are consistent with assignment of species C to a hydrolysis product. Since the EPR parameters for species C are distinguishable from hydrated vanadyl (Table 4), the hydrolysis product is assigned as a 1:1 complex of vanadyl with R-acac$^-$. This assignment also is in agreement with literature precedents in which VO(acac)$_2$ reacts with polydentate ligands and one acac$^-$ group is substituted whereas the second acac$^-$ group remains resistant to substitution.[60−63] The possibility of isomers in which the R-acac$^-$ ligand is coordinated in a monodentate manner[38] is less likely because such association is entropically unfavored and because of the relatively low concentrations of ligands used in these studies.

In summary, our structural assignments are (see Figure 1 and Scheme 1): the *trans*-VO(R-acac)$_2$·H_2O adduct (species A) has the H_2O molecule coordinated *trans* to V=O; the *cis*-VO(R-acac)$_2$·H_2O adduct (species B) has the H_2O molecule coordinated *cis* to V=O; and the 1:1 hydrolysis product (species C) may contain two or three coordinated water molecules and have an overall charge of +1 or neutral (if H^+ is lost from one

(58) Crans, D. C.; Mahroof-Tahir, M.; Keramidas, A. D. *Mol. Cell. Biochem.* **1995**, *153*, 17−24.

(59) Jiang, F.; Anderson, O. P.; Miller, S. M.; Chen, J.; Mahroof-Tahir, M.; Crans, D. C. *Inorg. Chem.* **1998**, *37*, 5439−5451.

(60) Crans, D. C.; Keramidas, A. D.; Amin, S. S.; Anderson, O. P.; Miller, S. M. *J. Chem. Soc., Dalton Trans.* **1997**, 2799−2812.

(61) Schmidt, H.; Bashirpoor, M.; Rehder, D. *J. Chem. Soc., Dalton Trans.* **1996**, 3865−3870.

(62) Cornman, C. R.; Kampf, J.; Lah, M. S.; Pecoraro, V. L. *Inorg. Chem.* **1992**, *31*, 2035−2043.

(63) Taguchi, H.; Isobe, K.; Nakamura, Y.; Kawaguchi, S. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 2030−2035.

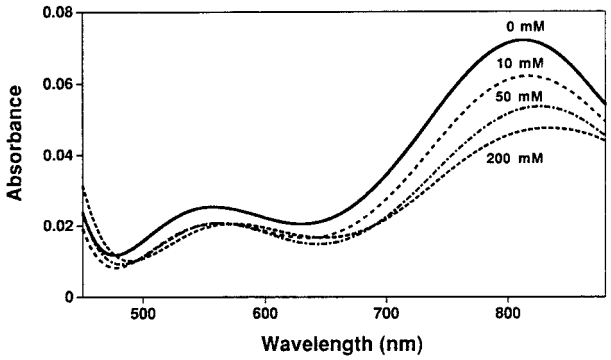
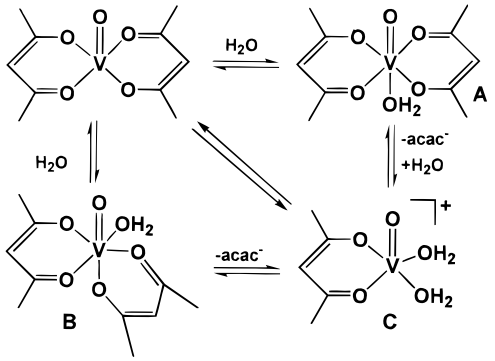


Figure 8. Visible spectra of solutions of 1.0 mM $VO(acac)_2$ and 1.0 mM Hacac plus increasing concentrations of NaF (0 (pH 5.8), 10 (pH 5.8), 50 (pH 5.7), and 200 mM (pH 5.7)). Although the experiment was carried out by the addition of an aqueous solution of NaF, dilution is corrected for in the spectra shown here.

Scheme 1. Species A, B, and C



of the coordinated water molecules). At this time we have not attempted to distinguish between the structural possibilities for species C.

Aqueous $VO(acac)_2$ and $VO(R\text{-}acac)_2$ in the Presence of Halide Salts. The effects of halide salts on $VO(acac)_2$ and derivatives in aqueous solution were important to determine given the need to add NaCl to the administration ligand to disguise the taste of the vanadium compounds.[52] The effects of addition of NaF, NaCl, and NaBr to aqueous solutions of crystalline $VO(R\text{-}acac)_2$ were examined by visible spectroscopy and by EPR spectroscopy at 20 °C. Since the results with NaF were most dramatic, these are described first. The visible spectra for solutions of $VO(acac)_2$ in the presence of 10, 50, and 200 mM NaF are shown in Figure 8. At 200 mM NaF, the absorbances for the $VO(acac)_2$ species had decreased by 50%. The lowering of the concentration of observable $VO(acac)_2 \cdot H_2O$ adduct shows that either a new complex with similar absorbance maxima but smaller extinction coefficients had formed or the speciation in solution had changed. Attempts were made to clarify these issues using the weakly coordinating perchlorate ion. Although the findings go beyond the scope of this work, suffice it to say that the addition of high concentrations of perchlorate both caused color changes for solutions of $VO(acac)_2$ and decreased absorbances. These results are consistent with the observation of a significant salt effect on speciation of the $VO(acac)_2$ system. It is likely that halides also exhibit such an effect. Similar but more pronounced effects were observed for the $VO(Me\text{-}acac)_2$ and the $VO(Et\text{-}acac)_2$ systems upon addition of perchlorate.

The addition of 10 mM NaF reduced the absorbance at 820 nm to approximately 85% for $VO(acac)_2$, 50% for $VO(Et\text{-}acac)_2$, and 80% for $VO(Me\text{-}acac)_2$. Solutions of 1.0 mM $VO(Et\text{-}acac)_2$ in the presence of 50 mM HEt-acac were prepared, and 10 mM NaF, 85 mM NaCl, and 85 mM NaBr were introduced to compare the effects of the different halide ions (Figure 9). NaF produced the greatest effects on the visible spectra, which is consistent with the fact that fluoride anion binds more strongly to vanadyl anion than other halides.[64,65] As illustrated by the addition of NaCl and NaBr, all salts induce changes in the extinction coefficient of the absorbance band at 820 nm, but also affect the λ_{max} of the absorbance band at 600 nm. These results are consistent with both formation of a new complex and salt effects, and further information was sought using EPR spectroscopy.

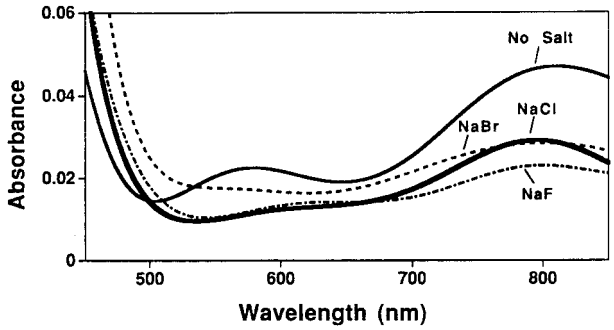


Figure 9. Visible spectra of solutions of 1.0 mM $VO(Et\text{-}acac)_2$ with 85 mM NaBr (pH 4.4), 85 mM NaCl (pH 4.3), or 10 mM NaF (pH 4.3).

Population differences, but no new species, were observed in the EPR spectra of solutions of $VO(acac)_2$ with up to 85 mM NaCl at ambient temperature. Since the complexes with *trans*-coordinated H_2O or *trans*-coordinated Cl^- are likely to have similar EPR parameters, a new EPR signal would not be anticipated. This expectation is supported by the known lability of these types of complexes with coordinated Cl^-, which can exchange readily with other donor ligands.[64] Furthermore, literature reports attest to the fact that chloride ion only poorly competes with H_2O molecules for coordination to the vanadium[66] and that resolved chlorine hyperfine coupling is not observed even when complexes form.[67,68] Given the inconclusiveness of these studies with respect to the formation of new complexes with Cl^-, EPR studies also were conducted with NaBr and NaF salts (Figure 10). As anticipated, the NaF studies showed the most dramatic effects. A 1.0 mM solution of $VO(Et\text{-}acac)_2$ in the presence of 50 mM HEt-acac and NaF (2.5 mM) gave an EPR spectrum with both isomer ratios and parameters (Figure 10d) different from those in the absence of fluoride (Figure 7d). The minor signal had parameters identical to those of C ($A_0 = 101 \times 10^{-4}$ cm^{-1}; $g_0 = 1.970$); the major signal had parameters different from those of any other species described in Table 4, and we will refer to it as species D ($A_0 = 105 \times 10^{-4}$ cm^{-1}; $g_0 = 1.970$). A solution containing a less than equimolar concentration of NaF showed the presence of species A (minor), C (major), and D (minor) (Figure 10b) and disputes the possibility that different parameters are simply due to changes in solution properties. The concentration of species D increases with increasing concentration of fluoride, supporting the interpretation that the new complex contains fluoride (species D).

(64) Selbin, J. *Chem. Rev.* **1965**, *65*, 153−175.
(65) Selbin, J.; Holmes, L. H., Jr.; McGlynn, S. P. *J. Inorg. Nucl. Chem.* **1963**, *25*, 1359−1369.
(66) Zeltmann, A. H.; Morgan, L. O. *Inorg. Chem.* **1971**, *10*, 2739−2745.
(67) McPherson, G. L.; Freedman, M. R. *Inorg. Chem.* **1976**, *15*, 2299−2301.
(68) Jezierski, A.; Raynor, J. B. *J. Chem. Soc., Dalton Trans.* **1981**, 1−7.

Table 5. Relative Compound Composition and Compound Effectiveness in Lowering Plasma Glucose Level

species	four vanadium compounds
A	$VO(acac)_2 > VO(malto)_2 > VO(Et\text{-}acac)_2 > VOSO_4$
B	$VO(malto)_2 > VO(acac)_2 \sim VO(Et\text{-}acac)_2 \sim VOSO_4$
C	$VO(acac)_2 > VO(Et\text{-}acac)_2 > VO(malto)_2 > VOSO_4$
monomeric VO^{2+}	$VOSO_4 > VO(Et\text{-}acac)_2 > VO(acac)_2 \sim VO(malto)_2$
EPR-silent vanadium(IV)	$VOSO_4 \sim VO(Et\text{-}acac)_2 > VO(malto)_2 > VO(acac)_2$
vanadium(V)	$VO(Et\text{-}acac)_2 > VOSO_4 > VO(malto)_2 > VO(acac)_2$
efficacy in lowering plasma glucose level (ref 17)	$VO(acac)_2 > VO(Et\text{-}acac)_2 > VO(malto)_2 > VOSO_4$

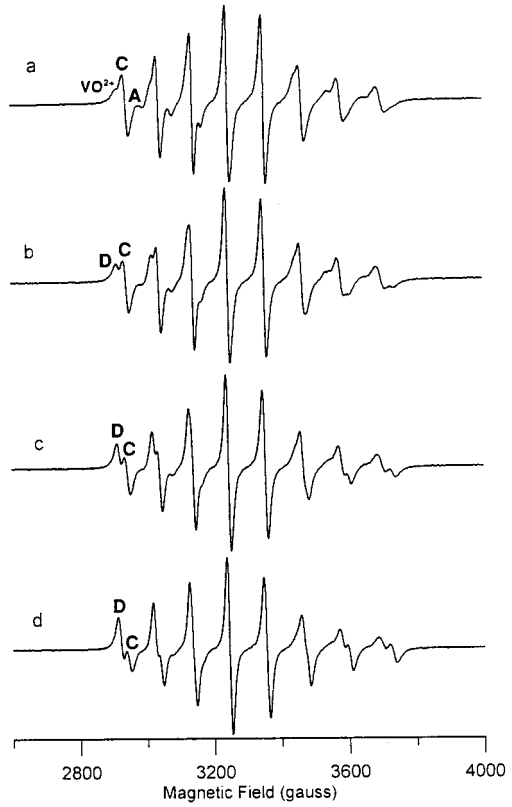


Figure 10. EPR spectra of 1.0 mM $VO(Et\text{-}acac)_2$ with 50 mM HEt-acac and varying concentrations of NaF: (a) 0 mM NaF (t = 10 min, pH = 4.8); (b) 0.3 mM NaF (t = 10 min, pH = 4.7); (c) 1.0 mM NaF (t = 10 min, pH = 4.6); (d) 2.5 mM NaF (t = 10 min, pH = 4.6).

We conclude that addition of halides both affects population ratios of solution $VO(R\text{-}acac)_2$ species and forms new halide-containing complexes. Although, EPR spectra failed to show a new signal for a Cl^--coordinated complex, a new complex was observed in solutions with F^-. The visible spectroscopy of these solutions also supported formation of a halide-containing complex (see also ref 52) as well as salt effects. These observations are in agreement with previous reports of complexes such as $VOF_2(bipy)$ and $VOF_2(phen)$.[69]

Oxidation to Vanadium(V). No evidence for vanadium(V) was found in fresh solutions of $VO(acac)_2$ even when spectral accumulation was increased to 100 000 scans. However, significant concentrations of vanadium(V) were observed for freshly prepared $VO(Et\text{-}acac)_2$ and $VO(Me\text{-}acac)_2$ solutions, which reflects the observed lower stability of these vanadium(IV) complexes. However, for all three derivatives, the quantitative studies described above indicate that not only does oxidation of vanadium(IV) to vanadium(V) take place but also oligo- or polymerization of the vanadium(IV) leads to EPR-silent vanadium(IV) species. The concentration of vanadium(V) was higher in the presence of 85 mM NaCl than in the absence of added salt, which indicates that NaCl reduces the stability of the vanadium(IV) complexes in all cases.[70,71] The concentration of EPR-silent vanadium(IV) species on the other hand decreased in the presence of 85 mM NaCl, consistent with the disruption of such species by the halide ions.[70]

Insulin-Mimetic Properties of $VO(acac)_2$ and $VO(Et\text{-}acac)_2$. The effects of $VO(acac)_2$ and $VO(Et\text{-}acac)_2$ on glycemia in streptozotocin-induced rats were examined and compared to the effects of $VOSO_4$ and $VO(malto)_2$. The full details of the pharmacological study were reported elsewhere.[17] In general, an *in vivo* animal study with one compound is difficult to

(69) Chakravorti, N. C.; Sarkar, A. R. *J. Fluorine Chem.* **1976**, *81*, 421−428.

(70) The quantitation of species was determined by quantitative EPR spectroscopic analysis of solutions containing 85 mM NaCl and 1.00 mM of the crystalline $VO(R\text{-}acac)_2$. The solutions were analyzed immediately after dissolution (at 10 min), after 24 h at ambient temperature, and after 48 h at ambient temperature. The data obtained by EPR spectroscopy are quantitated from total spins (EPR-silent species include both vanadium(IV) and vanadium(V)) and were as follows: Crystalline $VOSO_4$ showed VO^{2+} 0.30 mM at 10 min and pH 4.7 (±0.5), 0.25 mM at 24 h and pH 4.1 (±0.3), and 0.20 mM at 48 h and pH 3.9 (±0.3). The EPR-silent species were 0.70, 0.75, and 0.80 mM. No evidence for any other species was observed. $VO(malto)_2$: at 10 min, pH was 6.6 (±0.3) and speciation was A 0.04 mM (4%), B 0.71 mM (71%), C 0.00 mM (0%), VO^{2+} 0.00 mM (0%), and EPR-silent species 0.25 mM (25%); at 24 h, pH was 4.3 (±0.2) and speciation was A 0.01 mM (1%), B 0.40 mM (40%), C 0.03 mM (3%), VO^{2+} 0.00 mM (0%), and EPR-silent species 0.56 mM (56%); at 48 h, pH was 4.2 (±0.2) and speciation was A <0.005 mM (<0.5%), B 0.34 mM (34%), C 0.06 mM (6%), VO^{2+} 0.00 mM (0%), and EPR-silent species 0.60 mM (60%). $VO(Et\text{-}acac)_2$: at 10 min, pH was 4.6 (±0.2) and speciation was A 0.01 mM (1%), B 0.00 mM (0%), C 0.08 mM (8%), VO^{2+} 0.11 mM (11%), and EPR-silent species 0.80 mM (80%); at 24 h, pH was 4.4 (±0.2) and speciation was A <0.005 mM (<0.5%), B 0.00 mM (0%), C 0.04 mM (4%), VO^{2+} 0.11 mM (11%), and EPR-silent species 0.85 mM (85%); at 48 h, pH was 4.4 (±0.2) and speciation was A <0.003 mM (<0.3%), B 0.00 mM (0%), C 0.02 mM (2%), VO^{2+} 0.08 mM (8%), and EPR-silent species 0.90 mM (90%). $VO(acac)_2$: at 10 min, pH was 6.4 (±0.3) and speciation was A 0.68 mM (68%), B 0.00 mM (0%), C 0.22 (22%), VO^{2+} 0.00 mM (0%), and EPR-silent species 0.10 mM (10%); at 24 h, pH was 5.7 (±0.2) and speciation was A 0.57 mM (57%), B 0.00 mM (0%), C 0.28 mM (28%), VO^{2+} 0.00 mM (0%), and EPR-silent species 0.15 mM (15%); at 48 h, pH was 5.6 (±0.2) and speciation was A 0.48 mM (48%), B 0.00 mM (0%), C 0.32 mM (32%), VO^{2+} 0.00 mM (0%), and EPR-silent species 0.20 mM (20%). Although the results listed above were for 1.00 mM solutions, similar results were obtained with solutions at different times and at different concentrations.

(71) ^{51}V NMR spectra were recorded to determine how much of the compounds had oxidized to vanadium(V). Subtracting the amounts of measured vanadium(V) from the amounts of EPR-silent species determined above gives the amounts of EPR-silent vanadium(IV) in solution. $VOSO_4$ showed 0.25 mM vanadium(V) at 10 min and pH 4.7 (±0.5), 0.30 mM vanadium(V) at 24 h and pH 4.1 (±0.3), and 0.60 mM vanadium(V) at 48 h and pH 3.9 (±0.3). $VO(malto)_2$ showed 0.10 mM (10%) vanadium(V) at 10 min and pH 6.6 (±0.3), 0.40 mM (40%) vanadium(V) at 24 h and pH 4.3 (±0.2), and 0.45 mM (45%) vanadium(V) at 48 h and pH 4.2 (±0.2). $VO(Et\text{-}acac)_2$ showed 0.40 mM (40%) vanadium(V) at 10 min and pH 4.6 (±0.2), 0.55 mM (55%) vanadium(V) at 24 h and pH 4.4 (±0.2), and 0.65 mM (65%) vanadium(V) at 48 h and pH 4.4 (±0.2). $VO(acac)_2$ showed 0.10 mM (10%) vanadium(V) at 10 min and pH 6.4 (±0.3), 0.15 mM (15%) vanadium(V) at 24 h and pH 5.7 (±0.2), and 0.20 mM (20%) vanadium(V) at 48 h and pH 5.6 (±0.2). Repeat measurements (using different stock solutions, different instrumental settings, etc.) resulted in quantitative data within 5% of those reported.

compare to other reported studies given the many biological variables. In light of the chemical studies in this paper, and the reported differences in compound efficacies in the study with four different vanadium(IV) compounds,[17] the vanadium speciation[70,71] was studied for a solution similar to those administered to the rats.

The populations of the relevant species in aqueous 85 mM NaCl were measured using EPR spectroscopy[70] and ^{51}V NMR spectroscopy.[71] Since the administration solutions were changed every 2 days, 3 time points were selected to provide a cross section of information regarding the composition of the compounds used for animal treatment. The solution composition was determined immediately after preparation ($t = 10$ min), after 24 h, and after 48 h. The concentration of each EPR-observable species was determined by double integration and computer simulation,[70] and the concentration of vanadium(V) was determined by quantitative ^{51}V NMR spectroscopy.[71] The concentration of vanadium species in 85 mM NaCl solutions followed the general stability patterns as anticipated from the studies described earlier in this paper. Briefly, $VO(acac)_2$ was more stable with respect to oxidation than either $VO(Et\text{-}acac)_2$ or $VO(malto)_2$ as evidenced by both the greater concentrations of vanadium(IV) species in the $VO(acac)_2$ system and the greater concentration of vanadium(V) species formed in the $VO(Et\text{-}acac)_2$ and $VO(malto)_2$ systems. Furthermore, the predominant adducts for $VO(acac)_2$, $VO(malto)_2$, and $VO(Et\text{-}acac)_2$ were the *trans* adduct, the *cis* adduct, and the EPR-silent vanadium(IV) species, respectively.

Since the type of compound administered is important for the effects induced, the question was asked: Would the vanadium speciation in the aqueous administration liquid trace the observed compound effectiveness of plasma glucose lowering? The effectiveness of compounds in reducing the final glucose levels in STZ-diabetic rats after 12 weeks was as follows: $VOSO_4 < VO(malto)_2 < VO(Et\text{-}acac)_2 < VO(acac)_2$ (Table 5). Considering the fact that $VOSO_4$ does not form species A, B, or C, for the sake of completion we included three additional types of vanadium, namely, the hydrated monomeric VO^{2+}, the EPR-silent vanadium(IV), and the oxidized vanadium(V) species. Since the concentrations of species were measured at three different times, these studies provided additional information regarding compound properties. In the freshly prepared solutions the highest concentration of the major species is formed, and as time proceeds this species is depleted as the respective decomposition products form.[70,71] Species A is identified to be the major species on dissolution of $VO(acac)_2$, species B is the major species on dissolution of $VO(malto)_2$, and the EPR-silent vanadium(IV) is the major type of vanadium on dissolution of $VO(Et\text{-}acac)_2$ (species C is the major observable EPR-active species). In Table 5, the order of amounts of species A, B, C, VO^{2+}, EPR-silent vanadium(IV), and oxidation products is listed for the four vanadium compounds $VOSO_4$, $VO(malto)_2$, $VO(acac)_2$, and $VO(Et\text{-}acac)_2$. Interestingly, the concentration of the 1:1 hydrolysis product (species C) is in the order $VO(acac)_2 > VO(Et\text{-}acac)_2 > VO(malto)_2 > VO^{2+}$, which is the same order as observed for the *in vivo* effectiveness of this series of compounds (Table 5). None of the other species have similar populations. At the risk of oversimplifying, the observation that the population of the 1:1 hydrolysis product follows the observed *in vivo* insulin-mimetic action of the vanadium compounds may have implications for insulin-mimetic action of these compounds.

Studies with other compounds have been reported in the literature,[51,72,73] but the difficulties involved in comparison of effects of compounds in different studies, given type and varying levels of glycemia, residual insulinemia, rodent species, etc., prevent inclusion of other compounds in this comparison. However, it is likely that the stability pattern of a series of complexes in the administration fluid is similar to the stability pattern in cellular media. Thus the observation that populations of species parallel the compound efficacy may be of further mechanistic importance. Several proposed hypotheses explaining the insulin-mimetic properties of vanadium compounds involve formation of protein−vanadium complexes,[4,74] and the speciation described here may provide clues to the chemical nature of the vanadium component of such complexes.

Acknowledgment. D.C.C. thanks the Institutes for General Medical Sciences at the National Institutes of Health, and S.M.B. thanks the Foundation of Scientific and Medical Research (3.4513.93) for funding this work.

IC9905897

(72) Sakurai, H.; Fujii, K.; Watanabe, H.; Tamura, H. *Biochem. Biophys. Res. Commun.* **1995**, *214*, 1095−1101.
(73) Watanabe, H.; Nakai, M.; Komazawa, K.; Sakurai, H. *J. Med. Chem.* **1994**, *37*, 876−877.
(74) Tracey, A. S.; Crans, D. C. *ACS Symp. Ser*. **1998**, *711*.