Novel Approaches to Insulin Mimetic Vanadium Compounds

by

Marco Melchior

B. Sc. (Hons.), The University of Western Ontario, 1994

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

(Department of Chemistry)

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

OCTOBER 1999

© Marco Melchior, 1999
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Chemistry

The University of British Columbia
Vancouver, Canada

Date Nov 10, 1995
Abstract

Novel approaches to the synthesis of insulin mimetic vanadium compounds were explored. Derivatives of the potent insulin mimetic complex VO(ema)₂ or bis(ethylmaltolato)oxovanadium(IV) were successfully synthesized, including the highly lipophilic Hema (ethylmaltol) adduct [VO(ema)₂(Hema)]·H₂O, as well as [VOCI(Hema)₂]Cl·H₂O, a water-soluble salt form of VO(ema)₂. [VO(ema)₂(Hema)]·H₂O, VO(ema)₂ and [VOCI(Hema)₂]Cl·H₂O were characterized in the solid state by infrared spectroscopy, mass spectrometry and elemental analysis. [VO(ema)₂(Hema)]·H₂O and [VOCI(Hema)₂]Cl·H₂O are hydrolytically unstable and serve as VO(ema)₂ procomplexes and potential prodrugs. VO(ema)₂ was characterized in aqueous solution through a combination of spectrophotometry, electron paramagnetic resonance spectroscopy and potentiometry. VO(ema)₂ was found to be stable to hydrolysis.

Novel vanadium(III) complexes were synthesized, including V(ma)₃ or tris(maltolato)vanadium(III), V(ema)₃ or tris(ethylmaltolato)vanadium(III), V(koj)₃·H₂O or tris(kojato)vanadium(III) monohydrate and V(dpp)₃·12H₂O or tris(1,2-dimethyl-3-oxy-4-pyridinonato)vanadium(III) dodecahydrate, and were characterized in the solid state by infrared spectroscopy, mass spectrometry and elemental analysis. The X-ray structure determination of V(dpp)₃·12H₂O is reported. V(ma)₃, V(ema)₃ and V(koj)₃·H₂O were found to be hydrolytically stable at physiological pH through potentiometry. In the first examination of a vanadium(III) complex for insulin-enhancing activity, administration of V(ma)₃ was found to normalize blood glucose levels in STZ-diabetic rats.
Neutral oxovanadium(V) complexes including $V_2O_3(ema)_4$, $VO(fla)_2(OCH_3)$ or bis(flavonato)methoxyoxovanadium(V) and $[VO_2(dpp)]•Hdpp•H_2O$ were synthesized and characterized by infrared spectroscopy, mass spectrometry and elemental analysis.
# TABLE OF CONTENTS

Abstract

Table of Contents

List of Figures

List of Tables

List of Schemes

List of Abbreviations

Acknowledgments

Chapter 1  Vanadium Complexes as Insulin-Enhancing Agents

1.1 General Introduction

1.2 References

Chapter 2  Derivatives of VO(ema)₂

2.1 Introduction

2.2 Experimental

2.3 Results and Discussion

2.4 Conclusions

2.5 References

Chapter 3  The Synthesis of Novel Vanadium(III) Compounds

3.1 Introduction

3.2 Experimental

3.3 Results and Discussion

3.4 Conclusions

3.5 References
<table>
<thead>
<tr>
<th>Appendix II</th>
<th>Glucose Normalization by V(ma)₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>II.i Introduction</td>
<td>127</td>
</tr>
<tr>
<td>II.ii Experimental</td>
<td>128</td>
</tr>
<tr>
<td>II.iii Results and Discussion</td>
<td>129</td>
</tr>
<tr>
<td>II.iv Conclusions</td>
<td>133</td>
</tr>
<tr>
<td>II.v References</td>
<td>133</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Appendix III</th>
<th>X-ray Crystallographic Data for V(dpp)₃•12H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>134</td>
</tr>
</tbody>
</table>
List of Figures

| Figure 1.1 | Insulin-Enhancing Vanadyl Complexes. | 3 |
| Figure 1.2 | Reactions of Hma. | 5 |
| Figure 2.1 | Infrared Spectra of $[\text{VO}(\text{ema})_2(\text{Hema})]\cdot\text{H}_2\text{O}$, $\text{VO}(\text{ema})_2$ and $[\text{VOCl}(\text{Hema})_2]\cdot\text{H}_2\text{O}$. Key Vibrations Include $\nu_{\text{pyrone}}$ (a and b), $\nu_{\text{V=O}}$ (c), and $\nu_{\text{V-O}}$ (d). | 23 |
| Figure 2.2 | Positive Ion Detection Mode LSI Mass Spectrum of $[\text{VO}(\text{ema})_2(\text{Hema})]\cdot\text{H}_2\text{O}$ (L = ema). | 26 |
| Figure 2.3 | cis-$[\text{VO}(\text{ma})_2(\text{OCH}_2\text{CH}_2\text{OH})]$, a Proposed Model for $[\text{VO}(\text{ema})_2(\text{Hema})]\cdot\text{H}_2\text{O}$. | 27 |
| Figure 3.1 | Hma, Hkoj, Hema and Hdpp. | 32 |
| Figure 3.2 | Infrared Spectra of $\text{VO}(\text{ema})_2$, $\text{V(ema)}_3$ and Hema. Key Vibrations Include $\nu_{\text{pyrone}}$ (a and b), $\nu_{\text{V=O}}$ (c) and $\nu_{\text{V-O}}$ (d). | 37 |
| Figure 3.3 | Positive Ion Detection Mode LSI Mass Spectra for V(ema)$_3$ and $[\text{VO}(\text{Hema})_2(\text{Hema})]\cdot\text{H}_2\text{O}$ (L = ema). | 38 |
| Figure 3.4 | ORTEP Diagram of $\text{V(dpp)}_3\cdot12\text{H}_2\text{O}$ Showing the Crystallographic Numbering. Thermal Ellipsoids for Non-Hydrogen Atoms are Drawn at 33% Probability. | 43 |
| Figure 3.5 | ORTEP View Down the c Axis of the Unit Cell Packing of $\text{V(dpp)}_3\cdot12\text{H}_2\text{O}$. | 45 |
| Figure 4.1 | Calculated Speciation Diagrams for $\text{VO(ma)}_2$ at 10 mM and 0.1 mM (0.16 M NaCl, 25 °C). | 58 |
| Figure 4.2 | Beer's Law Plots for $\text{VO(ema)}_2$ (0.16 M NaCl, 25 °C). | 65 |
| Figure 4.3 | Plot of Absorbance$_{435}$ vs. $[\text{VO(ema)}_2]$ for 25 mM $\text{VO(ema)}_2$ (0.1 M HCl, 25 °C). | 66 |
| Figure 4.4 | Plots of Absorbance vs. Wavelength for the Variable pH Titration of $[\text{VO}^{2+}] = 968 \mu\text{M}$, $[\text{Hema}] = 94 \mu\text{M}$ (25 °C, 0.16 M NaCl). | 67 |
| Figure 4.5 | Plot of Absorbance$_{313}$ vs. $[\text{H}^+]^{-1}$ for $[\text{VO}^{2+}] = 968 \mu\text{M}$, $[\text{Hema}] = 94 \mu\text{M}$ (25 °C, 0.16 M NaCl). | 67 |
| Figure 4.6 | Plot of Absorbance vs. Wavelength for the Dilution | 69 |
of 5 mM V(ma)$_3$ (25 °C, 0.16 M NaCl).

**Figure 4.7** Plot of Absorbance$_{570}$ vs. [V(ma)$_3$] (25 °C, 0.16 M NaCl).

**Figure 4.8** Isotropic EPR Spectrum of 3 mM VO(ema)$_2$ in 50% Aqueous Glycerol (pH = 7.0, T = 298 K, ν = 9.5905 GHz).

**Figure 4.9** Experimental and Simulated Isotropic EPR Spectra of 1mM VO(ema)$_2$ in CH$_2$Cl$_2$ (T = 298 K, ν = 9.5855 GHz).

**Figure 4.10** Experimental and Simulated Anisotropic Frozen-solution EPR Spectra of 1mM VO(ema)$_2$ in CH$_2$Cl$_2$ (T = 177 K, ν = 9.5927 GHz).

**Figure 4.11** Plot of pH vs. V$_{NaOH}$ for 1.1 mM Hema (25 °C, 0.16 M NaCl).

**Figure 4.12** Plot of Z vs. pH for Hema 1.1 mM (■), 0.32 mM (□), 0.93 mM (△) and 0.84 mM (▲) (25 °C, 0.16 M NaCl). The Solid Line Corresponds to a Curve Fit for pK$_a$ (Hema) = 8.48(5) Based on Equation 4.1. The Dotted Line is Z = -0.5.

**Figure 4.13** Plot of Z vs. pH for V(koj)$_3$ at 1.8 mM (○), 1.8 mM (■), 1.3 mM (△)1.1 mM (▲) (0.16 M NaCl, 25 °C). The Area Between the Two Solid Lines Represents the Region where [V(koj)$_3$] > 95% Total Vanadium.

**Figure 4.14** Plot of Z vs. pH for V(ma)$_3$ at 2.3 mM (■), 1.6 mM (○) (0.16 M NaCl, 25 °C). The Area Between the Two Solid Lines Represents the Region where [V(ma)$_3$ (aq)] > 95% Total Vanadium.

**Figure 4.15** Plot of Z vs. pH for 2.6 mM VO(ema)$_2$ (■), 3.3 mM V(ema)$_3$ (+) and 1.1 mM Hema (○). The Region Between the Two Solid Lines Describes the Region of Predominance (> 95% formation) for the Respective Compounds (0.16 M NaCl, 25 °C).

**Figure 4.16** Plot of Z vs. pH for 2.6 mM VO(ema)$_2$ (●) and 1.1 mM [VOCl(Hema)$_2$Cl]Cl•H$_2$O (+) (0.16 M NaCl, 25 °C). The Region Between the Two Solid Lines Describes the Region of Predominance (> 95% Formation) for VO(ema)$_2$.

**Figure 4.17** Speciation Diagram for VO(ema)$_2$ (0.16 M NaCl, 25 °C).

**Figure 4.18** Speciation Diagram for V(ma)$_3$ (0.16 M NaCl, 25 °C).

**Figure 4.19** Visible Electronic Absorption Spectra of the Competition of VO(ema)$_2$ (aq) with H$_2$EDTA$^{2-}$ (aq) (0.16 M NaCl, 25 °C).

**Figure 5.1** Infrared Spectrum of [VO$_2$(dpp)]•Hdpp•H$_2$O.
Figure 5.2 Oxidation of V(ma)₃ in the Presence of Excess Hma
([V(ma)₃] = 1.77 mM, [Hma] = 35.5 mM, pO₂ = 1 atm, [KCl] = 1M,
[KPTH] = 0.05M, pH 4.0, 298 K).

Figure 6.1 Flavonol and Naturally Occurring Analogs.

Figure 6.2 The Modified Algar-Flynn-Oyamada Synthesis of Flavonol.

Figure 6.3 Infrared Spectra of VO(fla)₂(OCH₃) and Hfla.

Figure 7.1 One-Pot Synthesis of Pyromeconic Acid.

Figure 7.2 Tropolone and Naturally Occuring Derivatives.

Figure II.i Glucose Normalization of STZ-diabetic Wistar Rats by V(ma)₃
(n = 5) or VO(ma)₂ (n = 5) Administered by i. p. Injection (Symbols Represent
Individual Animals).

Figure II.ii Glucose Normalization of STZ-diabetic Wistar Rats by V(ma)₃
(n = 5) or VO(ma)₂ (n = 5) Administered by Oral Gavage (Symbols Represent
Individual Animals).
List of Tables

**Table 1.1** Toxicity of Representative Vanadium Compounds. 8

**Table 2.1** Elemental Analyses (found [calc.]) and Bulk Magnetic Moments (μ_eff, B. M.). 18

**Table 2.2** Selected Infrared Absorptions (KBr, ±4 cm^{-1}, Values in Parentheses Describe Changes from the Free Ligand Values, Δ cm^{-1}). 22

**Table 2.3** Selected Ions (Positive Ion Detection Mode LSIMS, % Relative Intensity) for [VO(ema)_2(Hema)]H_2O (1), VO(ema)_2 (2) and [VOCI(Hema)_2]Cl·H_2O (3). 25

**Table 3.1** Elemental Analyses (found [calc.]) and Bulk Magnetic Moments (B. M.). 35

**Table 3.2** Selected Infrared Vibrations (KBr, ± 4 cm^{-1}, Values in Parentheses Describe Changes from the Free Ligand Values, Δ cm^{-1}). 36

**Table 3.3** Selected Ions [Positive Ion Detection Mode LSIMS, m/z (% relative intensity)]. 39

**Table 3.4** Selected Bond Lengths (Å) and Angles (deg) in V(dpp)_3·12H_2O with Estimated Standard Deviations in Parentheses. 44

**Table 4.1** Visible Spectral Parameters. 63

**Table 4.2** Spin Hamiltonian Parameters for VO(ma)_2 and VO(ema)_2. 73

**Table 4.3** Conditional Acidity Constants (the Error in Parentheses Refers to 3α in the Last Digit) (0.16 M NaCl, 25 °C). 84

**Table 5.1** Elemental Analyses (found [calc.]). 95

**Table 5.2** Selected Infrared (KBr, ±4 cm^{-1}, Values in Parentheses Describe Reductions from the Free Ligand values, Δ cm^{-1}). 100

**Table 5.3** Selected Ions (Positive Ion Detection Mode LSIMS). 102

**Table 5.4** Oxidation Products of the Solid Oxidation of the V(III) Complexes. 103

**Table 6.1** Selected Ions (Positive Ion Detection Mode LSIMS and EIMS) for [VO(fia)_2(OCH_3)] 112
Table I.i Reference and Standard States for Stoichiometric and Absolute Equilibrium Constants. 124

Table III.i Selected Crystallographic Data for V(dpp)$_3$•12H$_2$O. 134

Table III.ii Bond Lengths (Å). 134

Table III.iii Bond Angles (deg). 135

Table III.iv Hydrogen bonds and C-H...O Interactions. 135
<table>
<thead>
<tr>
<th>Scheme</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scheme 2.1</td>
<td>20</td>
</tr>
<tr>
<td>Scheme 4.1</td>
<td>53</td>
</tr>
<tr>
<td>Scheme 4.2</td>
<td>55</td>
</tr>
<tr>
<td>Scheme 4.3</td>
<td>85</td>
</tr>
<tr>
<td>Scheme 4.4</td>
<td>87</td>
</tr>
<tr>
<td>Scheme 5.1</td>
<td>97</td>
</tr>
<tr>
<td>Scheme 5.2</td>
<td>98</td>
</tr>
<tr>
<td>Scheme 6.1</td>
<td>110</td>
</tr>
</tbody>
</table>
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>angstrom, $10^{-10}$ m</td>
</tr>
<tr>
<td>acac$^-$</td>
<td>acetylacetonate anion</td>
</tr>
<tr>
<td>Al(ma)$_3$</td>
<td>tris(maltolato)aluminum(III)</td>
</tr>
<tr>
<td>anal.</td>
<td>analysis</td>
</tr>
<tr>
<td>a. u.</td>
<td>arbitrary units</td>
</tr>
<tr>
<td>B. M.</td>
<td>Bohr Magneton</td>
</tr>
<tr>
<td>°C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>calc.</td>
<td>calculated</td>
</tr>
<tr>
<td>cat$^{2-}$</td>
<td>catecholate dianion</td>
</tr>
<tr>
<td>Cp$^-$</td>
<td>cyclopentadienyl anion</td>
</tr>
<tr>
<td>deg</td>
<td>degree</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulphoxide</td>
</tr>
<tr>
<td>dpp$^-$</td>
<td>1,2-dimethyl-3-hydroxy-4-pyridinonate anion</td>
</tr>
<tr>
<td>$\varepsilon$</td>
<td>extinction coefficient (UV-vis)</td>
</tr>
<tr>
<td>ED$_{50}$</td>
<td>efficacy dose for 50 % activity</td>
</tr>
<tr>
<td>EDTA$^{4-}$</td>
<td>N, N, N',N'-ethylenediaminetetraacetate tetraanion</td>
</tr>
<tr>
<td>EI</td>
<td>electron ionization</td>
</tr>
<tr>
<td>EIMS</td>
<td>electron ionization mass spectrometry</td>
</tr>
<tr>
<td>ema$^-$</td>
<td>ethylmaltolate anion</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>------</td>
<td>------------</td>
</tr>
<tr>
<td>EPR</td>
<td>electron paramagnetic resonance</td>
</tr>
<tr>
<td>fla^-</td>
<td>3-hydroxyflavonate anion</td>
</tr>
<tr>
<td>Hema</td>
<td>ethylmaltol, 3-hydroxy-2-ethyl-4-pyrene</td>
</tr>
<tr>
<td>H_2ema^+</td>
<td>ethylmaltolium cation</td>
</tr>
<tr>
<td>Hdpp</td>
<td>1,2-dimethyl-3-hydroxy-4-pyridinone</td>
</tr>
<tr>
<td>Hfla</td>
<td>flavonol, 3-hydroxyflavone</td>
</tr>
<tr>
<td>Hkoj</td>
<td>kojic acid, 5-hydroxy-2-hydroxymethyl-2-pyrene</td>
</tr>
<tr>
<td>Hma</td>
<td>maltol, 3-hydroxy-2-methyl-4-pyrene</td>
</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>i. p.</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>K</td>
<td>Kelvin, formation constant</td>
</tr>
<tr>
<td>K_a</td>
<td>acid dissociation constant</td>
</tr>
<tr>
<td>K_{ba}</td>
<td>basicity-adjusted formation constant</td>
</tr>
<tr>
<td>koj^-</td>
<td>kojate anion</td>
</tr>
<tr>
<td>KPTH</td>
<td>potassium hydrogen phthalate</td>
</tr>
<tr>
<td>( \lambda )</td>
<td>wavelength</td>
</tr>
<tr>
<td>( \lambda_{max} )</td>
<td>wavelength at peak maximum (UV-vis spectroscopy)</td>
</tr>
<tr>
<td>LD_{50}</td>
<td>lethal dose for 50% mortality</td>
</tr>
<tr>
<td>LD_{100}</td>
<td>lethal dose for 100% mortality</td>
</tr>
<tr>
<td>LSI</td>
<td>liquid secondary ion</td>
</tr>
<tr>
<td>LSIMS</td>
<td>liquid secondary ion mass spectrometry</td>
</tr>
<tr>
<td>( \mu_{eff} )</td>
<td>effective magnetic moment</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>----------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>M</td>
<td>molarity or metal ion</td>
</tr>
<tr>
<td>$M_v$</td>
<td>vanadium molarity</td>
</tr>
<tr>
<td>$ma^-$</td>
<td>maltolate anion</td>
</tr>
<tr>
<td>mL</td>
<td>millilitre</td>
</tr>
<tr>
<td>mmol</td>
<td>millimole</td>
</tr>
<tr>
<td>mol</td>
<td>mole</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>m/z</td>
<td>mass-to-charge ratio (in mass spectrometry)</td>
</tr>
<tr>
<td>$\nu$</td>
<td>vibration frequency (IR), microwave frequency (EPR)</td>
</tr>
<tr>
<td>Naglivan</td>
<td>bis(N-octylcysteineamido)oxovanadium(IV)</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>ORTEP</td>
<td>Oakridge Thermal Ellipsoid Program</td>
</tr>
<tr>
<td>pH</td>
<td>negative log of proton activity or proton concentration</td>
</tr>
<tr>
<td>$pK_a$</td>
<td>negative log of the acid dissociation constant</td>
</tr>
<tr>
<td>pro-ligand</td>
<td>a compound from which the ligand bound to the metal is derived</td>
</tr>
<tr>
<td>RT</td>
<td>room temperature</td>
</tr>
<tr>
<td>STZ</td>
<td>streptozotocin</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>V(acac)$_3$</td>
<td>tris(acetylacetonato)vanadium(III)</td>
</tr>
<tr>
<td>[V(cat)$_3$]$^3-$</td>
<td>tris(catecholato)vanadate(III)</td>
</tr>
<tr>
<td>vis</td>
<td>visible</td>
</tr>
<tr>
<td>VO(acac)$_2$</td>
<td>bis(acetylacetonato)oxovanadium(IV)</td>
</tr>
</tbody>
</table>
VO(dpp)$_2$  bis(1,2-dimethyl-3-oxy-4H-pyridin-4-onato)oxovanadium(IV)

VO(koj)$_2$  bis(kojato)oxovanadium(IV)

VO(ma)$_2$  bis(maltolato)oxovanadium(IV)

VO(metf)$_2$  bis(1,2-dimethybiguanidato)oxovanadium(IV)

VO(pic)$_2$  bis(picolinato)oxovanadium(IV)

VO(SALEN)  (N,N'-disalicylideneethylene diamino)oxovanadium(IV)

V-P  bis(pyrrolidine-N-carbodithiolato)oxovanadium(IV)

V(trencam)$^{3-}$  (tris(trihydroxyphenyl-triaminotriethylamino)vanadate(III)

UV  ultraviolet

X-Band  ~ 9 GHz (in EPR)

Z  proton stoichiometry (in potentiometry)

Z  number of molecules in the unit cell (in X-ray crystallography)
Acknowledgments

First, I would like to thank Prof. Chris Orvig for his endless patience, support, for allowing great scientific freedom and for encouraging creativity, not to mention his inspiring taste in music. I would like to thank Dr. Kathie Thompson for helpful discussions, encouragement and the odd pumpkin pie. I would also like to thank Dr. Graeme Hanson for the introduction to EPR. I would especially like to thank the late Dr. Steve Rettig for the X-ray determination of the V(dpp)$_3$$\cdot$12H$_2$O. I would also like to thank Violet Yuen and Prof. J. H. McNeill in the Faculty of Pharmaceutical Sciences for all aspects of the animal studies.

I would like to thank my colleagues in the group, past and present. Special thanks go to Dr. Ika, Leon, Pete, Mark, Grandpa and Ashley.

I extend a special thanks to the departmental support staff.

Finally, I acknowledge NSERC and the University, through a UGF, for financial support.
Chapter 1

Vanadium Complexes as Insulin-Enhancing Agents

1.1 General Introduction

This thesis examines the systematic variation of the properties of novel vanadium complexes, potentially useful as inorganic pharmaceuticals, based on the principles of coordination chemistry. This work serves as part of a collaborative effort in examining whether vanadium complexes have a role in the modern pharmacy. Vanadium is found at low levels in most living creatures and there is debate as to whether vanadium is an essential element for man; it is most likely not, or if so, it is essential at the trace levels usually present.\(^1\) Vanadium complexes possess a wide range of effects ranging from deleterious to potentially beneficial, depending on the properties of the vanadium complex, the dose, and the biological route of entry. A beneficial effect of vanadium complexes, first documented for inorganic vanadates(V),\(^2\) is a long term insulin-like ability to normalize blood glucose orally in animal models of diabetes. Because insulin is not orally active, orally active vanadium complexes are potentially useful in the treatment of *diabetes mellitus*.

Generally, metal-based drugs are "pro-drugs" undergoing some transformation to an active species *in vivo*. Anti-syphilitic arsenicals including Salvarsan are also pro-drugs and are activated by oxidation to As(V).\(^3\) Vanadium-based insulin mimics may also be considered to be pro-complexes, serving to maximize absorption of vanadium, with the vanadium necessarily released from the complex in order to exhibit the insulin-mimetic or insulin-enhancing effect.*

---

* Vanadium complexes do not function in the absence of insulin.
The *in vivo* speciation of vanadium complexes is still uncertain; however, a study of the *in vitro* speciation provides some insight into the *in vivo* speciation. Maximizing absorption for metal-based drugs is of utmost importance because therapeutic and toxic doses are often comparable for metal-based drugs. For example, for Li anti-depressants, the toxic dose represents three times the therapeutic dose.\(^4\) Inorganic vanadium salts are similar, becoming toxic at levels near the dose required for activity. Minimizing the vanadium dose and toxicity of the administered complex, while retaining activity, is a crucial aspect of the design of new complexes.

Other important properties of vanadium complexes as potential pharmaceutical agents include stability to hydrolysis and oxidation. Lower oxidation states are of interest because oxidants such as V(V) are generally undesirable due to the increased oxidative stress associated with both type I and type II *diabetes mellitus*.\(^5\) It is also desirable for a vanadium complex suitable for treatment of type II (non-insulin dependent) *diabetes mellitus* not to induce insulin secretion, serving instead as a partial hormone substitute or an insulin-enhancing agent.\(^6\)

Vanadyl (VO\(^{2+}\)) sulphate has been demonstrated to possess oral insulin-like activity similar to that of the vanadates(V), with lowered toxicity at comparable doses.\(^7,8\) Vanadyl salts are known to be poorly absorbed and therefore much less bioavailable compared to vanadates(V).\(^9\) Therefore, in an attempt to improve the bioavailability of vanadyl complexes, a variety of low molecular weight neutral vanadyl complexes with a variety of coordination environments have been examined.
as potential insulin-enhancing agents ranging from the common VO(O₄), to VO(N₂O₂),¹⁰⁻¹² VO(N₄),¹³ VO(S₄)¹⁴ and VO(N₂S₂) (Figure 1.1).¹⁵

**Figure 1.1** Insulin-Enhancing Vanadyl Complexes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Coordination Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO(ma)₂</td>
<td>VO(O₄)</td>
</tr>
<tr>
<td>VO(pic)₂</td>
<td>VO(N₂O₂)</td>
</tr>
<tr>
<td>V-P</td>
<td>VO(S₄)</td>
</tr>
<tr>
<td>VO(metf)₂</td>
<td>VO(N₄)</td>
</tr>
<tr>
<td>Naglivan</td>
<td>VO(N₂S₂)</td>
</tr>
</tbody>
</table>
The maltolate complex VO(ma)$_2$, bis(maltolato)oxovanadium(IV), which is simply and economically synthesized on a large scale, possesses equivalent oral glucose normalizing activity at significantly lower total doses of vanadium when compared to vanadyl sulphate.\textsuperscript{16} Additionally, VO(ma)$_2$ was found to induce long-term maintained glucose normalization with no evidence of toxicity when administered over a 6 month period to STZ-diabetic rats in drinking water.\textsuperscript{17,18} The structure of VO(ma)$_2$ has been described.\textsuperscript{19} The solution thermodynamics have been reported\textsuperscript{19} and independently corroborated.\textsuperscript{20} As well, a detailed EPR study in a variety of solvents\textsuperscript{21} and the solution oxidation in aqueous and non-aqueous media have been presented.\textsuperscript{22} This work builds on earlier studies and expands into new areas of the coordination chemistry of V(III) and V(IV) compounds.

Maltol and related 3-hydroxy-4-pyrones are natural products (acetogenins) which occur as fungal carbohydrate metabolites, as well as being produced as byproducts of fermentation. Maltol (Hma), ethylmaltol (Hema) and kojic acid (Hkoj) are all commercially available. Both maltol (Veltol™) and ethylmaltol (Veltol Plus™) are approved food additives in Canada and in the U.S. used to artificially enhance the flavour of baked goods. Both maltol and the metabolites of maltol, including maltol glucosides and sulphates, are relatively non-toxic. Possessing neither high activity nor toxicity, Hma is an excellent biological spectator ligand. 3-Hydroxy-4-pyrones combine a basic ketone ($pK_a \approx 0$, $pK_a = -0.71$ for Hma$^{23}$) with an acidic enol ($pK_a \approx 7.5-9$, $pK_a \approx 8.4$ for Hma$^{19,24-26}$).
The 3-hydroxy-4-pyrone ring possesses a high degree of electron delocalization, undergoing bromination and nitration as would an aromatic system. In addition, the pyrone carbonyl group does not react to from a phenylhydrazone or an oxime. 3-Hydroxy-4-pyrones react with nucleophiles and undergo a variety of cycloaddition reactions. For example, reaction of Hma with primary amines generates 3-hydroxy-4-pyridinones (Figure 1.2) which possess both a significantly more basic ketone ($pK_a \sim 3-4$) and enol ($pK_a \sim 10$). Both the protonated ($H_2L^+$) and deprotonated salts ($L^-$) of 3-hydroxy-4-pyrones and 3-hydroxy-4-pyridinones are stable and easily isolated. Capable of participating as hydrogen-bond acceptors or donors, pyrones and pyridinones often form hydrogen-bonds in the solid state.

**Figure 1.2** Reactions of Hma.

Maltol and related 3-hydroxy-4-pyrones, as well as the 3-hydroxy-4-pyridinones, are potential neutral bidentate ligands. This binding mode has only been documented for alkali metal coordination by Hkoj and alkyl derivatives of Hkoj.

References on page 9
in non-aqueous solvents. Coordination to lanthanides, involving both inner and outer sphere coordination of a 4-pyridinone, has been reported with the outer sphere association being the result of hydrogen-bonding between Nd$^{3+}$ bound water molecules, and 4-pyridinone. By far the most common coordination mode derived from 3-hydroxy-4-pyrones and 3-hydroxy-4-pyridinones is that of a bidentate monoanionic chelate. The coordination chemistry of 3-hydroxy-4-pyrones and 3-hydroxy-4-pyridinones complexes of main group metals including Al(III), Ga(III), In(III), Sn(IV) has been reported. A combination of water-solubility, hydrolytic stability and lipophilicity have led to wide-spread use of Al(ma)$_3$ in the study of aluminum neurotoxicity and to the examination of a $^{67}$Ga complex, tris(1-(p-methoxyphenyl)-2-methyl-3-oxy-4-pyridinonato)gallium(III), as a potential heart imaging agent. The coordination chemistry of Fe(III) with 3-hydroxy-4-pyrones and 3-hydroxy-4-pyridinones has been extensively explored with these ligands functioning as iron scavenging agents in potential treatments for iron overload. Numerous complexes of first row transition metal ions with these ligands have been reported including Fe, Cr, Cu and Zn, in addition to V. Relatively few complexes of the second and third row transition metals are known including a series of ternary complexes of Ru(II) as well as complexes of Tc and Re. Monocationic complexes of $^{99m}$Tc(IV) have been examined as potential, readily tailored, nuclear imaging agents.

In addition to VO(ma)$_2$, other vanadium complexes derived from Hma have been reported. These include a strongly anti-ferromagnetically coupled alkoxide dimer of V(IV) [VO(ma)(µ-OCH$_3$)]$_2$, stable ternary alkoxides of vanadium V(V).
including VO(ma)$_2$(OCH$_3$)$_2$ as well as the pyridine adduct VO(ma)$_2$(pyr). The K$^+$ and NH$_4^+$ salts of [VO$_2$(ma)$_2$] have also been synthesized and studied in solution as well as in the solid state with the X-ray structure determination of K[VO$_2$(ma)$_2$]•H$_2$O; however, NH$_4$[VO$_2$(ma)$_2$]•H$_2$O is not insulin-mimetic in the STZ diabetic animal model.

Other vanadyl 3-oxy-4-pyrone complexes include VO(koj)$_2$, bis(kojato)oxovanadium(IV), and VO(ema)$_2$, bis(ethylmaltolato)oxovanadium(IV). VO(koj)$_2$ has been the subject of solution and insulin-enhancing biological studies; it is insulin-mimetic, but is significantly less potent than VO(ma)$_2$. VO(ema)$_2$, a recent addition to the list of complexes suitable as insulin-enhancing agents, is also insulin-mimetic and possesses activity comparable to VO(ma)$_2$. VO(ema)$_2$ is currently in phase I clinical trials.

With many binary vanadyl complexes and vanadium(V) complexes of these ligands known, and the variety seemingly exhausted, new approaches to vanadium based insulin-enhancing agents were sought. These include the examination of V(III) complexes as well as the development of derivatives of the existing complex VO(ema)$_2$, both of which form major components of this thesis.

Vanadyl complexes such as VO(ema)$_2$ easily form ternary complexes. One major approach to complexes with potentially enhanced activity is the synthesis of ternary complexes, derivatives of VO(ema)$_2$ of the type VO(ema)$_2$(L), where L is a co-ligand.
No V(III) complex has been previously examined for insulin enhancing activity 
_in vivo or in vitro_. The observation that toxicity of a homologous series of vanadium 
complexes often decreases with decreasing oxidation state (Table 1.1) can be 
made. For example, for vanadium chlorides, significantly reduced toxicity was 
observed from VOCl₃, the most toxic, to VCl₂, the least toxic.⁵⁸ Such trends in 
toxicity are a combination of multiple factors including differences in biodistributions, 
oxidizing ability (oxidants are typically more toxic than reductants) and acidity (basic 
complexes are more likely to exhibit higher toxicities than acidic complexes) 
between the oxidation states. Similar relationships between oxidation state and 
toxicity are documented for Cr (Cr(VI) is significantly more toxic than Cr(III) due to 
differences in biodistribution).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rat Oral LD₅₀ (mmol kg⁻¹)</th>
<th>Rabbit Intravenous LD₁₀₀ (mmol kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCl₂</td>
<td>4.40</td>
<td>VOSO₄•2H₂O</td>
</tr>
<tr>
<td>VCl₃</td>
<td>2.24</td>
<td>NH₄VO₃</td>
</tr>
<tr>
<td>VCl₄</td>
<td>0.83</td>
<td>NaVO₃</td>
</tr>
<tr>
<td>VOCl₃</td>
<td>0.80</td>
<td>V₂O₅</td>
</tr>
</tbody>
</table>

V(III) is expected to form hydrolytically stable binary complexes derived from 
3-hydroxy-4-pyrone and 3-hydroxy-4-pyridinone ligands; however, these might be 
expected to be air-sensitive. V(IV) and V(V) form ternary and quaternary complexes

References on page 9
involving oxo, aquo and hydroxo ligands. For example, the major species of 
VO(ma)\textsubscript{2} in aqueous solution is considered to be solvated VO(ma)\textsubscript{2}(OH\textsubscript{2}), a ternary complex, based on EPR and UV-vis spectroscopy\textsuperscript{21}. Any new insight from the "simple" V(III) system might then be readily applicable to the oxovanadium(IV) and (V) systems.

Additional incentive for the examination of V(III) complexes was provided by the observation that, when accumulated in high concentrations in living organisms, vanadium is often in the low oxidation states V(III) and V(IV). For example, vanadium(III) is accumulated at molar levels by tunicates (sea-squirts) in which its function is unknown\textsuperscript{59}. Vanadium is also accumulated by toadstools of the family Amanita, as a rare 8-coordinate vanadium(IV) complex\textsuperscript{60,61}.

1.2 References


Chapter 2
Derivatives of VO(ema)₂

2.1 Introduction

This chapter describes the synthesis and characterization of derivatives of VO(ema)₂ in the solid state. Vanadium(IV) complexes typically contain the vanadyl (VO²⁺) moiety, one of the most stable diatomic ions known.¹ In aqueous solution, vanadyl complexes are typically octahedral, including the aquo ion [VO(H₂O)₅]²⁺.² In non-aqueous solution and in the solid state, square pyramidal complexes are stable and especially common.³⁻⁷ The chlorooxovanadium(IV) moiety [VOCI]⁺ can also form stable complexes including [CIVO(bipy)₂]Cl, a complex that can be isolated from aqueous solution.⁸⁻⁹

A vanadium(IV) compound which has shown excellent insulin-enhancing activity in vivo is VO(ema)₂, or bis(ethylmaltolato)oxovanadium(IV), a complex which is currently in phase I clinical trials.¹⁰

VO(ema)₂

The synthesis and characterization of derivatives of VO(ema)₂ are the major focus of this chapter. Because VO(ema)₂ is coordinatively unsaturated, coordination chemistry was to be used to generate complexes of the type VO(ema)₂(L) where L is a co-ligand. This approach was used rather than derivatization of ethylmaltol which can be difficult and time consuming.¹¹,¹² Attractive co-ligands were those capable of rendering VO(ema)₂ significantly more lipophilic and/or water-soluble. A complementary strategy is the synthesis of salts of VO(ema)₂, with salt formation a preferred method of enhancing the dissolution.

References on page 28
properties of a potential pharmaceutical. In addition, the judicious choice of counterions allows for further optimization of activity. For example, chloride administration been shown to decrease the deleterious effects of high vanadium doses, including growth inhibition.\textsuperscript{13}

2.2 Experimental

General Experimental

Materials and Methods. All chemicals were reagent grade and were used as received without further purification: VOSO\textsubscript{4}\textsubscript{•}3H\textsubscript{2}O (Aldrich), ethylmaltol (Pfizer), 37\% hydrochloric acid (Fisher). Water was distilled (Barnstead D8902 and D8904 Cartridges) and deionized (Corning MP-1 Megapure still).

Instrumentation. Infrared spectra were recorded as KBr disks in the range 4000-400 cm\textsuperscript{-1} on a Mattson Galaxy 5000 spectrophotometer and in the range 400-200 cm\textsuperscript{-1} on a Perkin-Elmer 783 spectrophotometer, with polystyrene as a reference. Mass spectra were obtained with a Kratos Concept II H32Q (Cs\textsuperscript{+}, LSIMS) or a Kratos M50 (EIMS) spectrometer. Analyses for C, H, N and Cl were performed in this department by Mr. Peter Borda. Room temperature magnetic susceptibilities were measured on a Johnson Matthey magnetic susceptibility balance. Diamagnetic corrections were based on Pascal's Constants.\textsuperscript{14}

Synthesis

(Ethylmaltol)bis(ethylmaltolato)oxovanadium(IV) monohydrate,

[VO(ema)\textsubscript{2}(Hema)]\textsubscript{•}H\textsubscript{2}O. Vanadyl sulphate trihydrate (19.70 g, 90.72 mmol) and ethylmaltol (25.43 g, 182.0 mmol) were suspended in 0.3 L of water at 45 \textdegree C. Following an induction period of 15-20 minutes, a fine yellow powder precipitated
rapidly, and the suspension (pH ~1) was cooled to room temperature. The solid was collected by filtration and air-dried (21.90 g, 43.51 mmol, 72% yield based on Hema). The complex can be dissolved in methanol, ethanol, benzene, toluene, acetone, acetonitrile, tetrahydrofuran, DMSO, DMF, chloroform, dichloromethane and diethyl ether at RT. [VO(ema)$_2$(Hema)]•H$_2$O can be dissolved with difficulty in water at RT, but can be readily dissolved in water (~ 25 mM) at temperatures exceeding RT and below 90 °C. [VO(ema)$_2$(Hema)]•H$_2$O gives neutral aqueous solutions. [VO(ema)$_2$(Hema)]•H$_2$O cannot be readily dissolved in water in mildly acidic conditions (pH 1) even at elevated temperatures. From aqueous solutions and suspensions of [VO(ema)$_2$(Hema)]•H$_2$O, VO(ema)$_2$ can be extracted into methylene chloride, ethyl acetate or n-octanol, with partitioning favouring the non-aqueous phase. [VO(ema)$_2$(Hema)]•H$_2$O is substantially air and moisture stable under ambient conditions but trace decomposition is observed over protracted periods of time (6 months or more).

Bis(ethylmaltolato)oxovanadium(IV), VO(ema)$_2$. Yellow

[VO(ema)$_2$(Hema)]•H$_2$O (10.00 g, 19.87 mmol) was added to 0.5 L boiling water under Ar, yielding, after six hours, a homogenous fine blue-green powder. The suspension, which was pH neutral, was filtered while hot and the solid collected and air-dried (5.66 g, 16.4 mmol, 82 % yield based on V). VO(ema)$_2$ is indefinitely air and moisture stable under ambient conditions. VO(ema)$_2$ can be dissolved in water (~ 4 mM), methanol, chloroform and methylene chloride. From aqueous solutions and suspensions of VO(ema)$_2$, in the presence of excess Hema, VO(ema)$_2$ can be extracted into methylene chloride, ethyl acetate or n-octanol, with partitioning
favouring the non-aqueous phase. VO(ema)₂ gives neutral aqueous solutions when dissolved in water.

**Chlorobis(ethylmaltol)oxovanadium(IV) chloride monohydrate,**

**[VOCl(Hema)₂]Cl·H₂O.** Solid blue-green VO(ema)₂ (0.545 g, 1.58 mmol) on a Schlenk filter connected to a water aspirator, was treated with a flow of concentrated HCl (aq) vapor, from a 250 mL reservoir filled with 125 mL of concentrated HCl (aq), in the absence of oxygen for 24 hours. This treatment resulted in a pale blue solid that spontaneously turned bright green over 2 days in air. The bright green powder was washed with cold acetone and dried *in vacuo* (0.700 g, 1.61 mmol, 100% yield based on V). [VOCl(Hema)₂]Cl·H₂O is stable *in vacuo*, and is indefinitely air and moisture stable under ambient conditions. [VOCl(Hema)₂]Cl·H₂O is completely water soluble giving acidic aqueous solutions (pH ~1 for a 0.120 M solution), indefinitely stable to oxidation.

**Table 2.1** Elemental Analyses (found [calc.]) and Bulk Magnetic Moments *(μₑff, B. M.)*.

<table>
<thead>
<tr>
<th>Compound</th>
<th>C</th>
<th>H</th>
<th>Cl</th>
<th>μₑff</th>
</tr>
</thead>
<tbody>
<tr>
<td>[VO(ema)₂(Hema)]·H₂O</td>
<td>50.09 [50.11]</td>
<td>4.81 [4.73]</td>
<td>-</td>
<td>1.71</td>
</tr>
<tr>
<td>VO(ema)₂</td>
<td>48.71 [48.63]</td>
<td>4.09 [3.97]</td>
<td>-</td>
<td>1.70</td>
</tr>
</tbody>
</table>

References on page 28
2.3 Results and Discussion

Starting from ethylmaltol and vanadyl sulphate, there are three complexes easily prepared in high yield on a large scale using aqueous conditions without the use of any pharmaceutically unacceptable reagents. VO(ema)₂ has been previously synthesized;¹⁰ however, a novel synthesis with comparable yields is presented. Two novel derivatives of VO(ema)₂ [VO(ema)₂(Hema)]·H₂O and [VOCI(Hema)₂]Cl·H₂O were discovered. [VO(ema)₂(Hema)]·H₂O is a very lipophilic compound, soluble in a variety of non-polar solvents. [VOCI(Hema)₂]Cl·H₂O is completely water-soluble. These complexes possess exciting properties for potential inorganic pharmaceuticals and present attractive alternatives to the common oxovanadium(IV) complexes used so far in the study of the insulin-enhancing activity of vanadium.

[VO(ema)₂(Hema)]·H₂O precipitates upon mixing vanadyl sulphate trihydrate and ethylmaltol in water following a brief induction period. Complexes analogous to [VO(ema)₂(Hema)]·H₂O proved elusive for other common pyrones, including maltol and kojic acid. There are few reported complexes similar to [VO(ema)₂(Hema)]·H₂O, an example being a complex which can be formulated as [VO(pic)₂(Hpic)]·2H₂O.¹⁵ In the synthesis of [VO(ema)₂(Hema)]·H₂O, it is necessary for the reagents to dissolve prior to the precipitation and the pure complex is best obtained under reaction conditions which are substoichiometric with respect to ethylmaltol. Suspension of solid [VO(ema)₂(Hema)]·H₂O in boiling water results in facile conversion to solid blue VO(ema)₂ in high isolated yield. In an alternative conversion, VO(ema)₂ is recovered from the recrystallization of

References on page 28
[VO(ema)$_2$(Hema)]$\cdot$H$_2$O in toluene/hexanes as a brown soluble solid. The aqueous solubility of VO(ema)$_2$ is usually high but can be effected by the chemical history of the complex. For example, VO(ema)$_2$ generated from the recrystallization of [VO(ema)$_2$(Hema)]$\cdot$H$_2$O in toluene/hexanes can be dissolved easily in toluene or methanol, while solid VO(ema)$_2$ formed from heating solid [VO(ema)$_2$(Hema)]$\cdot$H$_2$O in water is a recalcitrant solute and cannot be readily dissolved in toluene or methanol.

The synthesis of a highly water soluble hydrochloride salt of VO(ema)$_2$, [VOCI(Hema)$_2$]Cl$\cdot$H$_2$O, was also successfully accomplished. Treating solid blue VO(ema)$_2$ with concentrated hydrochloric acid vapors in the absence of dioxygen yields a mixture of solid ethylmaltolium chloride and aquavanadyl chloride (Scheme 2.1) with a gain of approximately four equivalents of HCl and water, as determined gravimetrically. The composition of the intermediate mixture was confirmed by infrared spectroscopy and by extraction of aquavanadyl chloride with diethyl ether from the solid reaction mixture. The two chlorides, aquavanadyl chloride and ethylmaltolium chloride, react over 2 days in a solid-solid reaction generating bright green [VOCI(Hema)$_2$]Cl$\cdot$H$_2$O quantitatively (Scheme 2.1). No solution synthesis of this highly stable complex was successful. The synthesis of complexes analogous to [VOCI(Hema)$_2$]Cl$\cdot$H$_2$O proved unsuccessful for VO(dpp)$_2$, VO(acac)$_2$, VO(pic)$_2$ and VO(SALEN) with unreactive hydrolysis products being generated for the first three complexes. VO(SALEN) did not react with acid or water under the reaction conditions used.
Scheme 2.1

\[
\text{VO(ema)}_2 (s) + 4\text{HCl} (g) + 4\text{H}_2\text{O} (g) \rightarrow [\text{VO(H}_2\text{O})_4]\text{Cl}_2 (s) + 2 [\text{H}_2\text{ema}]\text{Cl} (s)
\]

\[
[\text{VO(H}_2\text{O})_4]\text{Cl}_2 (s) + 2 [\text{H}_2\text{ema}]\text{Cl} (s) \rightarrow [\text{VOCI(Hema)}_2]\text{Cl}\cdot\text{H}_2\text{O} (s) + 2 \text{HCl} (g) + 3 \text{H}_2\text{O} (g)
\]

Characterized in the solid state by IR, MS, EA and bulk magnetic susceptibilities, \(\text{VO(ema)}_2\), [\(\text{VO(ema)}_2\text{(Hema)}\)]\(\cdot\text{H}_2\text{O}\) and [\(\text{VOCI(Hema)}_2\)]\(\cdot\text{H}_2\text{O}\) are generally stable to oxidation and hydrolysis under ambient moisture and oxygen in the solid state. Bulk magnetic susceptibilities are unremarkable, being typical for monomeric oxovanadium(IV) complexes (Table 2.1). These complexes are not prone to contamination by any specific impurity and give good analyses (Table 2.1). The solution characterization of these compounds is described in Chapter 4.

**Infrared spectroscopy.** The infrared spectra of the vanadyl complexes show diagnostic differences (Table 2.2, Figure 2.1). \(\text{VO(ema)}_2\) exhibits pyrone stretching frequencies, inseparable combinations of the pyrone carbonyl and the high energy ring vibrations,\(^{16}\) at 1601 and 1551 cm\(^{-1}\), shifted from the free ligand Hema by -43 and -62 cm\(^{-1}\), respectively. [\(\text{VO(ema)}_2\text{(Hema)}\)]\(\cdot\text{H}_2\text{O}\) possesses vibrations attributable to both Hema (1634, 1581 cm\(^{-1}\)) and \(\text{ema}^-\) (1596, 1562 cm\(^{-1}\)) coordination. Both coordination modes result in a reduction of the pyrone stretching frequencies from the free ligand, with \(\text{ema}^-\) coordination leading to significantly lower pyrone stretching frequencies (Table 2.2). The infrared spectrum distinguishes [\(\text{VO(ema)}_2\text{(Hema)}\)]\(\cdot\text{H}_2\text{O}\) from the alternative formulation [\(\text{VO(ema)}_2\text{(H}_2\text{O})\)]\(\cdot\text{Hema}\) in which coordinated \(\text{H}_2\text{O}\) replaces the Hema ligand.
Pyrone stretching frequencies intermediate in energy between Hema and VO(ema)\textsubscript{2}, present for [VO(ema)\textsubscript{2}(Hema)]\textsubscript{•}H\textsubscript{2}O, are consistent with Hema coordination and indicate that the alternative formulation [VO(ema)\textsubscript{2}(H\textsubscript{2}O)]\textsubscript{•}Hema is less likely. [VOCl(Hema)\textsubscript{2}]Cl\textsubscript{•}H\textsubscript{2}O also exhibits pyrone stretching frequencies intermediate in energy between Hema and VO(ema)\textsubscript{2} (Table 2.2) consistent with Hema coordination. The pyrone stretching frequencies (1634 cm\textsuperscript{-1}, 1581 cm\textsuperscript{-1}) of [VOCl(Hema)\textsubscript{2}]Cl\textsubscript{•}H\textsubscript{2}O are not significantly different from the pyrone stretching frequencies attributed to Hema coordination in the infrared spectrum of [VO(ema)\textsubscript{2}(Hema)]\textsubscript{•}H\textsubscript{2}O (1634 cm\textsuperscript{-1}, 1581 cm\textsuperscript{-1}). Additionally, a sharp peak at 3292 cm\textsuperscript{-1} attributed to the low energy OH vibration of a bound enol group is observed for [VOCl(Hema)\textsubscript{2}]Cl\textsubscript{•}H\textsubscript{2}O. A second sharp vibration at 3382 cm\textsuperscript{-1} in the infrared spectrum of [VOCl(Hema)\textsubscript{2}]Cl\textsubscript{•}H\textsubscript{2}O is consistent with either water or the hydronium ion. The absence of a sharp OH vibration in the infrared spectrum of [VO(ema)\textsubscript{2}(Hema)]\textsubscript{•}H\textsubscript{2}O suggests monodentate coordination of the third ligand.

All three compounds exhibit vanadium oxo stretching frequencies, $\nu_\text{V=O}$, at energies typical for oxovanadium complexes (950-1000 cm\textsuperscript{-1}). VO(ema)\textsubscript{2} possesses $\nu_\text{V=O} = 995$ cm\textsuperscript{-1}, typical of 5-coordinate square pyramidal complexes including VO(ma)\textsubscript{2}.\textsuperscript{3} The vanadium oxo stretching frequencies of [VO(ema)\textsubscript{2}(Hema)]\textsubscript{•}H\textsubscript{2}O and [VOCl(Hema)\textsubscript{2}]Cl\textsubscript{•}H\textsubscript{2}O, 971 cm\textsuperscript{-1} and 974 cm\textsuperscript{-1}, respectively, are not significantly different and are within the range of octahedral oxovanadium(IV) complexes including VO(ma)\textsubscript{2}(pyridine)\textsuperscript{3} and trans-[VOCl(pyrazole)\textsubscript{4}]Cl.\textsuperscript{17} Both [VO(ema)\textsubscript{2}(Hema)]\textsubscript{•}H\textsubscript{2}O and VO(ema)\textsubscript{2} possess vanadium alkoxide stretching frequencies $\nu_{\text{V-O}}$ at approximately 720 cm\textsuperscript{-1}; these are not observed for
[VOCI(Hema)$_2$]Cl·H$_2$O or for Hema. The presence of the vanadium alkoxide stretches confirms bidentate ema$^+$ coordination. [VOCI(Hema)$_2$]Cl·H$_2$O exhibits vanadium halide stretches $\nu_{V-Cl}$ at 315 cm$^{-1}$ and 320 cm$^{-1}$; these are not observed for the other oxovanadium complexes or for Hema.

**Table 2.2** Selected Infrared Absorptions (KBr, ±4 cm$^{-1}$, Values in Parentheses Describe Changes from the Free Ligand Values, $\Delta$ cm$^{-1}$).

<table>
<thead>
<tr>
<th></th>
<th>[VO(ema)$_2$(Hema)]·H$_2$O</th>
<th>VO(ema)$_2$</th>
<th>[VOCI(Hema)$_2$]Cl·H$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\nu_{OH}$</td>
<td>-</td>
<td>-</td>
<td>3382</td>
</tr>
<tr>
<td>$\nu_{OH}$</td>
<td>obscured</td>
<td>-</td>
<td>3292</td>
</tr>
<tr>
<td>$\nu_{C=O}$ and $\nu_{ring}$</td>
<td>1634 (-10)</td>
<td>1634 (-10)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1596 (-48)</td>
<td>1601 (-43)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1581 (-31)</td>
<td>1582 (-31)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1562 (-51)</td>
<td>1551 (-62)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1528</td>
<td>1502</td>
<td>1515</td>
</tr>
<tr>
<td></td>
<td>1464</td>
<td>1467</td>
<td>1471</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1456</td>
</tr>
<tr>
<td>$\nu_{\equiv V}$</td>
<td>971</td>
<td>995</td>
<td>974</td>
</tr>
<tr>
<td>$\nu_{V-O}$</td>
<td>721</td>
<td>724</td>
<td>-</td>
</tr>
<tr>
<td>$\nu_{V-Cl}$</td>
<td>-</td>
<td>-</td>
<td>315</td>
</tr>
</tbody>
</table>

References on page 28
Figure 2.1 Infrared Spectra of [VO(ema)$_2$(Hema)]·H$_2$O, VO(ema)$_2$ and [VOCl(Hema)$_2$]Cl·H$_2$O. Key Vibrations Include $\nu_{pyrone}$ (a and b), $\nu_{v=\sigma}$ (c), and $\nu_{v-O}$ (d).

Mass spectrometry. The positive ion detection mode LSI mass spectra of [VO(ema)$_2$(Hema)]·H$_2$O and VO(ema)$_2$ are very similar (Table 2.3, Figure 2.2). Both show a 100% relative intensity [V(ema)$_2$]$^+$ ion (m/z = 329) as the predominant ion. The parent molecular ion of VO(ema)$_2$ is observed as the (M+1)$^+$ protonated [HVO(ema)$_2$]$^+$ (m/z = 346), which is present in the mass spectra of both complexes with comparable intensities. In addition, ligand dissociation leads to an intense [VO(ema)]$^+$ ion (m/z = 206). The mass spectrum of [VO(ema)$_2$(Hema)]·H$_2$O
(Figure 2.2) exhibits an intense \([\text{V}(\text{ema})_3]^+\) ion (\(m/z = 468\)), which occurs with lower intensity in the mass spectrum of \([\text{VO}(\text{ema})_2]\). There is no parent molecular ion observed for \([\text{VO}(\text{ema})_2(\text{Hema})]\cdot\text{H}_2\text{O}\) in the mass spectrum, indicating either that \([\text{VO}(\text{ema})_2(\text{Hema})]\cdot\text{H}_2\text{O}\) easily dissociates to the \([\text{HVO}(\text{ema})_2]^+\) ion or that \([\text{VO}(\text{ema})_2(\text{Hema})]\cdot\text{H}_2\text{O}\) reacts readily to form the \([\text{V}(\text{ema})_3]^+\) ion under the conditions used in LSIMS. Numerous high molecular weight associated ions are observed for both \([\text{VO}(\text{ema})_2(\text{Hema})]\cdot\text{H}_2\text{O}\) and \([\text{VO}(\text{ema})_2]\), including intense \([\text{V}_2\text{O}_2(\text{ema})_3]^+\) and \([\text{V}_2\text{O}(\text{ema})_4]^+\) ions (Figure 2.2). The mass spectrum of \([\text{VOCl}(\text{Hema})_2]\text{Cl}\cdot\text{H}_2\text{O}\) shows only hydrolysis products. No parent molecular ion is observed for \([\text{VOCl}(\text{Hema})_2]\text{Cl}\cdot\text{H}_2\text{O}\), which displays a mass spectrum similar to those of the other oxovanadium(IV) complexes, although the major ions are less intense. However, numerous solvated ions resulting from associations of water or the matrix (thioglycerol), absent in the mass spectra of the other oxovanadium(IV) complexes, are observed in the mass spectrum of \([\text{VOCl}(\text{Hema})_2]\text{Cl}\cdot\text{H}_2\text{O}\). For example, in the mass spectrum of \([\text{VOCl}(\text{Hema})_2]\text{Cl}\cdot\text{H}_2\text{O}\), the \([\text{VO}(\text{ema})]^+\) ion is accompanied by the \([\text{VO}(\text{ema})(\text{H}_2\text{O})_4]^+\), \([\text{VO}(\text{ema})(\text{H}_2\text{O})_5]^+\) and \([\text{VO}(\text{ema})(\text{H}_2\text{O})_6]^+\) ions. In fact, the predominant ion in the mass spectrum of \([\text{VOCl}(\text{Hema})_2]\text{Cl}\cdot\text{H}_2\text{O}\) is the \([\text{VO}(\text{ema})(\text{H}_2\text{O})_6]^+\) ion (\(m/z = 314\)). Ions involving the association of six water molecules are particularly intense with \([\text{VO}(\text{ema})(\text{H}_2\text{O})_6]^+\), \([\text{VO}(\text{ema})(\text{H}_2\text{O})_5]^+\), and \([\text{HVO}(\text{ema})_2(\text{H}_2\text{O})_6]^+\) all prominent.

References on page 28
Table 2.3 Selected Ions (Positive Ion Detection Mode LSIMS, % Relative Intensity) for [VO(ema)₂(Hema)]⁺H₂O (1), VO(ema)₂ (2) and [VOCI(Hema)₂]Cl·H₂O (3).

<table>
<thead>
<tr>
<th>Ion</th>
<th>m/z</th>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[VO(ema)]⁺</td>
<td>206</td>
<td>50</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>[VO(ema)(H₂O)₄]⁺</td>
<td>279</td>
<td>-</td>
<td>-</td>
<td>&lt;5</td>
</tr>
<tr>
<td>[VO(ema)(H₂O)₅]⁺</td>
<td>296</td>
<td>-</td>
<td>-</td>
<td>&lt;5</td>
</tr>
<tr>
<td>[VO(ema)(H₂O)₆]⁺</td>
<td>314</td>
<td>-</td>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td>[V(ema)₂]⁺</td>
<td>329</td>
<td>100</td>
<td>100</td>
<td>17</td>
</tr>
<tr>
<td>[HVO(ema)₂]⁺</td>
<td>346</td>
<td>33</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>[V₂O₂(ema)₂]⁺</td>
<td>412</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[V₂O₃(ema)₂]⁺</td>
<td>428</td>
<td>10</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>[V(ema)₂(H₂O)₆]⁺</td>
<td>437</td>
<td>-</td>
<td>-</td>
<td>&lt;5</td>
</tr>
<tr>
<td>[HVO(ema)₂(H₂O)₈]⁺</td>
<td>454</td>
<td>-</td>
<td>-</td>
<td>&lt;5</td>
</tr>
<tr>
<td>[V(ema)₃]⁺</td>
<td>468</td>
<td>20</td>
<td>10</td>
<td>&lt;5</td>
</tr>
<tr>
<td>[V₂(ema)₃]⁺</td>
<td>519</td>
<td>-</td>
<td>-</td>
<td>&lt;5</td>
</tr>
<tr>
<td>[V₂O₂(ema)₃]⁺</td>
<td>551</td>
<td>10</td>
<td>10</td>
<td>&lt;5</td>
</tr>
<tr>
<td>[V₂(ema)₄]⁺</td>
<td>658</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td>[V₂O(ema)₄]⁺</td>
<td>674</td>
<td>&lt;10</td>
<td>-</td>
<td>&lt;5</td>
</tr>
<tr>
<td>[V₂(ema)₅]⁺</td>
<td>797</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Although single crystals proved elusive, some limited generalizations can be made regarding the structures in the absence of X-ray diffraction data. The structure of VO(ema)$_2$ is not expected to differ significantly from that of VO(ma)$_2$, an irregular square pyramid.\textsuperscript{3} The 5-coordinate distorted square pyramid is very common for oxovanadium(IV) complexes with additional examples provided by VO(acac)$_2$\textsuperscript{4,7} and K$_2$[VO(cat)$_2$]·EtOH·H$_2$O.\textsuperscript{5}

[VOCl(Hema)$_2$]Cl·H$_2$O is likely a cis-chloroxovanadium(IV) complex. Cis-chloroxovanadium(IV) forms complexes with many neutral ligands including 2,2'-bipyridine,\textsuperscript{8,9} 1,10-phenanthroline,\textsuperscript{8,18} macrocyclic pyridine amine macrocycles,\textsuperscript{19} and ureas.\textsuperscript{20} The cis-chloroxovanadium(IV) complexes of bidentate and polydentate ligands are typically octahedral and possess one chloro ligand, an example being [VOCl(bipy)$_2$]Cl.\textsuperscript{9} Complexes of the monodentate oxygen donors are typically
square pyramidal and possess an additional chloro ligand \textit{trans} to the first, an example being the tetramethylurea (tmu) complex $[\text{VOCl}_2(\text{tmu})_2]$.\textsuperscript{20} Far less common \textit{trans}-chloroxovanadium(IV) complexes include the tetrakis(vinylimidazole)\textsuperscript{21} and tetrakis(pyrazole) complexes.\textsuperscript{17}

**Figure 2.3** \textit{cis}-[$\text{VO(ema)}_2(\text{OCH}_2\text{CH}_2\text{OH})]$\textsuperscript{25} a Proposed Model for $[\text{VO(ema)}_2(\text{Hema})]\cdot\text{H}_2\text{O}$.

$[\text{VO(ema)}_2(\text{Hema})]\cdot\text{H}_2\text{O}$ is probably octahedral based on the IR evidence which indicates two bound bidentate ema$^-$ ligands and suggests an additional monodentate Hema ligand. Vanadyl commonly forms outer sphere complexes involving hydrogen bonding to the vanadyl oxygen atom.\textsuperscript{2,6,22-24} Because ethylmaltol is a good hydrogen bond donor, hydrogen bonding between the ethylmaltol ligand and the vanadyl moiety should be possible. A \textit{cis} to vanadyl arrangement of the additional ligand is necessary in order for the hydroxyl group of the ethylmaltol ligand to participate in hydrogen-bonding with the vanadyl oxygen.

27 References on page 28
atom. A very similar interaction to this proposed hydrogen-bonding was structurally documented for the oxovanadium(V) maltolate complex cis-[VO(ma)$_2$(OCH$_2$CH$_2$OH)]
(Figure 2.3). In this structure the dangling hydroxyl group of the 2-hydroxyethanolato ligand was found to participate in hydrogen-bonding to the vanadyl moiety.\textsuperscript{25}

2.4 Conclusions

[VO(ema)$_2$(Hema)]$\cdot$H$_2$O, VO(ema)$_2$ and [VOCl(Hema)$_2$]Cl$\cdot$H$_2$O were successfully synthesized. [VO(ema)$_2$(Hema)]$\cdot$H$_2$O and [VOCl(Hema)$_2$]Cl$\cdot$H$_2$O are rare examples of transition-metal complexes possessing a coordinated 3-hydroxy-4-pyrene. In addition, [VO(ema)$_2$(Hema)]$\cdot$H$_2$O and [VOCl(Hema)$_2$]Cl$\cdot$H$_2$O are novel stable complexes suitable for use in insulin enhancing studies, because the former is highly lipophilic while the latter is completely water soluble.

2.5 References


Chapter 3

The Synthesis of Novel Vanadium(III) Compounds

3.1 Introduction

Vanadium(III) is the lowest oxidation state of vanadium stable in water, with vanadium(II) complexes reducing water to dihydrogen. Vanadium(III) forms almost exclusively octahedral complexes in aqueous solution, including the aquo ion $\text{V(H}_2\text{O)}_6^{3+}$; however, seven-coordinate compounds are common in solution including $\text{[V(EDTA)(H}_2\text{O)]}$\textsuperscript{2+}. Labile with respect to ligand substitution reactions and highly susceptible to hydrolysis,\textsuperscript{3,4} vanadium(III) typically forms the most hydrolytically stable complexes with basic oxygen donors including catecholates.\textsuperscript{5,6} Vanadium(III) complexes are reducing in aqueous solution and are oxidized slowly by dioxygen.\textsuperscript{7} In the solid state, a variety of geometries are found with octahedral the most common for complexes relevant to aqueous media including the aquo complex $\text{[V(H}_2\text{O)}_6]^{3+}$,\textsuperscript{1} and the aquo chloro species $\text{trans-[VCl}_2\text{(H}_2\text{O)}_4]$\textsuperscript{8}. Vanadium(III) complexes are commonly oxygen-sensitive in the solid state but notable exceptions are known including $\text{V(pic)}_3\cdot\text{H}_2\text{O}$,\textsuperscript{9,10} and $\text{[V(urea)}_3]$\textsuperscript{11} $\text{V(III)}$ compounds ($d^2$) are spectroscopically quiet being typically EPR silent, due to a triplet ground state, and NMR silent.\textsuperscript{12}

No previous vanadium(III) complex has been examined as a potential insulin enhancing agent, so a key aspect of this work was the synthesis of novel vanadium(III) complexes for the purpose of testing for biological activity. By analogy to other trivalent metal oxypyrinato and oxypyridinonato complexes, vanadium(III) might be expected to form the stable binary complexes with these ligands. This
chapter deals with the synthesis of novel vanadium(III) complexes under aqueous conditions and their characterization.

**Figure 3.1** Hma, Hkoj, Hema and Hdpp.

3.2 Experimental

Synthesis

The general experimental method is described in Chapter 2.2. Maltol (Pfizer), kojic acid (Lancaster), 40% aqueous methylamine (Aldrich) and sodium dithionite (Fisher) were reagent grade and were used as received without further purification.

Tris(maltolato)vanadium(III), V(ma)₃. Vanadyl sulphate trihydrate (10.83 g, 49.87 mmol) and maltol (18.81 g, 149.1 mmol) were suspended at 60 °C in 0.3 L water under Ar. Reduction with sodium dithionite (25.00 g, 143.6 mmol) yielded a dark red microcrystalline solid that was collected by filtration, washed with water, and air-dried. The anhydrous compound was obtained by resuspension of the microcrystalline solid in water, heating to 60 °C overnight, collecting the fine red powder by filtration and subsequent drying in vacuo (13.76 g, 32.28 mmol, 65 % yield based on V). V(ma)₃ is robust with respect to solid state oxidation in the presence of dioxygen. V(ma)₃ is soluble in water, methanol, ethanol, benzene,
toluene, acetone, acetonitrile, tetrahydrofuran, DMSO, DMF, chloroform, and dichloromethane. The complex can be extracted from aqueous solutions with methylene chloride or n-octanol.

\textbf{Tris(ethyImaltolato)vanadium(III), V(ema)_3.} Yellow

[VO(ema)_2(Hema)]H_2O, (0.287 g, 0.573 mmol), the synthesis of which is described in Chapter 2, was dissolved in 50 mL water at 65 °C, and sodium dithionite (0.200 g, 1.14 mmol) was added under Ar. A fine red powder precipitated and the reaction mixture was stirred overnight and cooled; the precipitate was collected by filtration and dried \textit{in vacuo} (0.246 g, 0.526 mmol, 92% yield based on V). V(ema)_3 is very sensitive to oxidation in the solid state in the presence of dioxygen. This complex possesses a solubility profile similar to V(ma)_3. V(ema)_3 possesses greater solubility in the less polar solvents including toluene and benzene, and lower water solubility. V(ema)_3 can be extracted from aqueous solutions with methylene chloride, ethylacetate or n-octanol.

\textbf{Tris(kojato)vanadium(III) monohydrate, V(koj)_3•H_2O.} Vanadyl sulphate trihydrate (2.13 g, 9.81 mmol) and kojic acid (4.26 g, 29.9 mmol) were stirred until both dissolved in 50 mL water at 55° C under Ar. Addition of sodium dithionite (5.40 g, 31.0 mmol) to the green solution yielded, upon cooling, an orange powder which was collected by filtration, air-dried and subsequently dried exhaustively \textit{in vacuo} (3.60 g, 7.35 mmol, 75 % yield based on V). V(koj)_3•H_2O is moderately susceptible to oxidation in the solid state in the presence of dioxygen. V(koj)_3•H_2O is soluble only in water, from which it cannot be extracted.
Tris(1,2-dimethyl-3-oxy-4-pyridinonato)vanadium(III) dodecahydrate,
V(dpp)$_3$$\cdot$12H$_2$O. Vanadyl(IV) sulphate trihydrate (10.83 g, 49.87 mmol) and maltol (18.81 g, 149.1 mmol) were stirred at 65 °C in 0.3 L water under a positive flow of Ar. Aqueous 40% methylamine (40 mL, 58 mmol) was added, resulting in dissolution of all reagents. Sodium dithionite (10.00 g, 57.43 mmol) was added as a solid and the reaction mixture was stirred overnight. The reaction mixture was cooled to room temperature and the resulting brown crystalline solid was isolated by filtration and dried in a stream of Ar (15.57 g, 22.84 mmol, 45 % yield based on V). V(dpp)$_3$$\cdot$12H$_2$O can be dissolved in water or methanol.

X-ray crystallographic analysis of V(dpp)$_3$$\cdot$12H$_2$O. All measurements were performed by the late Dr. Steven J. Rettig on a Rigaku/ADSC CCD area detector with graphite monochromated Mo-K$\alpha$ radiation. The structure was solved by direct methods$^{13}$ and expanded using Fourier techniques.$^{14}$ The non-hydrogen atoms were refined isotropically. The hydrogen atoms were refined isotropically. Neutral atom scattering factors were taken from Cromer and Waber.$^{15}$ Anomalous dispersion effects were included in $F_{\text{calc}}$;$^{16}$ the values of $\Delta f$ and $\Delta f''$ were those of Creagh and McAuley.$^{17}$ The values for the mass attenuation coefficients are those of Creagh and Hubbell.$^{18}$ All calculations were performed using the teXscan crystallographic package of Molecular Structure Corporation.$^{19}$

References on page 46
Table 3.1  Elemental Analyses (found [calc.]) and Bulk Magnetic Moments (B. M.).

<table>
<thead>
<tr>
<th>Compound</th>
<th>C</th>
<th>H</th>
<th>N</th>
<th>$\mu_{\text{eff}}$ (B. M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V(ma)$_3$</td>
<td>50.52 [50.72]</td>
<td>3.56 [3.55]</td>
<td>-</td>
<td>2.7</td>
</tr>
<tr>
<td>V(ema)$_3$</td>
<td>53.31 [53.86]</td>
<td>4.46 [4.52]</td>
<td>-</td>
<td>2.6</td>
</tr>
<tr>
<td>V(koj)$_3$$\cdot$$\text{H}_2\text{O}$</td>
<td>43.92 [43.97]</td>
<td>3.48 [3.57]</td>
<td>-</td>
<td>2.7</td>
</tr>
<tr>
<td>V(dpp)$_3$$\cdot$$12\text{H}_2\text{O}$</td>
<td>37.19 [37.01]</td>
<td>6.98 [7.10]</td>
<td>6.03 [6.17]</td>
<td>2.5</td>
</tr>
</tbody>
</table>

3.3 Results and Discussion

The syntheses of the V(III) complexes are easily accomplished in relatively large quantities in high yields under aqueous conditions by dithionite (hydrosulphite) reduction of a suitable oxovanadium(IV) starting material. This method of generation of V(III) complexes has been employed in the facile synthesis of vanadium(III) diketonates in aqueous solvent mixtures.\(^{20}\) The “one-pot” synthesis of V(dpp)$_3$$\cdot$$12\text{H}_2\text{O}$ was adapted from the previously reported syntheses of Al(dpp)$_3$$\cdot$$12\text{H}_2\text{O}$ and Ga(dpp)$_3$$\cdot$$12\text{H}_2\text{O}$.\(^{21}\)

The complexes were characterized in the solid state by IR, MS, EA, bulk magnetic susceptibilities, and in the case of V(dpp)$_3$$\cdot$$12\text{H}_2\text{O}$, by a single crystal X-ray structure determination. Room temperature magnetic moments are unremarkable for the V(III) compounds; each possesses a bulk magnetic moment lower than the spin only value (2.83 B. M.), typical of vanadium(III) complexes (Table 3.1).\(^{22,23}\) The complexes are not prone to contamination by any impurity, except water, and give good analyses (Table 3.1).
Table 3.2 Selected Infrared Vibrations (KBr, ± 4 cm⁻¹, Values in Parentheses Describe Changes from the Free Ligand Values, Δ cm⁻¹).

<table>
<thead>
<tr>
<th></th>
<th>V(ma)₃</th>
<th>V(ema)₃</th>
<th>V(koj)₂•H₂O</th>
<th>V(dpp)₃•12H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>νₐ and νᵣᵉⁿᵍ</td>
<td>1605 (-44)</td>
<td>1600 (-44)</td>
<td>1613 (-47)</td>
<td>1606 (-24)</td>
</tr>
<tr>
<td></td>
<td>1571 (-41)</td>
<td>1567 (-45)</td>
<td>1556 (-56)</td>
<td>1550 (-16)</td>
</tr>
<tr>
<td></td>
<td>1501</td>
<td>1502</td>
<td>1514</td>
<td>1501</td>
</tr>
<tr>
<td></td>
<td>1464</td>
<td>1470</td>
<td>1471</td>
<td>1461</td>
</tr>
<tr>
<td>νᵥ₋ₒ</td>
<td>722</td>
<td>715</td>
<td>758</td>
<td>706</td>
</tr>
</tbody>
</table>

Infrared spectroscopy. The infrared spectra of the vanadium(III) complexes are, as expected, dominated by ligand vibrations characteristic of bidentate monoanionic 3-oxy-4-pyronato and 3-oxy-4-pyridinonato ligands. Significant reductions of the pyrone and pyridinone stretching frequencies, inseparable combinations of the ligand C=O and high energy ring vibrations,²⁴ are observed when the IR spectra of the V(III) complexes are compared to the IR spectra of the respective free ligands (Table 3.2, Figure 3.2). The pyrone vibrations shift approximately 40 cm⁻¹ to lower energy when the ligand is deprotonated and bound to V(III). The reduction in the pyridinone stretching frequencies, on forming the V(III) complex (~ 20 cm⁻¹), is significantly less than the reduction observed in the formation of the oxypyronato complexes (~ 40 cm⁻¹). Additionally, the appearance of V-O stretches (Figure 3.2) at approximately 700 cm⁻¹ distinguish the complexes from the free ligands and confirm the bidentate nature of the ligand binding. The key feature
of the infrared spectra of the vanadium(III) complexes is the complete absence of a V=O vibration. The infrared absorption spectra of V(ema)_3 and VO(ema)_2 are similar with the exception of the V=O vibration in the latter complex. The changes in the pyrone stretching frequencies (when compared to Hema), -43 and -62 cm^{-1} for VO(ema)_2, are similar to those observed for V(ema)_3. The waters of hydration in V(dpp)_3\cdot12H_2O are observed as a broad band at 3430 cm^{-1} with no defined structure.

**Figure 3.2** Infrared Spectra of VO(ema)_2, V(ema)_3 and Hema. Key Vibrations Include ν_{pyrone} (a and b), ν_{V=O} (c) and ν_{V-O} (d).
Figure 3.3 Positive Ion Detection Mode LSI Mass Spectra for

\[ \text{[VO(ema)\textsubscript{2}(Hema)]}^+\text{H}_2\text{O} \] and \( \text{V(ema)}\textsubscript{3} \) \((L = \text{ema})\).

\[ \text{[VO(ema)\textsubscript{2}(Hema)]}^+\text{H}_2\text{O} \]

\[ \text{[VOL]}^+ \]  \[ \text{[HVOL}\textsubscript{2}]^+ \]  \[ \text{[VL}\textsubscript{3}]^+ \]

Relative Intensity

\[ \text{m/z} \]

\( 73 \quad 137 \quad 160 \quad 229 \quad 256 \quad 289 \quad 370 \quad 420 \quad 468 \quad 526 \quad 535 \)

\[ \text{[V}_2\text{OL}\textsubscript{4}]^+ \]  \[ \text{[V}_2\text{O}_2\text{L}\textsubscript{3}]^+ \]  \[ \text{[V}_2\text{L}\textsubscript{5}]^+ \]

\[ \text{m/z} \]

\( 551 \quad 634 \quad 654 \quad 696 \quad 707 \quad 717 \quad 740 \quad 797 \quad 797 \quad 850 \quad 893 \quad 893 \quad 930 \quad 930 \quad 978 \quad 1077 \quad 1120 \quad 1193 \quad 1300 \)

\[ \text{V(ema)}\textsubscript{3} \]

\[ \text{[VOL]}^+ \]  \[ \text{[VL]}^+ \]  \[ \text{[VL}\textsubscript{3}]^+ \]

Relative Intensity

\[ \text{m/z} \]

\( 73 \quad 137 \quad 160 \quad 229 \quad 256 \quad 289 \quad 370 \quad 420 \quad 468 \quad 526 \quad 535 \)

\[ \text{[V}_2\text{L}\textsubscript{5}]^+ \]

\[ \text{m/z} \]

\( 707 \quad 797 \quad 850 \quad 893 \quad 930 \quad 1077 \quad 1120 \quad 1193 \quad 1300 \)

References on page 46
Table 3.3 Selected Ions [Positive Ion Detection Mode LSIMS, m/z (% relative intensity)].

<table>
<thead>
<tr>
<th>Ion</th>
<th>V(ma)₃</th>
<th>V(ema)₃</th>
<th>V(koj)₃·H₂O</th>
<th>V(dpp)₃·12H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>[VOL]⁺</td>
<td>192 (25)</td>
<td>206 (25)</td>
<td>208 (20)</td>
<td>205 (25)</td>
</tr>
<tr>
<td>[VL₂]⁺</td>
<td>301 (100)</td>
<td>329 (100)</td>
<td>333 (100)</td>
<td>327 (100)</td>
</tr>
<tr>
<td>[VL₃]⁺</td>
<td>426 (33)</td>
<td>465 (33)</td>
<td>474 (33)</td>
<td>468 (33)</td>
</tr>
<tr>
<td>[V₂O₂L₃]⁺</td>
<td>509 (&lt;10)</td>
<td>551 (&lt;10)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[V₂L₄]⁺</td>
<td>-</td>
<td>658 (&lt;10)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[V₂L₅]⁺</td>
<td>727 (&lt;10)</td>
<td>797 (10)</td>
<td>807 (10)</td>
<td>792 (&lt;10)</td>
</tr>
</tbody>
</table>

**Mass spectrometry.** The positive ion detection mode LSI mass spectra of the V(III) complexes exhibit characteristic ions with similar intensities (Table 3.3). The dominant feature in the mass spectra of all the vanadium(III) complexes is a 100% relative intensity ion with m/z characteristic of [VL₂]⁺. The parent molecular mass occurs as the [VL₃]⁺ ion, which possesses a lower relative intensity than that of the [VL₂]⁺ ion. High molecular weight associated species are observed with [V₂L₅]⁺ a particularly intense and diagnostic ion. The [V₂L₅]⁺ ion has been shown in previous work to be characteristic of trivalent metal ion tris-oxypyridinonato and -oxypyronato complexes.²⁵-²⁸ The mass spectrum of V(dpp)₃ additionally demonstrates the absence of mixed ligand complexes which may have been expected from a synthesis involving the *in situ* synthesis of the ligand.

References on page 46
Subtle mass spectral differences exist between the complexes of different oxidation states. For example, [VO(ema)₂(Hema)]⁺H₂O and V(ema)₃ exhibit similar mass spectra differing only in the high molecular weight associated ions (Figure 3.3). The [HVO(ema)₂]⁺ ion is significantly less intense in the mass spectrum of V(ema)₃ than in that of [VO(ema)₂(Hema)]⁺H₂O (Figure 3.3). In addition, the oxovanadium(IV) complex shows a less intense [V₂(ema)₃]⁺ ion than is seen for the V(III) complex, and a [V₂O(ema)₄]⁺ ion which is absent for the vanadium(III) complex (Figure 3.3).

Crystal structure of V(dpp)₃·12H₂O. V(dpp)₃·12H₂O crystallizes easily from saturated aqueous solutions at 4 °C, as large brown crystals robust to solvent loss and oxidation. Key bond lengths and angles are summarized in Table 3.4 (p. 44). The complex is isomorphous with the previously reported Al(dpp)₃·12H₂O, Ga(dpp)₃·12H₂O, In(dpp)₃·12H₂O, and Fe(dpp)₃·12H₂O. The structure consists of V(dpp)₃, a facial trigonally-symmetric compressed octahedron of three rigidly planar 1,2-dimethyl-3-oxy-4-pyridinonato ligands (Figure 3.4), enclosed in an exoclathrate hydrate structure, in which the complex is excluded from a hydrogen-bonded network provided by twelve water molecules (Figure 3.5). The M(dpp)₃·12H₂O structure is able to accommodate a variety of disparate trivalent metal ions through subtle modifications. In V(dpp)₃·12H₂O, there is a compression of the octahedron along the trigonal axis; the compression is seen in a large exocyclic angle O(1)-V(1)-O(2) of 97.74(6)° as well as in a relatively small bite angle O(1)-V(1)-O(2) of 80.57(6)°. This compression is similar to that reported for the iron homolog Fe(dpp)₃·12H₂O. In fact the bite angle, as well as the V(1)-O(1) and
V(1)-O(2) lengths for V(dpp)₃•12H₂O and Fe(dpp)₃•12H₂O are not significantly different. Fe(dpp)₃•12H₂O possesses a bite angle of 80.8(1)° and respective metal oxygen bond lengths of 1.998(4) Å and 2.038(4) Å or 2.046(3) Å.⁳⁰,³¹

The small bite angle associated with five-membered chelate rings is clearly seen when comparing V(dpp)₃•12H₂O to V(acac)₃, with the respective bite angles of 80.57(6)° for V(dpp)₃•12H₂O versus 88° for V(acac)₃.³² Oxypyridinonates are similar to catecholates, forming complexes of similar stability but differing in net charge. The bond lengths, 2.0067(14) Å for V(1)-O(1) and 2.0354(14) Å for V(1)-O(2), and the bite angle, 80.57(6)°, in this complex are well within the range of the V(III) catecholates, including [V(cat)₃]³⁻,⁵ and the macrocyclic catecholate [V(trencam)]³⁻.⁶ [V(cat)₃]³⁻ and [V(trencam)]³⁻ possess, respectively, mean V-O bond lengths of 2.013(9) Å and 1.996(7) Å as well as mean bite angles of 81.3(9)° and 80.75(8)°.⁵,⁶

The pyridinone still possesses carbonyl character which is seen in a significantly lengthened V(1)-O(2) distance versus the enolate V(1)-O(1) distance (2.0354(14) Å and 2.0067(14) Å, respectively). The ligands are strictly planar and display a polarization of the ligand with the difference between the O(1)-C(2) and O(2)-C(2) lengths, the hydroxy and ketone groups, reduced to 0.061 Å from 0.088 Å in the free ligand.³³ This is a significantly smaller averaging than is seen for the Al(III) complex (0.028 Å)²⁵ and the Ga(III) complex (0.038 Å).²⁸ Removal of all twelve waters is difficult, being accomplished only in vacuo at high temperature and greatly increasing the susceptibility of V(dpp)₃•12H₂O to oxidation. The hydrate structure provides moderate protection from ambient oxidation.
The V(dpp)$_3$ unit is chiral possessing point C$_3$ symmetry. One enantiomer stack is partnered with a stacking arrangement of the other enantiomer in the unit cell (Figure 3.5). The two enantiomers are bridged by two (H$_2$O)$_6$ rings connected to the respective enantiomer by two waters. One water, in the second coordination sphere, bridges two V(dpp)$_3$ units in the same enantiomer stack, linking the ketone O(2) of one unit to the enolate O(1) of the next, and provides connectivity in the stacking arrangement (Figure 3.5). The basic character of the V(dpp)$_3$ moiety is seen in the formation of this hydrogen-bond. The polarized second coordination sphere water passes on the excess basicity which it has gained to an additional water connecting the (H$_2$O)$_6$ ring. The ring possesses a structure nearly identical to that of common ice; ice in the low pressure form ice I$_h$. All the O-H...O bonds in the (H$_2$O)$_6$ rings are homodromic running counterclockwise, with the (H$_2$O)$_6$ rings possessing crystallographically imposed S$_6$ symmetry.
Figure 3.4 ORTEP Diagram of V(dpp)$_3$$\cdot$12H$_2$O Showing the Crystallographic Numbering. Thermal Ellipsoids for Non-Hydrogen Atoms are Drawn at 33% Probability.
Table 3.4 Selected Bond Lengths (Å) and Angles (deg) in V(dpp)$_3$·12H$_2$O with Estimated Standard Deviations in Parentheses.

<table>
<thead>
<tr>
<th>Bond Lengths</th>
<th>Bond Angles</th>
</tr>
</thead>
<tbody>
<tr>
<td>V(1)-O(1)</td>
<td>2.0067(14)</td>
</tr>
<tr>
<td>O(1)-C(2)</td>
<td>1.355(2)</td>
</tr>
<tr>
<td>N(1)-C(1)</td>
<td>1.363(2)</td>
</tr>
<tr>
<td>N(1)-C(6)</td>
<td>1.470(3)</td>
</tr>
<tr>
<td>C(1)-C(7)</td>
<td>1.482(3)</td>
</tr>
<tr>
<td>C(3)-C(4)</td>
<td>1.412(3)</td>
</tr>
<tr>
<td>V(1)-O(2)</td>
<td>2.0354(14)</td>
</tr>
<tr>
<td>O(2)-C(3)</td>
<td>1.294(2)</td>
</tr>
<tr>
<td>N(1)-C(5)</td>
<td>1.370(3)</td>
</tr>
<tr>
<td>C(1)-C(2)</td>
<td>1.390(3)</td>
</tr>
<tr>
<td>C(3)-C(4)</td>
<td>1.359(3)</td>
</tr>
<tr>
<td>C(2)-C(3)</td>
<td>1.405(3)</td>
</tr>
<tr>
<td>O(5)-V(1)-O(6)</td>
<td>104.4(1)</td>
</tr>
<tr>
<td>N(1)-C(5)-C(4)</td>
<td>121.8(2)</td>
</tr>
</tbody>
</table>
Figure 3.5 ORTEP View Down the c Axis of the Unit Cell Packing of V(dpp)₃•12H₂O.
Solid state hydrolysis. The vanadium(III) complexes also react in the solid state with hydrochloric acid vapours. Unlike VO(ema)_2, which forms a 3-hydroxy-4-pyrone complex [VOCl(Hema)_2]Cl•H_2O, V(ema)_3 and V(ma)_3 generate a mixture of the hydrolysis products VCl_3•6H_2O and ethylmaltolium chloride. The solid state hydrolysis product of V(ema)_3 is completely water soluble as well as air- and moisture-sensitive. The solid state hydrolysis product of V(ema)_3 is significantly more air-stable than V(ema)_3 and is conveniently handled in moist air for prolonged periods. Vanadium(III) aqua chloride, VCl_3•6H_2O, consists of the octahedral cis-[VCl_2(H_2O)_4]^+ moiety. A variety of stable complexes of the type Ct_n[VCl_2(H_2O)_4]Cl_{n+1}, containing a cation (Ct) and chloride incorporated in the structure, are known including KVCl_4•6H_2O, RbVCl_4•4H_2O, Cs_2VCl_5•4H_2O, and Cs_3VCl_6•4H_2O, all of which have been characterized by X-ray diffraction. The solid state hydrolysis product of V(ema)_3 is likely to be similar with ethylmaltolium chloride replacing the alkali metal chloride.

3.4 Conclusions

A series of novel vanadium(III) complexes was successfully synthesized: V(ma)_3, V(koj)_3•H_2O, V(ema)_3 and V(dpp)_3•12H_2O. Of these, V(ma)_3 possesses suitable stability for use in insulin enhancing studies because it is robust to oxidation.

3.5 References


Chapter 4

Solution Studies of V(III) and V(IV) Complexes

4.1 Introduction

This chapter deals with the solution studies of VO(ema)₂, and the novel derivatives of VO(ema)₂ [VO(ema)₂(Hema)]•H₂O and [VOCl(Hema)₂]Cl•H₂O, as well as the solution studies of the vanadium(III) complexes V(ema)₃, V(ma)₃ and V(koj)₃•H₂O primarily through the use of potentiometry and UV-vis spectrophotometry. As well, the solution behaviour of VO(ema)₂ was probed by EPR spectroscopy. The separation of solid and solution states is deliberate because solvation is paramount in aqueous solution, while lattice interactions dominate in the solid state. Solution properties, including hydrolytic stability, solubility and partitioning behaviour, can determine the activity of a potential inorganic pharmaceutical, particularly as it relates to bioavailability. Since [VO(ema)₂(Hema)]•H₂O and [VOCl(Hema)₂]Cl•H₂O contain the first examples of Hema coordination (albeit in the solid state), neutral 3-hydroxy-4-pyrone coordination to both VO²⁺ and V³⁺ was of particular interest.

Several common experimental methods are available for probing the hydrolysis of oxovanadium(IV) compounds and vanadium(III) complexes. The most widely used is potentiometry; a precise and readily automated method. The majority of metal complex hydrolysis reactions involve a transfer of protons. No information beyond stoichiometry is obtained through potentiometry. The information from equilibrium pH measurement is somewhat limited compared to measurements of

References on page 89
equilibrium free metal or free ligand concentrations. In addition, relatively high metal ion concentrations must be used (0.1-1 mM) to detect appreciable proton transfer. The hydrolysis of V(III) has not been studied by potentiometry as extensively as for the other trivalent metals ions, generally because of the tendency of V$^{3+}$ (aq) to hydrolyze and to oxidize in aqueous solution. V(H$_2$O)$_6^{3+}$, with a pK$_a$ of 2.6,$^1$ is extensively hydrolyzed at pH 2. The lack of conveniently prepared and stored primary standards of V$^{3+}$ (aq) also acts as an impediment. VO$^{2+}$ primary standards are commercially available. The only determinations of vanadium(III) binding constants published in the last five years have been those of water and sulphate binding.$^1$

UV-vis spectrophotometry gives specific information regarding the electronic structure of species in solution, because both vanadyl (d$^1$) and V(III) (d$^2$) complexes possess diagnostic ligand field transitions. Charge transfer transitions for complexes such as VO(ma)$_2$ are common. Electronic transitions for the vanadyl molecular ion are typically interpreted in terms of the molecular orbital scheme of Ballhausen and Gray.$^2$ Equilibrium constants may be determined by UV-vis spectrophotometry, provided the species follow Beer's Law. Spectrophotometry has also been widely used in the study of V(III) complexes in solution. The visible electronic absorption spectrum gives specific electronic information for V(III) (d$^2$). In fact, V(III) is one of the classical examples used in introductions to ligand field transitions. Recent examples of the use of d-d transitions in the solution study of V(III) complexes involve the use of low energy near-IR d-d transitions to differentiate between six and seven coordinate V(III) complexes of aminocarboxylates.$^3,4$ For ligands including
catecholates, the d-d transitions of V(III) complexes are often obscured by intense charge transfer transitions. Polymeric hydroxides of V(III), including $[\text{VOV}]^{4+}$ (aq) also possess diagnostic strong charge transfer transitions.$^1$

Vanadyl ($d^1$) can also be studied by EPR. V(III) ($d^2$) is not readily studied by EPR due to spin-orbit coupling.$^5$ X-band (~9 GHz) EPR gives specific information regarding the electronic structure of the vanadyl complexes. However V(IV) superhyperfine couplings (coupling of the unpaired electron to the chelating atoms) are typically smaller than experimental linewidths and are not observable.$^6$

The $\text{VO}^{2+}/\text{HL}/\text{H}^+$ equilibrium system is ternary and possesses a variety of stability constants for maximum metal ion ligand stoichiometry of one (Scheme 4.1). The basicity-adjusted stability constant ($K_{\text{ba1}}$) is the equilibrium constant determinable in variable pH experiments. The basicity-adjusted stability constant ($K_{\text{ba1}}$) is a composite of an aprotic association ($K_1$ or $K_{1^*}$) and a proton dissociation step ($K_{a1}$ or $K_{a1^*}$), and is independent of path (i.e. $K_{\text{ba1}} = K_1 K_{a1^*} = K_1 K_{a1}$). The leveling effect of water determines which deprotonations are observed directly (i.e. $pK_{a1}(\text{H}_3\text{O}^+) = 0$ (convention) < $pK_{a1^*} < pK_{a1}(\text{H}_2\text{O}) = 13.76$ (0.16 M)$^8$).

---

$^8$ Unless otherwise noted all components are aqueous. The term (aq) has been omitted for clarity and is defined in Appendix I for all components.
Scheme 4.1

\[
\begin{align*}
\text{HL} + \text{VO}^{2+} & \xrightarrow{K_1^*} [\text{VO(HL)}]^2^+ \\
\text{L}^- + \text{H}^+ + \text{VO}^{2+} & \xrightarrow{K_1} [\text{VO(L)}]^+ + \text{H}^+
\end{align*}
\]

\[K_{ba1} = K_1^* K_{a1}^* = K_{a1} K_1\]

[VO(L)]\(^+\) can form in several ways, depending on whether the metal ion plays an active or passive role in complex formation. Equivalently, hydrolysis to VO\(^{2+}\) can occur in several ways depending on whether H\(^+\) plays an active or passive role in hydrolysis. [VO(L)]\(^+\) can form through the association of VO\(^{2+}\) and L\(^-\), the product of passive deprotonation of the proligand (HL) described by the acid dissociation constant \(K_{a1}\). The association of L\(^-\) and VO\(^{2+}\) is described by the equilibrium constant \(K_1\) that is derived directly as the ratio of the constants \((K_{ba1}/K_{a1})\). Both \(K_{ba1}\) and \(K_{a1}\) can be independently determined experimentally by variable pH methods.

[VO(L)]\(^+\) can also form with the association of HL and V\(^{3+}\), described by \(K_1^*\), followed by a proton dissociation, described by the acid dissociation constant \(K_{a1}^*\). The association of the metal ion and HL typically assists in the formation of the [VO(L)]\(^+\) as deprotonation is promoted \((K_{a1}^* \gg K_{a1})\). For example, the vanadyl aqua ion \((pK_{a1}^* \sim 5.7)\) is approximately 8 orders of magnitude more acidic than water itself.
(pK_{a1}(H_2O) = 13.76^8). The deprotonation may also be inhibited by the metal ion (K_{a1} > K_{a1} as for Ru(II) pyrazole complexes^10) although this is far less common. The basicity-adjusted stability constant (K_{ba1}) is a composite of the HL association constant (K_{1^+}) and the acid dissociation constant (K_{a1^*}) which pair may not be easily separable by variable pH methods. When the association constant (K_{1^+}) is high enough for [VO(HL)]^+ to be fully formed, [VO(HL)]^+ behaves as a simple acid and K_{a1^*} is determined by variable pH methods. Because H_2O is conventionally ignored in the definition of the components, the association of VO^{2+} with HL describes a combination of inner sphere and outer sphere interactions. For instance, [VO(HL)]^{2+} is used to describe all of [VO(H_2O)_3(HL)]^{2+}, [VO(H_2O)_4(HL)]^{2+} and [VO(H_2O)_5]^{2+}•HL. For neutral weak acids (K_{a1} << 1 and K_{1^+} ~ 1), [VO(L)]^+ is expected to form almost exclusively via [VO(HL)]^{2+}. Usually, [VO(HL)]^{2+} is expected to be an intermediate not contributing significantly to the mass balance ([VO(HL)]^{2+} < 5\% [V]_{total}). Protonated intermediates similar to [VO(HL)]^{2+} are known to play an integral role in hydrolytic processes. Kinetic studies for the acid hydrolysis of bidentate chelates of trivalent metals, including V(acac)_3,^11 support proton-assisted hydrolysis involving long-lived [M(HL)L_2]^+ intermediates. Proton-assisted hydrolysis (metal ion-assisted deprotonation) has been well documented for Cr(III).^12-14 The deprotonation of glycine in the presence of Cr(III) was proposed to occur via a saturated Cr(H_2O)_6^{3+} glycine complex (Scheme 4.2),^15 with saturation attributed to strong hydrogen binding between Cr(H_2O)_6^{3+} and glycine.
Scheme 4.2 Proposed Mechanism for the Deprotonation of Glycine in the Presence of [Cr(H$_2$O)$_6$]$^{3+}$.

\[
\begin{align*}
[\text{Cr(H}_2\text{O)}_6]^{3+} + \text{NH}_3\text{CH}_2\text{CO}_2^- & \; \xrightarrow{K_1^*} \; [\text{Cr(H}_2\text{O)}_6\cdot\text{NH}_3\text{CH}_2\text{CO}_2] \\
[\text{Cr(H}_2\text{O)}_6\cdot\text{NH}_3\text{CH}_2\text{CO}_2] & \; \xrightarrow{} \; [\text{Cr(H}_2\text{O)}_4(\text{NH}_2\text{CH}_2\text{CO}_2)]^{2+} + \text{H}^+
\end{align*}
\]

The stability of [VO(L)]$^+$ (i.e. $K_{ba1}$ and $K_1$) can be greatly underestimated if a significant concentration of [VO(HL)]$^{2+}$ is overlooked because the equilibrium concentrations of VO$^{2+}$ and HL, which are generally not measured, are greatly overestimated. The presence of significant HL association can be easily detected if HL, VO$^{2+}$ or [VO(HL)]$^{2+}$ are measured along with the formation of [VO(L)]$^+$ but may otherwise be subtle.

Significant formation of [VO(HL)]$^{2+}$ can be predicted from the magnitude of the basicity-adjusted constant $K_{ba1}$ which provides an estimate of the minimum value of $K_1^*$. As $K_{ba1} = K_1^*K_{a1}^*$, the minimum value of $K_1^*$ equals $K_{ba1}$ when $K_{a1}^* = 1$ ($K_{a1}^* = K_a(\text{H}_3\text{O}^+) = 1$). Large values of $K_{ba1}$ ($K_{ba1} >> 1$) indicate significant association of HL. Generally, basicity-adjusted stability constants are significantly less than one. For maltol, $K_{ba1} > 1$ has only been observed for VO$^{2+}$ and Fe$^{3+}$. For Fe$^{3+}$ and Hma the reported values of log $K_1$ and p$K_{a1}$ are 11.5 and 8.62, respectively, leading to log $K_{ba1} = 2.9$. With a minimum value of log $K_1^* = 2.9$ for Fe$^{3+}$ and Hma association, [Fe(Hma)]$^{3+}$ is expected to be a major contributor to the mass balance. For Hma and VO$^{2+}$, the basicity-adjusted stability constant log $K_{ba1} = 0.34$ leads to a minimum association constant log $K_1^* = 0.34$. With a minimum value of log $K_1^* =
0.34 for VO\(^{2+}\) and Hma association, \([\text{VO(Hma)}]\(^{2+}\) is not expected to be a major contributor to the mass balance.

The presence of HL association can be determined using a variety of direct and indirect methods. Ideally, HL association would be directly observable by spectroscopic methods. No direct evidence of the existence of Hma complexes of vanadium in solution or in the solid state has been found in previous studies;\(^7\)\(^-\)\(^{16}\) however, indirect methods can prove useful for spectroscopically subtle associations. Suitable indirect methods include examinations of dilution behaviour as well as ligand competitions. These methods are generally useful in equilibrium model testing and are not limited to detecting behaviour like HL association. Linear dilution behaviour is diagnostic for saturation. Saturation can result from the highly favourable association of HL (\(K_1^* \gg 1\)) leading to complete formation of \([\text{VO(HL)}]\(^{2+}\). \([\text{VO(HL)}]\(^{2+}\) in a saturated system dilutes linearly because the ligand to metal stoichiometry is essentially constant. In a saturated system, \([\text{VO(HL)}]\(^{2+}\) behaves as a simple acid with an apparent acid dissociation constant \(pK_{a1}^*\) determinable by variable pH methods. Linear dilution behaviour, although desirable for a potential inorganic pharmaceutical, is generally unexpected for complexes with high ligand to metal stoichiometries. Figure 4.1 illustrates the expected dramatic effect of dilution on VO(ma)\(_2\) based on literature stability constants.\(^{16}\) Although complexation is significant at 10 mM VO(ma)\(_2\) at pH 2, dilution to 0.1 mM VO(ma)\(_2\) is expected to lead to nearly complete dissociation at equilibrium.

Ligand competition with strong chelators including EDTA\(^4-\), provides an additional useful method to confirm the validity of the determined stability constants.
determined by potentiometry. Discrepancies between equilibrium constants determined by variable pH methods and those determined by ligand competition can result, in part, from the neglected association of the proligand.

Ternary systems involving ligand to metal stoichiometries greater than one, as well as multiple deprotonations, present much more complicated systems but can generally be simplified because all the possible equilibria will not contribute to the mass balance.

The major goal of this chapter was to examine the hydrolytic stability of V(ema)$_3$, V(ma)$_3$ and V(koj)$_3$·H$_2$O, as well as VO(ema)$_2$ through potentiometry. An additional goal was to examine whether the association of neutral 3-hydroxy-4-pyrones was significant. The simplest model that could adequately describe the equilibria was to be developed. Model testing was to be accomplished using simple experiments including dilution experiments (dilution titrations or Beer's Law Plots) and ligand competition experiments.
Figure 4.1 Calculated Speciation Diagrams for VO(ma)$_2$ at 10 mM and 0.1 mM (0.16 M NaCl, 25 °C).

10 mM VO(ma)$_2$

0.1 mM VO(ma)$_2$

References on page 89
4.2 Experimental

Complexes were synthesized as detailed in Chapters 2.2 and 3.2. All manipulations were performed using standard inert atmosphere techniques. Vanadium(III) complexes, used in potentiometric and spectrophotometric studies, were stored in a Vacuum Atmosphere dry-box. CH$_2$Cl$_2$ was distilled and dried (CaH$_2$) prior to use. Na$_2$[H$_2$EDTA]•2H$_2$O (Aldrich) was used without further purification.

**Instrumentation** UV-Vis absorption spectra were recorded in 10 mm quartz cells with a Shimadzu UV-2100 spectrophotometer connected to a Julabu UC circulating bath (25.0 ± 0.1 °C) or an HP 8453 spectrophotometer connected to a Fisher ISOTEMP 1016D circulating bath (25.0 ± 0.1 °C). X-band (~9 GHz) EPR spectra were recorded as the first derivative of absorption on a Bruker ECS-106 EPR spectrometer. A flow-through cryostat in conjunction with a Eurotherm B-VT 2000 variable-temperature provided temperatures from 120-370K. The microwave frequency and magnetic field were calibrated with an EIP 625A microwave frequency counter and a Varian E500 gaussmeter, respectively. Computer simulations of isotropic and anisotropic EPR spectra were performed using the SOPHE simulation package$^{19,20}$ running on IBM AX-6000 and Sun SPARC station 10/30 Unix workstations.

**Potentiometric measurements.** All potentiometric measurements were performed under an inert atmosphere (Ar passed through 10% aqueous NaOH). All potentiometric measurements were performed with autotitrators consisting of either a Fisher Acumet 950 Fisher digital pH meter or an Orion EA 920 digital pH meter.
connected to model 665 Metrohm Dosimat autoburets and water jacketed vessels maintained at 25.0 ± 0.1 °C by a Julabo UC circulating bath. The autotitrators were automated with IBM PCs interfaced with both the autoburets and pH meters. Orion-Ross glass and calomel reference electrodes were used. Electrodes were calibrated by titrating a known amount of HCl with standardized NaOH. A plot of mV (measured) vs. pH (calculated) gave a working slope and intercept so that pH could be read as -log[H⁺] directly.** NaOH solutions were prepared from dilution of 50% NaOH with freshly boiled degassed water and standardized against potassium hydrogen phthalate.

Solutions were prepared for vanadyl titrations either by dilution of VO²⁺ atomic absorption standard (Sigma) or by dissolving solid VO(ema)₂, [VO(ema)₂(Hema)]·H₂O or [VOCI(Hema)₂]Cl·H₂O in an aqueous solution of appropriate concentration of NaCl, HCl and NaOH. Hema (Pfizer) was added to obtain the desired ligand to metal ratio. The vanadyl standard was titrated with standardized NaOH from pH 2 to pH 2.5 to obtain the excess acid by the method of Gran.21 The ratio of ligand to metal used in the titration of VO²⁺ and Hema was 1:10 < L:M < 1:1. The concentration range was 0.1 mM to 4 mM. A total of ten titrations were performed for Hema alone, with five titrations of combined VO²⁺ and Hema. Typically, 100 data points were collected in the buffer region of metal-ligand complexation. Equilibrium times between 1-3 minutes were used to ensure that equilibrium was achieved.

** pH is used to indicate -log[H⁺] throughout this chapter

References on page 89
For potentiometric measurements, solutions of the vanadium(III) complexes were prepared by dissolution of $\text{V(ema)}_3$, $\text{V(ma)}_3$ or $\text{V(koj)}_3\cdot\text{H}_2\text{O}$ in an aqueous solution with appropriate concentrations of NaCl (0.15 M NaCl) and HCl (0.01 M HCl), with the concentration of HCl determined by the method of Gran\textsuperscript{21} prior to the addition of the metal complex. For $\text{V(ema)}_3$, $\text{V(ma)}_3$ and $\text{V(koj)}_3\cdot\text{H}_2\text{O}$, all titrations were, by definition, at the 3:1 ligand to metal ratio. For spectrophotometric measurements, $\text{V(ema)}_3$, $\text{V(ma)}_3$ or $\text{V(koj)}_3\cdot\text{H}_2\text{O}$ were dissolved in the appropriate concentrations of HCl, NaCl and NaOH. $\text{V(koj)}_3\cdot\text{H}_2\text{O}$ was titrated six times in 0.16 NaCl. $\text{V(ema)}_3$ was titrated three times in 0.16 M NaCl. $\text{V(ma)}_3$ was titrated nine times in 0.16 NaCl. Generally, 100 data points were collected in the buffer region of metal-ligand complexation. Equilibrium times between 1-3 minutes were used to ensure that equilibrium was achieved.

Solutions for spectrophotometric measurements were made similarly to those used in the potentiometric measurements. Solutions with acidities between 1M–0.01 M HCl were made by dissolving the solid metal complexes in acid solutions, the concentrations of which were determined by titration with standardized NaOH. For solutions involving HCl concentrations greater than 1 M, the metal complexes were dissolved in the appropriate ratio of concentrated aqueous HCl and water. Under these conditions concentrations were not corrected for density changes.

**EDTA\textsuperscript{2-} competition.** Solutions were made containing a dissolved metal complex ($\text{VO(ema)}_2$ or $[\text{VO(ema)}_2(\text{Hema})]\cdot\text{H}_2\text{O}$) and $\text{Na}_2[\text{H}_2\text{EDTA}]\cdot\text{2H}_2\text{O}$ in 0.16 M NaCl under Ar. The pH was measured as for the section on potentiometry and was adjusted to pH 7.4 with the addition of standardized NaOH.
4.3 Results and Discussion

UV-vis spectroscopy. All three oxovanadium(IV) complexes VO(ema)$_2$, [VO(ema)$_2$(Hema)]$\cdot$H$_2$O, and [VOCI(Hema)$_2$]Cl$\cdot$H$_2$O give identical visible spectra in water at pH 7 at identical concentrations, indicating the formation of identical products under these conditions (Table 4.1). The visible spectra, which are identical to that of VO(ma)$_2$ under analogous conditions,$^{16}$ possess three transitions including an ema$^{-}$ to VO(IV) charge transfer with $\lambda_{\text{max}} = 445$ nm, as well as two d-d transitions. In the UV spectrum, an ema$^{-}$ $\pi-\pi^*$ transition is observed with $\lambda_{\text{max}} = 313$ nm.

The visible electronic absorption spectra are consistent with the formation of octahedral vanadyl complexes possessing an O$_5$ donor set,$^{22}$ such as VO(ema)$_2$(OH)$_2$. According to the molecular orbital scheme of Ballhausen and Gray$^2$ for octahedral vanadyl complexes (with C$_{4v}$ symmetry), the two d-d transitions are attributed to a low energy $^2\text{B}_2 \rightarrow ^2\text{E}$ ($d_{xy} \rightarrow d_{xz}$, $d_{yz}$) transition, with $\lambda_{\text{max}} = 872$ nm and $\varepsilon = 35(1) \text{ M}^{-1} \text{ cm}^{-1}$ at pH 7 for VO(ema)$_2$, and a less intense higher energy $^2\text{B}_2 \rightarrow ^2\text{B}_1$ ($d_{xy} \rightarrow d_{x^2-y^2}$) transition, with $\lambda_{\text{max}} = 622$ nm and $\varepsilon = 16(1) \text{ M}^{-1} \text{ cm}^{-1}$ at pH 7 for VO(ema)$_2$. 

References on page 89
Table 4.1 Visible Spectral Parameters.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conditions</th>
<th>(\lambda_{\text{max}}), nm ((\varepsilon), M(^{-1}) cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO(ema)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>pH 7</td>
<td>445 (118), 622 (16), 872 (35)</td>
</tr>
<tr>
<td>[VO(ema)&lt;sub&gt;2&lt;/sub&gt;(Hema)]•H(_2)O</td>
<td>pH 7</td>
<td>445 (118), 622 (16), 872 (35)</td>
</tr>
<tr>
<td>[VOCl(Hema)&lt;sub&gt;2&lt;/sub&gt;]Cl•H(_2)O</td>
<td>pH 7</td>
<td>445 (118), 622 (16), 872 (35)</td>
</tr>
<tr>
<td>VO(ema)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>conc. HCl</td>
<td>723 (19)</td>
</tr>
<tr>
<td>[VO(ema)&lt;sub&gt;2&lt;/sub&gt;(Hema)]•H(_2)O</td>
<td>conc. HCl</td>
<td>723 (19)</td>
</tr>
<tr>
<td>[VOCl(Hema)&lt;sub&gt;2&lt;/sub&gt;]Cl•H(_2)O</td>
<td>conc. HCl</td>
<td>400 (35), 723 (19)</td>
</tr>
<tr>
<td>VO(ema)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>CH(_2)Cl(_2)</td>
<td>400 (530), 542 (263), 617 (158)</td>
</tr>
<tr>
<td>[VO(ema)&lt;sub&gt;2&lt;/sub&gt;(Hema)]•H(_2)O</td>
<td>CH(_2)Cl(_2)</td>
<td>400 (530), 542 (263), 617 (158)</td>
</tr>
</tbody>
</table>

The LMCT and the ema\(^*\) \(\pi-\pi^*\) transition follow Beer's Law (Figure 4.2), diluting linearly to the origin within experimental error. In Figure 4.2, VO(ema)<sub>2</sub> at pH 7 dilutes linearly to detection limits, indicating that VO(ema)<sub>2</sub> remains intact even at \(\mu\)M concentrations. This stability to dilution is unexpected for a complex with a ligand to metal stoichiometry greater than one, and is highly desirable in a potential inorganic pharmaceutical. For VO(ema)<sub>2</sub> and [VO(ema)<sub>2</sub>(Hema)]•H\(_2\)O in CH\(_2\)Cl\(_2\), the ligand field transitions are obscured by charge transfer transitions (Table 4.1). The visible spectra of VO(ema)<sub>2</sub> and [VO(ema)<sub>2</sub>(Hema)]•H\(_2\)O in CH\(_2\)Cl\(_2\) are similar, indicating that only VO(ema)<sub>2</sub> persists in CH\(_2\)Cl\(_2\).
Differences occur in the visible spectra of VO(ema)$_2$, [VO(ema)$_2$(Hema)]•H$_2$O, and [VOCl(Hema)$_2$]Cl•H$_2$O dissolved in concentrated HCl (Table 4.1). In concentrated HCl, an additional high energy charge-transfer transition is observed for [VOCl(Hema)$_2$]Cl•H$_2$O attributed to a Cl$^-$ to VO(IV) charge transfer transition. The Cl$^-$ to VO(IV) charge transfer transition dilutes linearly with [VOCl(Hema)$_2$]Cl•H$_2$O concentration, at constant acidity, indicating the presence of an equilibrium with a constant stoichiometry. Neither VO(ema)$_2$ nor [VO(ema)$_2$(Hema)]•H$_2$O exhibit any charge transfer transitions when these complexes are dissolved in concentrated HCl, nor do acidified solutions of [VOCl(Hema)$_2$]Cl•H$_2$O dissolved in water.

The LMCT of VO(ema)$_2$ dilutes linearly with [VO(ema)$_2$] under conditions of acidic hydrolysis, indicating the presence of an equilibrium dominated by a single metal to ligand stoichiometry (Figure 4.3). The hydrolysis due to dilution is expected to be very significant under acidic conditions (Figure 4.1, p. 58).
Figure 4.2 Beer's Law Plots for VO(ema)\(_2\) (0.16 M NaCl, 25 °C).

Plot of Absorbance\(_{313}\) vs. [VO(ema)\(_2\)]

\[ \varepsilon_{313} = 1.13(1) \times 10^4 \text{ M}^{-1} \text{ cm}^{-1} \]

Plot of Absorbance\(_{445}\) vs. [VO(ema)\(_2\)]

\[ \varepsilon_{445} = 118(4) \text{ M}^{-1} \text{ cm}^{-1} \]
Figure 4.3 Plot of Absorbance$_{435}$ vs. [VO(ema)$_2$] for 25 mM VO(ema)$_2$ (0.1 M HCl, 25 °C).

The Hema π-π* transition shifts to lower energy (Figure 4.4) in the presence of vanadyl at low pH due to deprotonation; no change is observed in the absence of vanadyl. The increase in the ema* π-π* which accompanies this process varies linearly with [H$^+$]$^{-1}$ (Figure 4.5) indicating that only one equivalent of proton per metal is involved (i.e. simple stepwise deprotonations). The LMCT transition also increases linearly with [H$^+$]$^{-1}$ in the pH range 2-7. Additionally buffering is observed below pH 2 with dramatic deviations from linearity as the charge transfer decreases linearly with approximately [H$^+$]$^{1/2}$ indicating a proton to metal stoichiometry of less than one. This additional buffering, precluding the determination of equilibrium constants in this pH region using linear methods, is likely due to the protonation of Hema or depletion of water as the acid concentration becomes significant. Above pH 7, new intense charge transfer transitions in the visible spectrum of VO(ema)$_2$ are observed, consistent with hydrolysis to polyoxovanadates of V(IV).
Figure 4.4 Plots of Absorbance vs. Wavelength for the Variable pH Titration of \([\text{VO}^{2+}] = 968 \ \mu\text{M}, \ [\text{Hema}] = 94 \ \mu\text{M} \ (25 \ ^\circ\text{C}, 0.16 \ \text{M NaCl})].

![Absorbance vs. Wavelength](image)

Figure 4.5 Plot of Absorbance_{313} vs. \([\text{H}^+]^{-1}\) for \([\text{VO}^{2+}] = 968 \ \mu\text{M}, \ [\text{Hema}] = 94 \ \mu\text{M} \ (25 \ ^\circ\text{C}, 0.16 \ \text{M NaCl})].

![Absorbance vs. [H+]^{-1}](image)

The ligand field transitions (d-d bands) for the vanadium(III) complexes are obscured in neutral to moderately acidic aqueous solutions by intense charge
transfer transitions which dominate the electronic absorption spectra. Ligand field
transitions (d-d transitions) are observed in strongly acid conditions. The
absorptions due to charge transfer are significantly more intense than those
observed for the analogous oxovanadium(IV) complexes.

\textit{V(ma)\textsubscript{3}} (Figure 4.6), \textit{V(ema)\textsubscript{3}} and \textit{V(koj)\textsubscript{3}H\textsubscript{2}O}, in neutral aqueous conditions,
are intensely red due to an intense charge transfer transition, \(\lambda_{\text{max}}\) (log \(\varepsilon\)): 480 nm (2.7), which is not significantly different for the three complexes. \textit{V(dpp)\textsubscript{3}} is intensely
yellow in solution, also due to an intense charge transfer transition, \(\lambda_{\text{max}}\) (log \(\varepsilon\)): 400 nm (2.8). These transitions are solvent dependent. For \textit{V(ma)\textsubscript{3}}, the lowest energy
transition occurs in \textit{CH\textsubscript{2}Cl\textsubscript{2}} and \textit{CHCl\textsubscript{3}}, \(\lambda_{\text{max}}\) (log \(\varepsilon\)): 525 nm (3.5). In acetone and
toluene the transition occurs at intermediate energy, \(\lambda_{\text{max}}\) (log \(\varepsilon\)): 510 nm (2.3). The
highest energy transition occurs in the protic solvents methanol and water. The
complexes dilute linearly in neutral to moderately acidic aqueous conditions. At pH
7, \textit{V(ma)\textsubscript{3}} dilutes linearly to nearly 50 \(\mu\text{M}\) (Figure 4.7), and \textit{V(dpp)\textsubscript{3}} to detection
limits. For \textit{V(ma)\textsubscript{3}} and \textit{V(ema)\textsubscript{3}} the intensity of the charge transfer achieves a
maximum at a pH of approximately 7 indicating the greatest concentration of the
neutral complex at this pH. Hydrolysis is observed as a decrease in the intensity of
the charge transfer transition under both acidic and alkaline conditions.
Figure 4.6 Plot of Absorbance vs. Wavelength for the Dilution of 5 mM \text{V(\text{ma})_3} (25 ^\circ \text{C}, 0.16 \text{ M NaCl}).

Figure 4.7 Plot of Absorbance_{570} vs. [V(\text{ma})_3] (25 ^\circ \text{C}, 0.16 \text{ M NaCl}).
EPR spectroscopy. As found with VO(ma)$_2$, VO(ema)$_2$ in aqueous glycerol (1:1) displays two sets of qualitatively very similar eight line patterns (Figure 4.8, most clearly seen in the $m_I = 5/2$ resonance) resulting from hyperfine couplings of the vanadium nuclear spin ($I = 7/2$) to the unpaired electron. These overlapping spectra were attributed to ligand orientational isomers for octahedral VO(ma)$_2$(H$_2$O) (i.e. cis and trans aquaioxobismaltolato species) and VO(ema)$_2$ appears to behave similarly. Although this spectrum was not modelled based on the spin Hamiltonian, an isotropic $g$ value ($g_{iso}$) of 1.96 and an isotropic vanadium hyperfine coupling ($A_{iso}$) of approximately $-100 \times 10^{-4}$ cm$^{-1}$ can be estimated, based on the midpoint and on the distance between the $m_I = -\frac{1}{2}$ and $m_I = \frac{1}{2}$ transitions, respectively, for both species. Such parameters, although approximate, are well within the range of VO(O$_5$) coordination environments including VO(H$_2$O)$_5^{2+}$ (aq) and VO(ma)$_2$(OH)$_2$ (aq).$^7$

**Figure 4.8** Isotopic EPR Spectrum of 3 mM VO(ema)$_2$ in 50% Aqueous Glycerol (pH = 7.0, $T = 298$ K, $\nu = 9.5905$ GHz).
Figure 4.9 Experimental and Simulated Isotropic EPR Spectra of 1mM VO(ema)$_2$ in CH$_2$Cl$_2$ (T = 298 K, $\nu = 9.5855$ GHz).

Experimental

Simulated

References on page 89
Figure 4.10 Experimental and Simulated Anisotropic Frozen-solution EPR Spectra of 1mM VO(ema)$_2$ in CH$_2$Cl$_2$ (T = 177 K, $\nu = 9.5927$ GHz).

Experimental

Simulated
Table 4.2 Spin Hamiltonian Parameters for VO(ma)$_2$ and VO(ema)$_2$.

<table>
<thead>
<tr>
<th></th>
<th>$g_z$</th>
<th>$g_x^a$</th>
<th>$g_y^a$</th>
<th>$g_{iso}$</th>
<th>$A_z^b$</th>
<th>$A_x^b$</th>
<th>$A_y^b$</th>
<th>$A_{iso}^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO(ma)$_2$</td>
<td>1.944</td>
<td>1.984</td>
<td>1.978</td>
<td>1.971</td>
<td>-163.2</td>
<td>-49.2</td>
<td>-59.5</td>
<td>-90.05</td>
</tr>
<tr>
<td>VO(ema)$_2$</td>
<td>1.935</td>
<td>1.988</td>
<td>1.976</td>
<td>1.982</td>
<td>-170.0</td>
<td>-60.5</td>
<td>-60.0</td>
<td>-90.0</td>
</tr>
</tbody>
</table>

$^a$ The assignment of the x and y principle axes is arbitrary.

$^b$ Units for vanadium hyperfine coupling are $10^{-4}$ cm$^{-1}$.

The isotropic EPR spectrum of VO(ema)$_2$ in CH$_2$Cl$_2$ possesses only a single set of eight resonances consistent with the presence of a single square planar vanadyl species, the spectrum of which can be satisfactorily simulated based on the spin Hamiltonian parameters $g_{iso} = 1.971$ and $A_{iso} = -90.0 \times 10^{-4}$ cm$^{-1}$ (Figure 4.9). Very similar to the spin Hamiltonian parameters obtained for VO(ma)$_2$ (Table 4.2), these values are well within the range of VO(O$_4$) complexes in CH$_2$Cl$_2$.7

The anisotropic spectrum of a frozen solution of VO(ema)$_2$ in CH$_2$Cl$_2$ (Figure 4.10), displaying nearly axial symmetry, can be adequately simulated with the spin Hamiltonian parameters in Table 4.2. Small variations between the simulated and the experimental spectra in Figure 4.10 are likely the result of outer sphere coordination of CH$_2$Cl$_2$, with CH$_2$Cl$_2$ hydrogen-bonding to the vanadium oxo, or alternatively to the ligand oxygen atoms. A similar phenomenon was reported for VO(ma)$_2$ in CH$_2$Cl$_2$.7 Outer sphere bonding is well-established for vanadyl complexes and an additional example includes a hydrogen-bonded outer sphere complex of VO(acac)$_2$ and CHCl$_3$.23

References on page 89
**Potentiometry.** An indication of the hydrolytic stability of dissolved VO(ema)$_2$, as well as V(ma)$_3$, V(ema)$_3$ and V(koj)$_3$·H$_2$O, was the absence of precipitates of metal hydroxides at intermediate (pH 7) to acidic conditions.

Informative generalizations regarding the hydrolysis of a compound can be made simply from examining experimental titration curves. Hydrolysis behaviour is clear and obvious when experimental titration curves are expressed as $Z$ (Equation 4.1) vs. pH plots. In the definition of $Z$, $[H]_{\text{total}}$ is the total proton concentration, $[H]$ is the equilibrium proton concentration, $K_w$ is the ionic product of water and concentration $[HL]_{\text{total}}$ is the concentration of the acid or base examined. The quantity $Z$ gives the proton stoichiometry of acid-base reactions. $Z$ describes the release of protons by an acid ($Z < 0$) or the consumption of protons by a base ($Z > 0$) and gives the mean charge developed in the equilibrium system.

$$Z = ([H]_{\text{total}} - [H] + K_w[H]^{-1})/[HL]_{\text{total}} \quad \text{(equation 4.1)}$$

Figure 4.11 shows an experimental titration in terms of the common representation of pH vs. $V_{\text{NaOH}}$ for 1.1 mM Hema (0.16M NaCl, 25 °C). Figure 4.12 presents experimental titration curves with variable concentrations of Hema, including 1.1 mM, as plots of $Z$ (equation 4.1) versus pH (0.16M NaCl, 25 °C). While the $pK_a$ of Hema is obvious in the $Z$ vs. pH plot (Figure 4.12), it is not clear in the plot of pH vs. $V_{\text{NaOH}}$ (Figure 4.11).
**Figure 4.11** Plot of pH vs. $V_{\text{NaOH}}$ for 1.1 mM Hema ($25^\circ\text{C}$, 0.16 M NaCl).

**Figure 4.12** Plot of $Z$ vs. pH for Hema 1.1 mM (■), 0.32 mM (□), 0.93 mM (△) and 0.84 mM (□) ($25^\circ\text{C}$, 0.16 M NaCl). The Solid Line Corresponds to a Curve Fit for $pK_{a1}(\text{Hema}) = 8.48(5)$ Based on Equation 4.1. The Dotted Line is $Z = -0.5$. References on page 89
The Z vs. pH plot is essentially a speciation diagram for Hema based completely on experimental data. The degree of ionization of Hema is clear from the plot of Z vs. pH. For example, at Z = -0.5, ema\(^{-}\) is 50 % formed as 0.5 proton equivalents have been released. The pH region of predominance for Hema, the region of hydrolytic stability, is clear from the Z plot (0.05 < Z < -0.05 for 95 % Hema) with Hema hydrolytically stable below pH 7 (Figure 4.12). From the plot of Z vs. pH (Figure 4.12), Hema obviously possesses a single pK\(_a\). The pK\(_{a1}\) value of Hema was estimated to be 8.5 from the value pH at Z = -0.5. This value of the pH at Z = -0.5 provided a valuable initial guess in the iterative curve-fitting of Z to equation 4.1 which leads to a value of 8.48(5) for the pK\(_{a1}\) of Hema. Multiple concentrations of total Hema lead to coincident or parallel (a result of error in total acid concentration) Z plots, as expected for a simple acid (Figure 4.12).

\[
Z = \frac{- (K_{a1}) [H]^{-1}}{1 + (K_{a1}) [H]^{-1}} \quad \text{(equation 4.2)}
\]

Z plots form the basis for a qualitative discussion of the hydrolysis of VO(ema)\(_2\) as well as of V(ema)\(_3\), V(ema)\(_3\) and V(koj)\(_3\)H\(_2\)O, because the hydrolysis behaviour of the metal complexes is also clear in the Z vs. pH plots.

\[
Z = \frac{([H]_{\text{total}} - [H] + K_w[H]^{-1})/M_{\text{total}}}{(equation 4.3)}
\]

\[
Z_{\text{avg}} = \frac{([H]_{\text{total}} - [H] + K_w[H]^{-1} - [L^-])/M_{\text{total}}}{(equation 4.4)}
\]
Most hydrolysis processes of metal complexes involve the transfer of protons. Z is defined for a metal complex M by equations 4.3 and 4.4. Z (equation 4.3) describes the proton to metal complex stoichiometry of hydrolysis; \( Z_{\text{avg}} \) (equation 4.4) corrects this for proligand (HL) ionization. For ligands such as Hema with a \( pK_a \) of 8.48(5), at acidic to intermediate pHs, Z and \( Z_{\text{avg}} \) are equivalent, because the proligand (HL) ionization is not significant and the concentration of [L'] is very low.

Figure 4.13 shows Z vs. pH plots for titrations of V(koj)₃. The region where V(koj)₃ predominates is observed in Figure 4.13 to be from approximately pH 5.8 to 7 (Z = 0.05 to −0.05 for at least 95% V(koj)₃). Two regions where hydrolysis occurs are also clear from the Z plots corresponding to acidic (Z > 0) and alkaline hydrolysis (Z < 0) of the complex, where mean positive and negative charges, respectively, are developed on the complex. The Z plot in the region of acid hydrolysis lacks plateaus and other features, and is consistent with well-defined stepwise protonations. In the region of acid hydrolysis, and in the intermediate region where the complex is fully formed, Z vs. pH plots for V(koj)₃ at different total V(koj)₃ concentrations coincide within experimental error at a constant metal to ligand ratio (Figure 4.13). Hydrolysis independent of the total metal ion concentration indicates well-defined stepwise protonations and the absence of hydroxo complexes or binary complexes. V(koj)₃ behaves macroscopically as a simple acid in this pH range, with hydrolysis independent of the total V(koj)₃ concentration. The coincidence of Z vs. pH plots for different concentrations of V(koj)₃ is unexpected, because coordination complexes with high metal ligand stoichiometries such as V(koj)₃ are expected to show strong dilution effects, with dissociation favoured at low concentrations and complex...
formation favoured at high concentrations. This observation is based on a limited concentration range because the total concentration of \( V(koj)_3 \) was limited by solubility (< 10 mM) in addition to the high metal concentration (>1 mM) needed to detect appreciable amounts of \( H^+ \) liberated in the hydrolysis reactions. The validity of this observation is confirmed by a spectrophotometric examination of dilution (i.e. Beer's Law Plots) which was found to be linear.

**Figure 4.13** Plot of Z vs. pH for \( V(koj)_3 \) at 1.8 mM (O), 1.8 mM (■), 1.3 mM (∆) 1.1 mM (□) (0.16 M NaCl, 25 °C). The Area Between the Two Solid Lines Represents the Region where [\( V(koj)_3 \)] > 95% Total Vanadium.
The low pH region gives valuable information about the potential performance of V(koj)₃ as an insulin-enhancing agent. It is clear from the Z vs. pH plots (Figure 4.13) that V(koj)₃ will develop a high mean positive charge at pH < 3. Generally, the human stomach has a pH of 2-3 and highly charged complexes are not expected to be very bioavailable by passive diffusion. Therefore, V(koj)₃ is not expected to be highly bioavailable.

The alkaline hydrolysis of V(koj)₃ is dependent on the total concentration of V³⁺. V(koj)₃ is less susceptible to hydrolysis and develops a lower negative charge at high pH with higher total concentrations of V(koj)₃ (Figure 4.13). The Z plot in the region of alkaline hydrolysis is consistent with ill-defined hydrolysis, because Z begins to curl back on itself above pH 8, with the formation of polymeric vanadates of V(III). As seen in the plot of Z vs. pH (Figure 4.13), there is no well-defined stable hydrolysis such as to [V(koj)₃(OH)]⁻ for which a plateau at Z = -1, or an inflection point at Z = -0.5, would be expected.

The plots of Z vs. pH for V(ma)₃ (Figure 4.14) and V(koj)₃ (Figure 4.13) are very similar and possess regions of well-defined acidic hydrolysis (Z > 0) and ill-defined alkaline hydrolysis (Z < 0); however, V(ma)₃ has a wider pH region of stability (Z ~ 0 and V(ma)₃ ~ 95 % V_total) extending from pH 5.5 to 8 compared to that for V(koj)₃. In addition, compared to V(koj), V(ma)₃ develops a significantly lower positive charge at low pH and is more stable at physiological pH (7.4). The positive charge developed at pH 2 by V(ma)₃ remains relatively high (Figure 4.14).
**Figure 4.14** Plot of $Z$ vs. $pH$ for V(ma)$_3$ at 2.3 mM (■), 1.6 mM (○) (0.16 M NaCl, 25 °C). The Area Between the Two Solid Lines Represents the Region where [V(ma)$_3$] > 95% Total Vanadium.

V(ma)$_3$ and V(koj)$_3$ are similar, with acidic hydrolysis independent of total complex concentration (coincident $Z$ plots in the region of acid hydrolysis) and alkaline hydrolysis dependent on total metal concentration (lower magnitudes of $Z$ for higher total complex concentration at a given $pH$).

The hydrolysis behaviour of V(ema)$_3$ does not differ appreciably from that of V(ma)$_3$ (Figure 4.15). In fact, there are also many additional similarities between V(ema)$_3$ and VO(ema)$_2$. Both V(ema)$_3$ and VO(ema)$_2$ are neutral ($Z \sim 0$) at physiological $pH$ and display similar hydrolysis behaviours under conditions of acid hydrolysis (Figure 4.15). The degree of hydrolytic stability at $pH$ 7.4 is also comparable for VO(ema)$_2$ and V(ema)$_3$. V(ema)$_3$ is fully formed ($Z \sim 0$) in a somewhat larger $pH$ region ($pH$ 5.5-8) compared to that for VO(ema)$_2$ ($pH$ 5.5-7.5).
In addition, both VO(ema)\textsubscript{2} and V(ema)\textsubscript{3} develop large positive charges, approaching +2 at pH 2.

Key differences exist between V(ema)\textsubscript{3} and VO(ema)\textsubscript{2} when hydrolysis at high pH is examined. VO(ema)\textsubscript{2} is significantly more susceptible to alkaline hydrolysis than is V(ema)\textsubscript{3} which is clear in the Z plots at pH > 7 where VO(ema)\textsubscript{2} develops a significantly higher negative charge than V(ema)\textsubscript{3} (Figure 4.15).

Hydrolytic stability in this pH range (pH > 7) is not necessary for a complex suitable as an insulin-enhancing agent because this pH range is outside of physiological concentrations. Previous kinetic studies have indicated the presence of hydroxo complexes such as [VO(ema)\textsubscript{2}(OH)]\textsuperscript{+} formed from a bound water complex with a pK\textsubscript{a} of 7.6\textsuperscript{26}. Whether complexes such as [VO(ema)\textsubscript{2}(OH)]\textsuperscript{+} are present in high concentration can be determined from inspection of the plot of Z vs. pH. Anionic hydroxo complexes might be expected to alter adversely the bioavailability and retention of the parent complex. From the studies reported here, no mixed stable hydroxo complexes, such as [VO(ema)\textsubscript{2}(OH)]\textsuperscript{+}, form in high concentration for either VO(ema)\textsubscript{2} and V(ema)\textsubscript{3}. The plateaus or sigmoidal curves (as seen for Hema in Figure 4.15) in the plots of Z vs. pH, indicative of ternary hydroxo complexes, are absent in the plots for VO(ema)\textsubscript{2} and V(ema)\textsubscript{3}.

[VOCl(ema)\textsubscript{2}]Cl.H\textsubscript{2}O dissolved in water gives identical titration curves to those for VO(ema)\textsubscript{2} (Figure 4.16), indicating that the chloro ligand is not retained. Additionally potentiometry presents an alternative method to determine the Cl\textsuperscript{-} content of this compound, because one equivalent of H\textsuperscript{+} is bound per Cl\textsuperscript{-}. 

References on page 89
Figure 4.15 Plot of Z vs. pH for 2.6 mM VO(ema)$_2$ (■), 3.3 V(ema)$_3$ (+), and 1.1 mM Hema (O). The Region Between the Two Solid Lines Describes the Region of Predominance (> 95% formation) for the Respective Compounds (0.16 M NaCl, 25 °C).

Figure 4.16 Plot of Z vs. pH for 2.6 mM VO(ema)$_2$ (●) and 1.1 mM [VOCI(Hema)$_2$]Cl·H$_2$O (+) (0.16 M NaCl, 25 °C). The Region Between the Two Solid Lines Describes the Region of Predominance (> 95% Formation) for VO(ema)$_2$.  

References on page 89
The hydrolysis of each of V(koj)₃·H₂O, V(ema)₃, V(ma)₃ and VO(ema)₂ has been addressed and all of these complexes are clearly hydrolytically stable at physiological pH. The determination of the equilibrium constants that can be extracted are presented using the simplest model which adequately describes the experimental observations.

The intermediate to acidic pH range only was used in the determination of equilibrium constants. The region of alkaline hydrolysis was excluded. The non-ideal region below pH 2 was also excluded. In the determination of the stability constants with various models, the hydrolysis of the HL and the free metal ions were not found to contribute significantly to the mass balances and could be excluded below pH 7.

Although stability constants could be calculated with the aid of potentiometric-curve-fitting programs such as BEST²⁷ or SUPERQUAD²⁸ for VO(ema)₂ and the V(III) complexes, these were somewhat unsatisfactory.²⁹ First, it was possible to refine constants for components which were clearly not present in high concentration from the experimental titration curves, such as [VO(ema)₂(OH)]⁻ and [V(ema)₃(OH)]⁻. Second, the values of the basicity-stability constants indicated significant 3-hydroxy-4-pyrone association. For example, values of log $K_{ba1}$ = 4.00(20) for $V^{3+}$ and Hma and log $K_{ba1}$ = 3.71(15) could be refined for $V^{3+}$ and Hkoj. With a minimum value of log $K_{1}^{+}$ ~ 4 for $V^{3+}$ and Hma association, $[V(Hma)]^{2+}$ is expected to be a major contributor to the mass balance. In addition, when the constants determined were reintroduced into a speciation diagram such as Figure 4.1 (p. 60), the observed stability upon dilution was apparently underestimated. These inconsistencies were
considered to be the result of substantial association of the neutral 3-hydroxy-4-pyrone ligands, although whether this is predominantly an outer sphere or an inner sphere interaction is not clear.

The only constants which were extracted from the potentiometric data are conditional acidity constants (Table 4.3) defined in Scheme 4.3 valid for particular metal to ligand ratio, because the complexes behave as simple saturated acids. These acidity constants adequately describe the hydrolysis of the vanadium(III) complexes at a 3:1 ligand to metal ratio and VO(ema)₂ at a 2:1 ligand to metal ratio; ligand to metal ratios greater than these are expected to result in increases in acidity. Only pKₐ's greater than 2.5 could be determined with precision.

**Table 4.3** Conditional Acidity Constants (the Error in Parentheses Refers to 3σ in the Last Digit) (0.16 M NaCl, 25 °C).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ka₃ *</th>
<th>Ka₂ *</th>
<th>Ka₁ *</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO(ema)₂</td>
<td>-</td>
<td>3.9 (1)</td>
<td>~2.4</td>
</tr>
<tr>
<td>V(ma)₃</td>
<td>3.9 (1)</td>
<td>~2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>V(ema)₃</td>
<td>4.0 (1)</td>
<td>~2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>V(koj)₃</td>
<td>4.2 (1)</td>
<td>~2</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>
Scheme 4.3

\[
\begin{align*}
&\text{[V(HL)]}^{3+} + K_{a1}^* \text{H}^+ \rightarrow \text{[V(L)(HL)]}^{2+} + K_{a2}^* \text{H}^+ \rightarrow \text{[V(L)]}^+ + V(L) \quad (4.3)
\end{align*}
\]

HL = Hema, Hma, Hkoj

\[
\begin{align*}
&\text{[VO(Hema)]}^{2+} + K_{a1}^* \text{H}^+ \rightarrow \text{[VO(ema)(Hema)]}^+ + VO(ema)
\end{align*}
\]

The speciations of VO(ema)\(_2\) (Figure 4.17) and V(ma)\(_3\) (Figure 4.18) calculated from the conditional constants (Table 4.3) are independent of complex concentration. The exact nature of species such as [V(ma)\(_2\)(Hma)]\(^+\), beyond stoichiometry, remains unclear. Water, an exceptional ligand for V(III) and VO\(^{2+}\), could be retained for species such as [V(ma)\(_2\)(Hma)]\(^+\); additionally, the very unfavourable hydration entropy associated with both V\(^{3+}\) and VO\(^{2+}\) might be relieved by outer sphere association of Hma. The hydration entropy of VO\(^{2+}\) has been determined to be -133.9 J mol\(^{-1}\) K\(^{-1}\) and the hydration entropy of V\(^{3+}\) has been estimated to be -230 J mol\(^{-1}\) K\(^{-1}\). Inner sphere coordination of ligands such as Hma is also expected to relieve the high hydration entropy.
Figure 4.17 Speciation Diagram for VO(ema)$_2$ (0.16 M NaCl, 25 °C).

Figure 4.18 Speciation Diagram for V(ma)$_3$ (0.16 M NaCl, 25 °C).
Spectrophotometric H$_2$EDTA$^{2-}$ competition. Because the protonation equilibria appeared complex for VO(ema)$_2$, competition with H$_2$EDTA$^{2-}$ at pH 7.4 was used to determine the value of $\beta_{ba2}$ for VO(ema)$_2$. The stability of VO(ema)$_2$ could be determined independently of the variable pH behaviour. Significant changes in the visible electronic absorption spectrum of VO(ema)$_2$ are seen when compared to VO(EDTA)$_2$ with the ligand field transitions of VO(EDTA)$_2$ significantly higher in energy than those of VO(ema)$_2$ (Figure 4.19).

Scheme 4.4

\[
\begin{align*}
\text{VO(ema)}_2 + \text{H}_2\text{EDTA}^{2-} & \rightleftharpoons K [\text{VO(EDTA)}]^2 + 2\text{Hema} \\
\text{VO}^{2+} + \text{H}_2\text{EDTA}^{2-} & \rightleftharpoons K_{ba} [\text{VO(EDTA)}]^2 + 2\text{H}^+ \\
\text{VO}^{2+} + 2\text{Hema} & \rightleftharpoons \text{VO(ema)}_2 + 2\text{H}^+
\end{align*}
\]

\[
K = \frac{K_{ba} (\text{H}_2\text{EDTA}^{2-})}{\beta_{ba2} (\text{Hema})}
\]

\[
[\text{VO(ema)}_2] = A_{445}/\varepsilon_{445}(\text{VO(ema)}_2)
\]

\[
[[\text{VO(EDTA)}]^2] = [\text{VO(ema)}_2]_{\text{initial}} - [\text{VO(ema)}_2]
\]

\[
[\text{H}_2\text{EDTA}^{2-}] = [\text{H}_2\text{EDTA}^{2-}]_{\text{initial}} - [[\text{VO(EDTA)}]^2]
\]

\[
[\text{Hema}] = [\text{Hema}]_{\text{initial}} + 2[[\text{VO(EDTA)}]^2]
\]

Because $K_{ba}(\text{H}_2\text{EDTA}^{2-})^{31}$ is known, the value of $\beta_{ba2}$ can be determined based on the competition shown in Scheme 4.4. Because $[\text{VO(EDTA)}]^2$ does not possess any charge transfer transition in the visible region, the equilibrium constant $K$
(Scheme 4.4) was determined spectrophotometrically based on the loss of the ema⁻ to oxovanadium(IV) charge transfer at 445 nm upon the formation of VO(EDTA)²⁻ (Figure 4.19). In the absence of H₂EDTA²⁻ and oxygen, the charge transfer did not change over time. The equilibrium constant $K$ was determined to be 0.078(2) corresponding to a log $\beta_{ba2}$ of 3.64(2). Notably, the equilibrium favours the ema⁻ complex over the H₂EDTA²⁻ complex indicating that VO(ema)₂ is, in fact, very stable.

**Figure 4.19** Visible Electronic Absorption Spectra of the Competition of VO(ema)₂ with H₂EDTA²⁻ (0.16 M NaCl, 25 °C).

The value of log $\beta_{ba2}$ is larger than expected. A value of log $\beta_{ba2}$ of -0.61 was obtained for the VO(ma)₂ hydrolysis system.\(^{16}\) This discrepancy likely results from undetermined equilibria for the [VO(EDTA)]²⁻ and/or VO(ema)₂ hydrolysis systems including the formation of ternary hydrolysis products such as [VO(EDTA)(OH)]³⁻ or protonated complexes such as [VO(Hema)(ema)]⁺ and [VO(H₂EDTA)].
4.4 Conclusions

In conclusion, VO(ema)₂ is a uniquely stable oxovanadium(IV) complex. VO(ema)₂ is hydrolytically stable, competes effectively with common metal scavengers including H₂EDTA²⁻ and does not hydrolyze simply from dilution. The vanadium(III) complexes V(ma)₃, V(ema)₃ and V(koj)₃ possess stabilities similar to that of VO(ema)₂, being stable to hydrolysis as well as not hydrolyzing simply from dilution.

4.5 References


29) Melchior, M., unpublished results.


Chapter 5
Oxidation Reactions

5.1 Introduction

Three phases are important when addressing the oxidative stability of a potential insulin-enhancing agent: the solid state, the aqueous solution, and the aqueous suspension. A compound suitable as an insulin-enhancing agent would ideally be thermodynamically stable with respect to oxidation in these phases or, at least, would possess substantial robustness or inertness to oxidation. For example, VO(ma)$_2$ possesses favourable oxidative stability in that it is completely stable in the solid state and when it is suspended in water. Although, thermodynamically unstable with respect to oxidation to cis-[VO$_2$(ma)$_2$]$^-$ in aqueous solution at intermediate pH, VO(ma)$_2$ is oxidized slowly.

A series of oxidations of V(ma)$_3$, V(ema)$_3$, V(koj)$_3$•H$_2$O and V(dpp)$_3$•12H$_2$O, as well as VO(ema)$_2$, [VOCl(Hema)$_2$]Cl•H$_2$O and VO(ema)$_2$(Hema)•H$_2$O, was undertaken. First, a key question regarding V(III) complexes as insulin-enhancing agents concerns their stability to oxidation; V(III) complexes are generally perceived not to possess sufficient stability to oxidation to be useful insulin-enhancing agents. Second, oxidations provide a potential synthetic route to novel complexes. Because cis-[VO$_2$(ma)$_2$]$^-$ is both hydrolytically unstable and not insulin-mimetic, specific interest centred on the development of novel complexes of vanadium(V). Unlike neutral oxovanadium(IV) chelate complexes which have been extensively examined as insulin-enhancing agents, neutral oxovanadium(V) chelate complexes have rarely been examined for potential insulin-enhancing ability.
5.2 Experimental

The general experimental method is described in Chapter 2.2. VO(dpp)$_2$ was prepared following literature methods.$^4$ Hdpp was prepared according to literature methods.$^5$-$^7$

Oxidations (and alternative syntheses)

µ-Oxobis[bis(ethylmaltolato)oxovanadium(V)], V$_2$O$_3$(ema)$_4$.

[VO(ema)$_2$(Hema)]$\cdot$H$_2$O (503 mg, 1.21 mmol) was dissolved in 50 mL 1:1 methanol/water. The solution was stirred under ambient air for 1 month resulting in the precipitation of a purple microcrystalline solid that was collected by filtration and air-dried (320 mg, 0.453 mmol, 75% yield based on V). V$_2$O$_3$(ema)$_4$ is air- and moisture-stable and can be suspended in water without decomposition. V$_2$O$_3$(ema)$_4$ is diamagnetic.

Bis(maltolato)oxovanadium(IV), VO(ma)$_2$.

a. Red V(ma)$_3$ (10.0 g, 23.5 mmol) was oxidized by sitting at room temperature under ambient air and moisture for 1 month. The resulting solid was suspended in water (25 mL), and the remaining grey solid was collected by filtration and air-dried (7.07, 22.3 mmol, 95% yield).

b. Red V(ma)$_3$ (0.500 g, 1.17 mmol) was suspended in 10 mL of water and allowed to oxidize under ambient air and moisture for 1 month, with the resulting grey precipitate collected by filtration, and air-dried (0.359 g, 1.13 mmol, 97 % yield). The filtrate was strongly acidic (pH ~1).

Bis(ethylmaltolato)oxovanadium(IV), VO(ema)$_2$.

Intensely red V(ema)$_3$ (1.34 g, 2.86 mmol) was suspended in 10 mL of water and allowed to oxidize under
ambient air and moisture for 1 month, resulting in a mixture of yellow and blue-green solids. Allowing the mixture to sit for a further 3 months gave only a blue-green solid, which was collected by filtration and air-dried (0.920 g, 2.66 mmol, 93 % yield based on V). The filtrate was strongly acidic (pH ~1).

**Chlorobis(ethylmaltol)oxovanadium(IV) chloride monohydrate,**

\[ \text{VOCI(Hema)}_2\text{Cl}\cdot\text{H}_2\text{O} \]. Solid V(ema)$_3$ (1.07 g, 2.28 mmol) was first treated, on a Schlenk filter connected to a water aspirator, with a flow of concentrated HCl (aq) vapor, from a 250 mL reservoir filled with 125 mL concentrated HCl (aq) in the absence of oxygen for 24 hours; a pale green solid resulted (1.69 g). Allowing the pale green solid to oxidize under ambient air and moisture for 1 month resulted in the formation of a dark green solid. Trituration in diethyl ether resulted in a bright green solid that was collected by filtration and dried in vacuo (1.00 g, 2.29 mmol, 100% yield based on vanadium).

**Dioxo(1,2-dimethyl-3-oxy-4-pyridinonato)vanadium(V) (1,2-dimethyl-3-hydroxy-4-pyridinone) monohydrate,** [VO$_2$(dpp)]$\cdot$Hdpp$\cdot$H$_2$O.

a. V$_2$O$_5$ (32.5 mg, 0.179 mmol) was suspended in an aqueous solution of Hdpp (152 mg, 1.09 mmol) in 10 mL water. The suspension was stirred overnight at RT resulting in the formation of a very pale grey precipitate which was collected by filtration and dried in vacuo (125 mg, 0.330 mmol, 92 % yield based on V).

b. V(dpp)$_3$$\cdot$12H$_2$O (66.4 mg, 0.0974 mmol) was oxidized at 4 °C, by exposing it to air through a syringe needle for 2 months yielding, upon washing with water, a pale grey powder which was collected by filtration and dried in vacuo (42.3 mg, 0.0903 mmol, 93 % yield based on vanadium).

94 References on page 106
c. VO(dpp)$_2$ (80.0 mg, 0.233 mmol) was suspended in 2 mL of water at RT for 2 months, under air, resulting in a pale yellow solid which was collected by filtration and dried \textit{in vacuo} (82.5 mg, 0.218 mmol, 94 \% yield based on V).

\textbf{Table 5.1} Elemental Analyses (found [calc.]).

<table>
<thead>
<tr>
<th>Compound</th>
<th>C</th>
<th>H</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>V$_2$O$_3$(ema)$_4$</td>
<td>47.74 [47.61]</td>
<td>4.01 [4.00]</td>
<td>-</td>
</tr>
<tr>
<td>[VO$_2$(dpp)]$\cdot$Hdpp$\cdot$H$_2$O</td>
<td>44.53 [44.46]</td>
<td>4.78 [5.06]</td>
<td>7.24 [7.41]</td>
</tr>
</tbody>
</table>

\textbf{5.3 Results and Discussion}

A series of oxidations of V(ma)$_3$, V(ema)$_3$, V(koj)$_3$$\cdot$H$_2$O and V(dpp)$_3$$\cdot$12H$_2$O, as well as VO(ema)$_2$, [VOCl(Hema)$_2$]Cl$\cdot$H$_2$O and VO(ema)$_2$(Hema)]$\cdot$H$_2$O, in the three phases (solid state, aqueous suspension, and solution) important to insulin-enhancing agents was undertaken. The reactions typically involved prolonged reaction times. Although the autoxidations could be significantly increased (V(ma)$_3$ is oxidized to completion in 24 hr at 150 °C), long reaction times under ambient condition were used to describe the potential "shelf-life" of the vanadium complex. Complete stability to changes in oxidation state of approximately 6 months are desirable for a potential pharmaceutical agents. When the oxidation produced a previously established complex, characterization was by IR spectroscopy. When the oxidation produced a new complex, further characterization by EA and LSIMS of the isolated product was undertaken. Previously unpublished V(V) complexes including
V$_2$O$_3$(ema)$_4$ and [VO$_2$(dpp)]·Hdpp·H$_2$O were accessed from these oxidation reactions.

Isolation of the ethylmaltolate analogs of the maltolates VO(ma)$_2$(OCH$_3$)$_3$, NH$_4$[VO$_2$(ma)$_2$]·H$_2$O and K[VO$_2$(ma)$_2$]·H$_2$O using the literature preparation for these maltolates$^1$ proved unsuccessful. Although these analogs are likely formed, the greatly enhanced solubility of the ethylmaltolate analogs precluded isolation. The synthesis of V$_2$O$_3$(ema)$_4$, a complex analogous to V$_2$O$_3$(ma)$_4$,$^1$ proved successful using an alternate synthetic route. Rather than the hydrolysis of VO(ma)$_2$(OCH$_3$)$_3$ (the method used in the synthesis of V$_2$O$_3$(ma)$_4$),$^1$ V$_2$O$_3$(ema)$_4$ was synthesized in relatively high yield through the oxidation of [VO(ema)$_2$(Hema)]·H$_2$O in aqueous methanol and was characterized by LSIMS, IR and EA. Likely, the oxidation of [VO(ema)$_2$(Hema)]·H$_2$O in methanol/water generates VO(ema)$_2$(OCH$_3$)$_3$ in situ, which then hydrolyzes to V$_2$O$_3$(ema)$_4$ (Scheme 5.1). Although V$_2$O$_3$(ema)$_4$ has been previously synthesized,$^8$ a novel synthetic route is reported here. This method gives a significantly higher yield than the 20-40% yield obtained for V$_2$O$_3$(ma)$_4$ and produces a pure product which gives a good elemental analysis (Table 5.1). As was found for V$_2$O$_3$(ma)$_4$,$^1$ the ease with which V$_2$O$_3$(ema)$_4$ undergoes solvolysis in methanol or chloroform prevented the characterization of this complex in solution; however, V$_2$O$_3$(ema)$_4$ is air- and moisture-stable, and can be suspended in water and dilute acids without decomposition.
Although complexes such as $V_2O_3(ema)_4$ might not be expected to be hydrolytically stable, the reluctance of $V_2O_3(ema)_4$ to solvate and to dissolve leads to at least kinetic stability in water. Hydrolytically inert, although ultimately hydrolytically unstable, complexes can be useful insulin-enhancing complexes, as exemplified by the metformin complex $VO(metf)_2$ an insulin-enhancing complex possessing basic dimethylbiguanidate ligands. The stability of $V_2O_3(ema)_4$ is suitable for biological assays involving the administration of a solid complex or suspension, such as the oral gavage experiment described in Appendix II. A material formulated as $[VO_2(dpp)]^*Hdpp*H_2O$ is obtained in high yield from the oxidation of $V(dpp)_3*12H_2O$ in the solid state (under moist conditions at 4 °C with a restricted flow of oxygen), from the oxidation of $VO(dpp)_2$ suspended in water under air, as well as from the condensation of suspended $V_2O_5$ with excess aqueous Hdpp.
Isolation of V(V) dpp⁻ analogs of the maltolates VO(ma)₂(OCH₃), NH₄[VO₂(ma)₂]·H₂O and K[VO₂(ma)₂]·H₂O using the literature preparation for these maltolates¹ proved unsuccessful.

Scheme 5.2

\[
V_2O_5(s) + 2 \text{Hdpp} \quad \text{(aq)} \rightarrow 2 [VO_2(dpp)]\cdot\text{Hdpp}\cdot\text{H}_2\text{O} \quad \text{(s)}
\]

Consistent with the elemental analysis (Table 5.1), the formula of [VO₂(dpp)]·Hdpp·H₂O is further corroborated by infrared spectroscopy and, to a limited extent, by positive detection mode LSIMS. Vanadium(V) forms a variety of polymeric materials analogous to the metavanadates (Ct[VO₃] where Ct is a monovalent cation) and metavanadate monohydrates (Ct[VO₃]·H₂O) including VO₂(acac), a polymeric material that has been utilized recently in the synthesis of novel V(V) clusters.¹⁰ Like the metavanadates, [VO₂(dpp)]·Hdpp·H₂O is nearly colourless. The variant formed from the oxidation of VO(dpp)₂ in aqueous suspension, although pale yellow, possesses an identical composition (Table 5.1)

References on page 106
**Infrared spectroscopy.** In the infrared spectrum of V$_2$O$_3$(ema)$_4$ (Table 5.2), an oxovanadium V=O stretch is observed at 963 cm$^{-1}$, which is not significantly different from that observed for V$_2$O$_3$(ma)$_4$.\(^1\) Pyrone stretching frequencies, at significantly lower energy than in the free ligand, are observed for V$_2$O$_3$(ema)$_4$ at 1612 cm$^{-1}$ and 1574 cm$^{-1}$. Bridging oxo vibrations at 759 cm$^{-1}$, 737 cm$^{-1}$ and 600 cm$^{-1}$ are also observed for V$_2$O$_3$(ema)$_4$. The IR spectrum of [VO$_2$(dpp)]•Hdpp•H$_2$O (Figure 5.1) clearly shows the presence of an uncomplexed Hdpp molecule. For [VO$_2$(dpp)]•Hdpp•H$_2$O there are two absorptions in the pyridinone region of the IR spectrum, combinations of the carbonyl and ring vibrations, occurring with approximately equal intensity at 1631 cm$^{-1}$ and 1610 cm$^{-1}$ (Figure 5.1, Table 5.2). The first pyridinone vibration at 1631 cm$^{-1}$ is not significantly different from that in uncomplexed Hdpp, and is consistent with the presence of Hdpp not bound to vanadium. Although Hdpp is completely soluble in water, washing of [VO$_2$(dpp)]•Hdpp•H$_2$O with water does not remove the additional Hdpp molecule, indicating that this molecule is reasonably strongly associated with [VO$_2$(dpp)]. The second pyridinone vibration occurs at 1610 cm$^{-1}$ and is consistent with dpp$^-$ coordination, with the reduction of this stretching frequency ~-20 cm$^{-1}$ similar to that observed for VO(dpp)$_2$ and V(dpp)$_3$•12H$_2$O. The oxovanadium V=O stretching frequency observed in the IR spectrum of [VO$_2$(dpp)]•Hdpp•H$_2$O at 935 cm$^{-1}$ is within the range of the metavanadates (~880-940 cm$^{-1}$).\(^1\)
### Table 5.2

Selected Infrared (KBr, ±4 cm\(^{-1}\), Values in Parentheses Describe Changes from the Free Ligand Values, ∆ cm\(^{-1}\)).

<table>
<thead>
<tr>
<th></th>
<th>(V_2O_3(ema)_4)</th>
<th>([VO_2(dpp)]\cdot Hdp\cdot H_2O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\nu_{C=O}\text{ and }\nu_{ring})</td>
<td>1612 (-32)</td>
<td>1631 (0)</td>
</tr>
<tr>
<td></td>
<td>1573 (-40)</td>
<td>1610 (-21)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1563 (-4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1553 (-14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1510 (-3)</td>
</tr>
<tr>
<td>(\nu_{V=O})</td>
<td>963</td>
<td>936</td>
</tr>
<tr>
<td>(\nu_{V-O})</td>
<td>700</td>
<td>721</td>
</tr>
<tr>
<td></td>
<td>519</td>
<td>574</td>
</tr>
<tr>
<td>(\nu_{V-O-V})</td>
<td>759</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>737</td>
<td></td>
</tr>
<tr>
<td></td>
<td>600</td>
<td></td>
</tr>
</tbody>
</table>
Figure 5.1 Infrared Spectrum of [VO₂(dpp)]·Hdpp·H₂O.

Mass spectrometry. The positive ion detection mode LSIMS of V₂O₃(ema)₄ shows no parent ion. Only low intensity [V(ema)₂]⁺ (m/z = 329) and [HVO(ema)]₂⁺ (m/z = 346) ions are observed in the LSIMS of this compound (Table 5.3). A parent peak is observed in the positive detection mode LSIMS of [VO₂(dpp)]·Hdpp·H₂O with the ion [HVO₂(dpp)]⁺ occurring at m/z = 222 (Table 5.3); however, the intensity of this ion is very low. Far more intense ions are observed in the LSIMS (Table 5.3) including those corresponding to [V(dpp)₂]⁺ (m/z = 327, 100% relative intensity, the predominant ion), as well as [HVO(dpp)₂]⁺ (m/z = 344) and [VO(dpp)]⁺ (m/z = 205). Low intensity high molecular weight ions are also observed in the LSIMS including ions corresponding to [V(dpp)₃]⁺ (m/z = 465), [V₂O₂(dpp)₃]⁺ (m/z 548) and [V₂O(dpp)₄]⁺ (m/z = 670).
**Table 5.3** Selected Ions (Positive Ion Detection Mode LSIMS).

<table>
<thead>
<tr>
<th>Ion</th>
<th>m/z</th>
<th>Ion</th>
<th>m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>[V(ema)₂]⁺</td>
<td>329</td>
<td>[VO₂(dpp)]⁺·Hdpp·H₂O</td>
<td>202</td>
</tr>
<tr>
<td>[HVO(ema)₂]⁺</td>
<td>346</td>
<td>[HVO₂(dpp)]⁺</td>
<td>222</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[V(dpp)₂]⁺</td>
<td>327</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[HVO(dpp)₂]⁺</td>
<td>344</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[V(dpp)₃]⁺</td>
<td>465</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[V₂O₂(dpp)₃]⁺</td>
<td>548</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[V₂O(dpp)₄]⁺</td>
<td>670</td>
</tr>
</tbody>
</table>

**Solid state oxidations.** All of V(ma)₃, V(ema)₃, V(koj)₃·H₂O and V(dpp)₃·12H₂O are ultimately oxidized in the solid state (Table 5.4), with the stability (inertness) of the V(III) 3-oxy-4-pyrones complexes to oxidation decreasing with increasing alkyl substitution. [VO(ema)₂(Hema)]⁺·H₂O is prone to trace oxidation in the solid state but is not susceptible to oxidation in aqueous suspension, under ambient conditions. The other vanadyl complexes including VO(ema)₂ and [VOCl(Hema)₂]Cl·H₂O are indefinitely stable with respect to oxidation in the solid state, and aqueous suspension, under ambient conditions. V(ma)₃ is the most stable of the V(III) 3-oxy-4-pyrones described herein and can be left in the open air for several weeks without signs of oxidation (the development of a vanadyl oxo stretching frequency). Once oxidation has initiated for V(ma)₃, however, it is rapid.
V(ema)$_3$, is the least stable of the V(III) 3-oxy-4-pyrone discussed and readily oxidizes upon contact with air.

**Table 5.4** Oxidation Products of the Solid Oxidation of the V(III) Complexes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Phase</th>
<th>Product</th>
<th>$\nu_{v=0}$ (±4 cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V(ma)$_3$</td>
<td>solid</td>
<td>VO(ma)$_2$</td>
<td>993</td>
</tr>
<tr>
<td></td>
<td>suspension (H$_2$O)</td>
<td>VO(ma)$_2$</td>
<td>993</td>
</tr>
<tr>
<td>V(ema)$_3$</td>
<td>solid</td>
<td>Unknown</td>
<td>982</td>
</tr>
<tr>
<td></td>
<td>solid (w/HCl vapours)</td>
<td>[VOCl(Hema)$_2$]Cl•H$_2$O</td>
<td>973</td>
</tr>
<tr>
<td></td>
<td>suspension (H$_2$O)</td>
<td>VO(ema)$_2$</td>
<td>995</td>
</tr>
<tr>
<td>V(koj)$_3$•H$_2$O</td>
<td>solid</td>
<td>Unknown</td>
<td>982</td>
</tr>
<tr>
<td>V(dpp)$_3$•12H$_2$O</td>
<td>solid (RT)</td>
<td>VO(dpp)$_2$</td>
<td>965</td>
</tr>
<tr>
<td></td>
<td>solid (4°C)</td>
<td>[VO$_2$(dpp)]•Hdpp•H$_2$O</td>
<td>936</td>
</tr>
</tbody>
</table>

Whereas the solid state oxidation of V(ma)$_3$ generates a tractable product (VO(ma)$_2$) quantitatively, the solid state oxidation of V(ema)$_3$ does not generate VO(ema)$_2$ but instead generates an ill-defined product. The trace decomposition product of the oxidation of [VO(ema)$_2$(Hema)]•H$_2$O is similar to the solid state oxidation product of V(ema)$_3$. V(koj)$_3$•H$_2$O, intermediate in stability to oxidation between V(ma)$_3$ and V(ema)$_3$, also generates an intractable oxidation product similar to V(ema)$_3$. Based on elemental analyses, both V(ma)$_3$ and V(koj)$_3$•H$_2$O lose significantly more than two equivalents of ligand to oxidation processes.
In the presence of proton sources, V(ema)$_3$ can be oxidized cleanly in the solid state to well-defined products. For example, the solid state oxidation of V(ema)$_3$ hydrolyzed in the solid state with concentrated hydrochloric acid vapours generates [VOCl(Hema)$_2$]Cl•H$_2$O. When suspended in water under air, V(ema)$_3$ and V(ma)$_3$ slowly (~1 month for completion of oxidation) generate the corresponding oxovanadium complexes VO(ema)$_2$ and VO(ma)$_2$. The stability of solid V(ema)$_3$, as well as that of the other vanadium(III) complexes and [VO(ema)$_2$(Hema)]•H$_2$O is enhanced when the complexes are blanketed with water. Because these oxidations display induction periods, the solid state oxidations are likely radical processes. Water likely serves as an inhibitor of the oxidation leading to a chemical dead-end, with termination of the reaction occurring prior to propagation. An excess of water likely provides an alternative proton source (to the ligand) and thus inhibits oxidation.

Of the vanadium(III) complexes, V(dpp)$_3$•12H$_2$O generates the greatest variety of oxidation products, with the nature of the product depending on moisture and oxygen levels. The solid state oxidation of V(dpp)$_3$•12H$_2$O at RT generates VO(dpp)$_2$ rapidly (1-2 days) a complex which has been previously described.$^4$ At low temperature (4 °C), the solid state oxidation of V(dpp)$_3$•12H$_2$O was found to generate [VO$_2$(dpp)]+Hdpp•H$_2$O. Unlike VO(ma)$_2$ and VO(ema)$_2$ which can be suspended indefinitely without oxidation, VO(dpp)$_2$ is also susceptible to oxidation, when suspended in water under air, forming [VO$_2$(dpp)]+Hdpp•H$_2$O. VO(dpp)$_2$ is indefinitely stable in the solid state under ambient conditions.

References on page 106
Solution oxidation of V(III) compounds. In aqueous solution, the oxidation of the vanadium(III) complexes was probed spectrophotometrically and proved to be complicated and non-linear. Although the oxidation could not be modelled using any simple mathematical treatment, the lifetimes of the V(III) complexes could be estimated from an inflection point in the absorbance vs. time graphs, as the oxidations are typically biphasic (Figure 5.2). The first phase corresponds to the oxidation of V(III) to V(IV), with the second phase corresponding to the oxidation of V(IV) to V(V). V(III), a strong reductant, and V(V), a moderate oxidant, react rapidly. It is likely that the major oxidant of V(III) is in fact V(V) and not oxygen, with V(V) as the major oxidant accounting for the non-linear nature of the reaction kinetics.

Figure 5.2 Oxidation of V(ma)$_3$ in the Presence of Excess Hma ([V(ma)$_3$]$_{\text{total}}$ = 1.77 mM, [Hma]$_{\text{total}}$ = 35.5 mM, pO$_2$ = 1 atm, [KCl] = 1 M, [KPTH] = 0.05 M, pH 4.0, 298 K).
Few generalizations beyond the fact that the oxidations are slow can be made; V(ma)$_3$ and V(ema)$_3$ each possess a lifetime of approximately 16 hours at pH 7.4 under ambient pO$_2$. Lifetimes are greatly reduced in organic solvents such as methanol, in which V(ma)$_3$ and V(ema)$_3$ are least stable, possessing lifetimes on the order of only 10s at pO$_2$ = 1 atm. Although the oxidation is slow V(III), is unlikely to persist \textit{in vivo} where oxidants including O$_2$ and Fe(III), which oxidizes V(III) to V(IV), are present.

5.4 Conclusions

Of the vanadium(IV) complexes, only [VO(ema)$_2$(Hema)]$\cdot$H$_2$O displayed signs of trace oxidation in the solid state. The V(III) complexes V(ma)$_3$, V(ema)$_3$, V(koj)$_3$$\cdot$H$_2$O and V(dpp)$_3$$\cdot$12H$_2$O are all ultimately sensitive to oxidation in the solid state. Because the oxidation of V(ma)$_3$, the most oxidatively stable of the vanadium(III) complexes, is slow and is characterized by an induction period in which no appreciable oxidation occurs, it is most suitable for biological testing.

5.4 References


Chapter 6

Oxovanadium(V) and Flavonol

6.1 Introduction

Flavonoids are natural products that serve a variety of functions including plant pigmentation.\(^1\) Recent interest in the chemistry of flavonoids stems from the anti-oxidant\(^2\) and photophysical properties\(^3\) of flavonoids.

Figure 6.1 Flavonol and Naturally Occurring Analogs.

Flavonol (Hfla) is a synthetic flavonoid and a structural archetype for naturally occurring analogues such as quercetin and kaempferol (Figure 6.1). Hfla is very lipophilic and has the potential to form exceptionally lipophilic vanadium complexes. Polyhydroxy derivatives of Hfla including quercetin are water soluble, but are significantly more toxic than Hma. Hfla and derivatives are easily synthesized in the “one-pot” modified Algar-Flynn-Oyamada synthesis (Figure 6.2).\(^4\),\(^5\) The synthesis of Hma and derivatives is far more laborious.\(^6\),\(^7\) Few well-defined flavonato (fla\(^-\)) complexes of transition metals have been described including Cu(fla)(bipy)(ClO\(_4\))\(^8\) Cu(fla)(PPh\(_3\))\(_2\),\(^9\) both of which have been characterized crystallographically.

References on page 113
Complexes of fla⁻ are prone to oxidation, an example being Cu(fla)(PPh₃)₂ which was documented to undergo oxidation at fla⁻.⁹,¹⁰

**Figure 6.2** The Modified Algar-Flynn-Oyamada Synthesis of Flavonol.⁴,⁵

![Chemicalreaction](image)

**6.2 Experimental**

The general experimental method is described in Chapter 2. Flavonol (Aldrich) was used without further purification. VO(ma)₂(OCH₃) was synthesized according to the literature methods.¹¹

**Bis(flavonato)methoxyoxovanadium(V), VO(fla)₂(OCH₃)** To an intensely red solution of VO(ma)₂(OCH₃) (0.243 g, 69.8 mmol) in 25 mL methanol was added flavonol (0.500 g, 210 mmol) in 10 mL methanol at room temperature. A dark precipitate formed which was collected by filtration and dried *in vacuo* (0.359 g, 62.7 mmol, 90 % yield based on V). VO(fla)₂(OCH₃) is indefinitely moisture and air stable. VO(fla)₂(OCH₃) is completely insoluble in water under neutral, acidic or strongly basic conditions (3M KOH). Additionally, VO(fla)₂(OCH₃) is completely insoluble in MeOH and C₆H₆. VO(fla)₂(OCH₃) is diamagnetic. EA (found [calc.]) C 64.68 [65.04], 3.67 [3.70].
6.3 Results and Discussion

The first vanadium complex of fla\(^{-}\), VO(fla)\(_2\)(OCH\(_3\))\(_2\), was isolated and characterized in the solid state by IR, EA and MS. VO(fla)\(_2\)(OCH\(_3\)) was synthesized in high yield utilizing a metathesis reaction involving the maltolato complex VO(ma)\(_2\)(OCH\(_3\)) and excess Hfla (Scheme 6.1). Unlike the maltolate complex VO(ma)\(_2\)(OCH\(_3\)), VO(fla)\(_2\)(OCH\(_3\)) resists dissolution even under harsh aqueous conditions (i.e. refluxing 3M KOH) and is insoluble in common organic solvents including benzene and methanol. VO(fla)\(_2\)(OCH\(_3\)) is not susceptible to contamination by any impurity and gives good elemental analyses. VO(fla)\(_2\)(OCH\(_3\)) was intended as a starting material for the syntheses of a variety of [VO(fla)\(_2\)]\(^{\textsc{i}}\) salts as these salts are potentially lipophilic and water soluble; however the synthesis of [VO(fla)\(_2\)]\(^{\textsc{i}}\) salts from VO(fla)\(_2\)(OCH\(_3\)) was not successful due to lack of solubility of VO(fla)\(_2\)(OCH\(_3\)). Like VO(ma)\(_2\)(OCH\(_3\)), VO(fla)\(_2\)(OCH\(_3\)) undergoes a variety of solvolysis reactions and forms ill-defined intensely green products in the presence of chlorinated solvents (CH\(_2\)Cl\(_2\), CHCl\(_3\)).

Scheme 6.1 Synthesis of VO(fla)\(_2\)(OCH\(_3\))

\[
\begin{align*}
2 \quad \text{Hfla} & \quad + \quad \text{VO(ma)\(_2\)(OCH\(_3\))} & \quad \xrightarrow{\text{MeOH}} \quad \text{VO(fla)\(_2\)(OCH\(_3\))} & \quad (\text{s}) \\
& \quad + \quad 2 \text{Hma}
\end{align*}
\]
**Infrared spectroscopy.** A reduction of approximately 100 cm\(^{-1}\) is observed for the most intense of the flavone vibrations when the IR spectrum of VO(fla\(_2\))(OCH\(_3\)) (1498 cm\(^{-1}\)) is compared to that of Hfla (1607 cm\(^{-1}\)) (Figure 6.3). In addition, the low energy OH vibration of free Hfla at 3162 cm\(^{-1}\) is absent for VO(fla\(_2\))(OCH\(_3\)). The V=O stretching frequency of VO(fla\(_2\))(OCH\(_3\)) at 971 cm\(^{-1}\) is not significantly different from that observed for VO(ma\(_2\))(OCH\(_3\)).\(^{11}\) Additionally, V-O vibrations are observed for VO(fla\(_2\))(OCH\(_3\)) at 756 cm\(^{-1}\) and 521 cm\(^{-1}\).

Figure 6.3 Infrared Spectra of VO(fla\(_2\))(OCH\(_3\)) and Hfla.
**Mass spectrometry.** No parent ion is observed for VO(fla)$_2$(OCH$_3$) in the positive ion detection mode LSI mass spectrum. The predominant ion is [V(fla)$_2$]$^+$ at m/z = 525. Other intense ions include the [VO(fla)]$^+$ ion as well as [V(fla)$_3$]$^+$ and the series of high-molecular weight [V$_2$O$_2$(fla)$_3$]$^+$, [V$_2$O$_3$(fla)$_3$]$^+$ and [V$_2$O$_4$(fla)$_4$]$^+$ ions (Table 6.1). The EI MS of VO(fla)$_2$(OCH$_3$) does exhibit a parent molecular ion [VO(fla)$_2$(OCH$_3$)]$^+$ at m/z = 572 in addition to a more intense [VO(fla)$_2$]$^+$ at m/z = 541.

**Table 6.1** Selected Ions (Positive Detection Mode LSIMS and EIMS) for VO(fla)$_2$(OCH$_3$).

<table>
<thead>
<tr>
<th>LSIMS</th>
<th>EIMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion</td>
<td>m/z</td>
</tr>
<tr>
<td>[VO(fla)]$^+$</td>
<td>304</td>
</tr>
<tr>
<td>[V(fla)$_2$]$^+$</td>
<td>525</td>
</tr>
<tr>
<td>[VO(fla)$_2$]$^+$</td>
<td>541</td>
</tr>
<tr>
<td>[V$_2$O$_2$(fla)$_3$]$^+$</td>
<td>608</td>
</tr>
<tr>
<td>[V$_2$O$_3$(fla)$_3$]$^+$</td>
<td>624</td>
</tr>
<tr>
<td>[V$_2$O$_4$(fla)$_4$]$^+$</td>
<td>640</td>
</tr>
<tr>
<td>[V(fla)$_3$]$^+$</td>
<td>762</td>
</tr>
<tr>
<td>[V$_2$(fla)$_3$O$_2$]$^+$</td>
<td>845</td>
</tr>
</tbody>
</table>

References on page 113
The structure of VO(fla)$_2$(OCH$_3$)$_2$, although not determined, is likely to resemble that of the maltolato analog VO(ma)$_2$(OCH$_3$)$_2$.$^{12}$

### 6.4 Conclusions

In conclusion, a robust oxovanadium(V) complex VO(fla)$_2$(OCH$_3$)$_2$ has been synthesized and characterized in the solid state. Due to limited solubility this complex is not highly suitable for use as an insulin-enhancing agent.

### 6.5 References


References on page 113


Chapter 7

Summary and Suggestions for Future Work

7.1 Summary

Novel hydrolytically stable V(III) complexes suitable for insulin enhancing biological studies were successfully synthesized including V(ma)₃, V(ema)₃, V(koj)₃•H₂O and V(dpp)₃•12H₂O. Of these, V(ma)₃ and V(ema)₃ are highly lipophilic. In addition, V(ma)₃, V(ema)₃, V(koj)₃•H₂O and V(dpp)₃•12H₂O do not hydrolyze simply from dilution; a great advantage for potential insulin-enhancing agents. However, these compounds suffered from the common disadvantage of V(III) complexes, that of air sensitivity in the solid state as well as in solution. [VO(ema)₂(Hema)]•H₂O, a novel derivative of the potent insulin mimic VO(ema)₂, possesses similar solubility and lipophilicity to V(ema)₃ as well as sufficient air stability in the solid state suitable for use as a pharmaceutical agent. [VO(ema)₂(Hema)]•H₂O is hydrolytically unstable, generating VO(ema)₂ in aqueous solution, and serves as a VO(ema)₂ procomplex and a potential prodrug. VO(ema)₂ is hydrolytically stable and, like the vanadium(III) complexes does not hydrolyze simply from dilution. A novel approach to increase the water solubility of VO(ema)₂ was the solid state synthesis of [VOCI(Hema)₂]Cl•H₂O, a highly soluble salt of VO(ema)₂. [VOCI(Hema)₂]Cl•H₂O is hydrolytically unstable and also serves as a VO(ema)₂ procomplex and a potential prodrug. [VOCI(Hema)₂]Cl•H₂O and [VO(ema)₂(Hema)]•H₂O are the first examples of 3-hydroxy-4-pyrone coordination to a transition metal ion. Novel oxovanadium(V) complexes of dpp⁻ and fla⁻ were also

References on page 121
synthesized; however, because of poor solubility, these oxovanadium(V) complexes are likely not suitable as insulin-enhancing agents.

7.2 Suggestions for Further Work

The V(III) complexes synthesized were rather robust to oxidation, although all the V(III) complexes are ultimately air-sensitive in the solid state. V(ma)$_3$, the most robust to solid state oxidation, is the most suitable for further study regarding both the coordination chemistry and biological activity. Because the V(III) complexes tended to increase in air sensitivity with increasing alkyl substitution (i.e. V(ema)$_3$ is much more air sensitive than V(ma)$_3$), a potentially significantly more air stable V(III) compound might be the complex of pyromeconic acid, the simplest 3-hydroxy-4-pyrone ligand (Figure 7.1). Of the synthetic routes available for pyromeconic acid, a convenient preparation involves the adaptation of the one-pot synthesis of 3-hydroxy-4-pyrones$^{1,2}$ with pyromeconic acid synthesized from commercially available furfuryl alcohol.

**Figure 7.1** One-Pot Synthesis of Pyromeconic Acid.

\[
\text{OH} \quad \xrightarrow{[\text{ox}]} \quad \xrightarrow{[\text{H}^+]} \quad \text{OH}
\]

furfuryl alcohol \hspace{1cm} pyromeconic acid

Additional ligands are known to make robust, although not indefinitely air-stable V(III) compounds and may provide alternatives to the V(III) complexes presented in this thesis. Although V(acac)$_3$ possesses satisfactory hydrolytic
stability, subac somewhat air sensitive. Substituted acetylacetonate ligands, especially phenyl derivatives, are known to form significantly more robust V(III) complexes and these might be suitable for examination as insulin-enhancing agents. Tropolones, closely related to 3-hydroxy-4-pyrones both chemically and biologically, are known to form robust tris(tropolonato)vanadium(III) complexes. In addition, tropolone forms stable binary vanadyl complexes as well as an odd complex formulated as VO(trop)Cl•Htrop. An examination of parallels between the tropolone and 3-hydroxy-4-pyrene coordination chemistry of oxovanadium(IV) and vanadium(III) would be interesting especially regarding the synthesis of a tropolone complex analogous to [VOCI(Hema)₂]Cl•H₂O. Tropolones occur in nature (Figure 7.2) as water soluble fungal metabolites.

**Figure 7.2** Tropolone and Naturally Occuring Derivatives.8,9

![Tropolone and Naturally Occuring Derivatives](image)

Tropolone  Stipitatic Acid  Anhydrosepedonin

Ligands with great promise of forming air-stable V(III) insulin enhancing agents include basic imine carboxylates. V(pic)₃•H₂O is a highly air-stable V(III) complex in the solid state; however, it is hydrolytically unstable. The substitution of more basic ligands for pic⁻ including commercially available imidazole-
2-carboxylate may improve on the hydrolytic stability of \( V(\text{pic})_3 \cdot \text{H}_2\text{O} \) while retaining the air stability.

The novel oxovanadium(IV) complexes \([\text{VOCl(Hema)}_2]\text{Cl} \cdot \text{H}_2\text{O}\) and \([\text{VO(ema)}_2(\text{Hema})]\text{H}_2\text{O}\) present viable alternatives to the oxovanadium(IV) complexes commonly used in studies of the insulin-enhancing activity of vanadium. Future glucose normalization studies involving the treatment of STZ-diabetic rats with \([\text{VOCl(Hema)}_2]\text{Cl} \cdot \text{H}_2\text{O}\) or \([\text{VO(ema)}_2(\text{Hema})]\text{H}_2\text{O}\) will likely prove interesting. \([\text{VOCl(Hema)}_2]\text{Cl} \cdot \text{H}_2\text{O}\) and \([\text{VO(ema)}_2(\text{Hema})]\text{H}_2\text{O}\) also provoke interest in neutral 3-hydroxy-4-pyrone binding.

A general method used in the synthesis of the rare examples of neutral Hacac complexes (including the monodentate Hacac complex \( \text{MnBr}_2(\text{Hacac})_2 \)) and the bidentate Hacac complexes \( \text{CrCl}_2(\text{acac})(\text{Hacac}), \text{CoCl}_2(\text{Hacac}), \text{ZnCl}_2(\text{Hacac}) \) and \( \text{NiBr}_2(\text{Hacac})_2 \) is the use of neat Hacac (I) as the solvent and either inert metal ions including \( \text{Cr(III)} \) or relatively weak Lewis acids including \( \text{MnBr}_2 \) and \( \text{ZnCl}_2 \). Similar methods to those used in the synthesis of Hacac complexes can be applied to the synthesis of novel complexes containing neutral 3-hydroxy-4-pyrone ligands in the future. The use of Hema (I) as a solvent for these studies is attractive due to the relatively low melting point of Hema.

Oxovanadium(V) complexes of fla do not show great promise as orally active insulin-enhancing agents; however, the synthetic methodology developed in Chapter 6 involving the flavonol metathesis of a maltolato complex provides a simple synthetic route to other binary fla complexes.

References on page 121
The only other vanadium oxidation state that has not been examined for activity is V(II). V(II) complexes of maltol and related 3-hydroxy-4-pyrones are unknown and were not considered to be suitable as insulin-enhancing agents, due to the expected high reactivity of these complexes; however, relatively stable complexes of V(II) include $\text{Vl}_2^{15}$ and $[\text{V(pic)}_3]^-.^{16}$ There may be applications, although limited, of relatively non-toxic strong reducing agents including V(III) and V(II) complexes in medicinal inorganic chemistry. Such applications include use as reductants in the \textit{in situ} reduction of $^{99m}\text{TcO}_4^-$ in the preparation of nuclear imaging agents in which toxic (compared to vanadium complexes) reducing agents including SnCl$_2$ and dithionite are currently employed.

A possible extension of the coordination chemistry of insulin-enhancing vanadium complexes would be the inclusion of vanadium’s neighbours in the periodic table, Cr and Ti. The coordination chemistry of Ti(IV) and V(IV) is very similar. The biodistribution processes of Ti(IV) and V(IV) are also very similar with transferrin binding strongly to both Ti(IV)$^{17,18}$ and V(IV)$^{19}$ Enzyme inhibition by titanyl sulphate has been suggested to originate from 5-coordinate Ti(IV)$^{20}$ similar to the transition state model of phosphatase inhibition by vanadate. Titanium(IV) might be expected to form neutral diamagnetic TiO(ma)$_2$ and TiO(ema)$_2$ complexes which possess structures similar to that of [TiO(acac)$_2$]$^2$.\textsuperscript{21} TiO(ma)$_2$ and TiO(ema)$_2$ would likely be air stable in aqueous solution, as the oxidation chemistry of V(IV) is not accessible for Ti(IV). Ti(IV) is also unlikely to change oxidation state \textit{in vivo}. Ti(IV) complexes would be expected to possess some insulin-enhancing ability unless redox processes are, in fact, essential for activity.

References on page 121
One of the few parallel examinations of the activity of Ti(IV) and V(IV) complexes involves the metallocene dichlorides CpMCI\textsubscript{2} of Ti(IV) or V(IV), which were both found to possess carcinostatic activity\textsuperscript{22,23}. Because metallic Ti is well-tolerated physiologically and Ti(IV) complexes including salicylates, oxides, peroxides and tannates are exceptionally non-toxic, uses in modern medicine include the metal in surgical implants and Ti(IV) complexes as topical agents in the treatment of skin disorders\textsuperscript{24–28}. Additionally, TiO\textsubscript{2} is currently approved as a pharmaceutically acceptable whitening agent as well as being used as a food additive in the production of confections, dairy products and bread flours. Because low levels of Ti are released from surgical implants, coordination chemistry modelling the \textit{in vivo} behaviour becomes highly relevant. Peroxotitanates(IV), including K\textsubscript{2}TiO\textsubscript{5}(dipicolinate)•5H\textsubscript{2}O\textsuperscript{29} are stable and may possess the high activity of the peroxovanadium(V) complexes. Ti(IV) complexes may also be used in the study of the vanadium complexes, as TiO(ma)\textsubscript{2} and TiO(ema)\textsubscript{2} may also provide suitable diamagnetic matrices for obtaining well resolved powder EPR spectra of magnetically dilute VO(ma)\textsubscript{2} or VO(ema)\textsubscript{2}. Ti(III) complexes, like V(III) complexes, are easily accessed by dithionite reduction in water\textsuperscript{5}. Although likely to be air-sensitive, Ti(ma)\textsubscript{3} and Ti(dpp)\textsubscript{3}•12H\textsubscript{2}O should be stable and easily synthesized, and would most likely have properties similar to those of the V(III) system. The aqueous chemistry of Ti(III) is rarely studied. Ti(dpp)\textsubscript{3}•12H\textsubscript{2}O would likely prove an isostructural addition to the series of known M(dpp)\textsubscript{3}•12H\textsubscript{2}O complexes. Compared to V(III), additional spectroscopic methods are available for the study of Ti(III) (d\textsuperscript{1}) including EPR. Additionally, the solution oxidation of Ti(III) complexes would likely
be more simple than that of V(III), because autocatalytic oxidations are unlikely due to the lack of oxidation states greater than IV for Ti. The study of the oxidation processes of Ti(III) complexes may clarify the oxidation chemistry of the analogous V(III) compounds. A variety of diamagnetic matrices suitable for solid state EPR of Ti(III) complexes are available including the analogous Ga(III) and Al(III) complexes. Ti(III) complexes, like V(III) complexes, may also possess biological activity.

Although the biological activity of Cr(ma)$_3$ has been documented, the inertness of Cr(III) may be of great use in probing the hydrolysis of trivalent metal maltolates, especially regarding Hma coordination. Although Cr(III) forms more stable complexes with nitrogen donors than do V(III) and Ti(III), complexes formed with maltol and related ligands are likely to be similar. Cr(dpp)$_3$•12H$_2$O might prove to be another isostructural addition to the series of known exoclathrate M(dpp)$_3$•12H$_2$O complexes.

7.3 References


Appendix I

Stability Constants and the Nature of the Reference and Standard States

There are two major categories of stability constants, absolute and stoichiometric determined in aqueous solution; they differ in the definition of the reference and standard states (Table I.i).

Table I.i Reference and Standard States for Stoichiometric and Absolute Equilibrium Constants.

<table>
<thead>
<tr>
<th>Constant</th>
<th>Reference State</th>
<th>Activity Coefficients</th>
<th>Standard States</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute (activity</td>
<td>Infinite dilution</td>
<td>Unity</td>
<td>Hypothetical solutions of unit activity and the properties of the reference state</td>
</tr>
<tr>
<td>products)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stoichiometric</td>
<td>Ionic medium</td>
<td>Constant</td>
<td>Hypothetical solutions of unit concentration* and the properties of the reference</td>
</tr>
<tr>
<td>(concentration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>products)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For metal ions of high charge, such as $V^{3+}$, infinitely dilute solutions are not experimentally convenient, and stoichiometric constants are almost exclusively employed. Ionic strengths are typically chosen to be directly applicable to an area of interest without correction. Common ionic strengths used include 0.16 M, modelling physiological saline, and 0.60 M, modelling sea-water.

* The choices of concentration scales include molarities, molalities and solute mole fractions.
Conditional or relative stability constants are apparent constants, restricted to specific conditions, often implemented for complex multicomponent systems. Restricting conditions include pH or the concentration of a specific interfering compound (often the “inert” electrolyte). Complicated or indeterminable thermodynamic behaviour can be conveniently absorbed into a conditional constant.

Conventional approximations are made when dealing with aqueous solutions and are built into the definition of the standard states, which are hypothetical and somewhat arbitrary. Water activity is 1 unless depleted by high solute concentration; water concentration is constant unless depleted by high solute concentration and is absorbed into the stability constants. Hydration energies are also separately absorbed into the stability constants. The hydration energy of the proton is arbitrarily set at 0 by convention. The hydration energy for all ions and compounds are also absorbed into the aqueous stability constants by setting the hydration energy equal for all ions and compounds equal to that of the proton.

\[ X + n \text{H}_2\text{O} = X(\text{H}_2\text{O})_n \quad K \]  

In equation l.i, K is set at 1 by convention for $H^+$ (aq). $H^+$ (aq) consists of $X = H^+, H(\text{H}_2\text{O})^+, H(\text{H}_2\text{O})_2^+...H(\text{H}_2\text{O})_n^+$. The pKₐ of $H^+$ (aq) has been set at 0. For a metal ion $M^{z+}, K$ is also set at 1 and $M^{n+}$ (aq) involves $X = \text{naked } M^{z+}, M(\text{H}_2\text{O})^{z+}, M(\text{H}_2\text{O})_2^{z+}...M(\text{H}_2\text{O})_n^{z+}$. Any remaining compound $X$, a complex for example, is dealt with similarly and termed $X$ (aq). Since the hydration energies are already contained in the aqueous stability constants, direct comparison of constants in a cation-cation
competition is valid (i.e. $V^{3+}(aq)$ and $H^+(aq)$). Aqueous stability constants are composites of hydration and ligation interactions. Trends in aqueous stability constants are also a composite of hydration and ligation trends.
Appendix II

Glucose Normalization by $V(\text{ma})_3$

II.i Introduction

The ability of $V(\text{ma})_3$, administered either by intraperitoneal injection or by oral gavage, to normalize blood glucose in STZ-diabetic male Wistar rats was examined. The antibiotic streptozotocin (STZ) is a $\beta$-cell-specific cytotoxin that reduces the ability to secrete insulin and leads to elevated glucose levels in blood and urine.\(^1\) Intraperitoneal injection (i. p.) involves injection of a solution directly into the intraperitoneal cavity. Intraperitoneal injection is generally used as an initial assay, because complexes are typically more active when administered this way. A compound active by i. p. injection is then examined for glucose normalization by oral gavage. Oral gavage involves oral administration of the complex as an aqueous suspension or solution. The initial probing doses for each method were the ED$_{50}$ doses for VO(\text{ma})$_2$.\(^{\dagger}\)

Two aspects of the ability of a complex to normalize blood glucose are the frequency of response (activity) and the sustainability of the response. The insulin-enhancing activity is measured as the frequency of response or the percentage of animals achieving a state of euglycemia upon treatment with the complex. The sustainability of response describes the quality of the response. A sustained response, in which the euglycemic state is maintained over a period of 48-72 hours

\(^{\dagger}\) The ED$_{50}$ dose represents the dose at which 50% of the animals tested respond.
is considered superior to one involving only a brief glucose-lowering to a euglycemic state.

II.ii Experimental

Animal studies was carried out by Violet Yuen in the laboratory of Prof. J. H. McNeill of the Faculty of Pharmaceutical Sciences, U. B. C. Samples of V(ma)₃ were prepared as described in Chapter 3 of this thesis and were formulated in our laboratory. The control complex, VO(ma)₂, was prepared according to literature methods² and was formulated in the Faculty of Pharmaceutical Sciences. Streptozotocin (Sigma), halothane (Wyeth-Ayerst), and gum arabic (Sigma) were used without further purification.

Thirty male Wistar rats weighing 190-220 g were obtained from the Animal Care Unit of U. B. C. and were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care. Animals were acclimatized for 7-14 days. A single tail-vein injection of STZ (60 mg mL⁻¹ kg⁻¹) under light halothane anaesthesia was then used to induce a diabetes-like state. The induced diabetic state was confirmed 3 days following administration of STZ by a blood glucometer test (Ames Glucometer, Miles Inc. and Glucostix, Miles Canada Inc.). Animals with blood glucose levels ≥ 13 mM were designated diabetic. Animals were not fasted prior to glucose administration. Animals were housed, two animals per cage, on a 12h light: 12h dark schedule and given food (Purina Lab Chow) and fluid ad libitum.

Animals were divided into diabetic (D) and treated (DT) groups. For the i. p. administration experiments, the control diabetic (D) group (n = 5) was treated only with saline; treated (DT) animals were given a single i. p. dose of 0.1 mmol kg⁻¹ in
saline (0.9 % w/w NaCl) of VO(ma)2 (n = 5) or V(ma)3 (n = 5) under Ar. For the oral gavage experiment, the control diabetic (D) group (n = 5) was given 3% w/w gum arabic: diabetic treated (DT) animals were given a single dose of 0.6 mmol kg\(^{-1}\) VO(ma)2 (n = 5) or V(ma)3 (n = 5) suspended in 3% w/w gum arabic by oral gavage.

A volume of 50 \(\mu\)L of blood was collected immediately prior to administration of the compound and at 2, 4, 6, 8, 12, 16, 20, 24, 48 and 72 hours following compound administration. Blood was collected for plasma analysis by nicking the tip of the tail and expressing the blood into heparinized capillary tubes. The blood was centrifuged at 10 000 \(g\) for 15 min, and blood glucose levels were determined immediately (Boehringer Mannheim kits, glucose oxidase method).

The response in the biological assay was defined either as a return from an initial hyperglycemic state ([blood glucose] \(\geq\) 13 mM) to a euglycemic state ([blood glucose] < 9 mM), or as a reduction of the blood glucose concentration to 50% of the initial value.

II.iii Results and Discussion

Due to the dichotomy of the response and the low number or rats (n = 5 in each experiment) employed in the glucose lowering experiments, the experimental data, without averaging, is presented as suggested by Thompson et al.\(^{3}\) The plasma glucose time course data for individual animals treated by i. p. injection with V(ma)3 or VO(ma)2 at equivalent vanadium doses (0.1 mmol kg\(^{-1}\)) is shown in Figure II.i. For i. p. treatment, the control STZ-diabetic group (n = 5), treated with saline, remained hyperglycemic throughout the time course of the experiment.
Figure II.i Glucose Normalization of STZ-diabetic Wistar Rats by V(ma)$_3$ (n = 5) or VO(ma)$_2$ (n = 5) Administered by i. p. Injection (Symbols Represent Individual Animals).
Initially, both the $\text{V(ma)}_3$ treated animals ($n = 5$) and the $\text{VO(ma)}_2$ treated animals ($n = 5$) were clearly hyperglycemic (Figure II.i). Of the animals treated with $\text{V(ma)}_3$ by i. p. injection ($n=5$), 60% of the initially hyperglycemic animals achieved a sustained state of euglycemia (Figure II.i). Of the animals treated with $\text{VO(ma)}_2$ by i. p. injection ($n=5$), 80% of the initially hyperglycemic animals achieved a sustained state of euglycemia (Figure II.i).

The plasma glucose time course data for individual animals treated by oral gavage with $\text{V(ma)}_3$ or $\text{VO(ma)}_2$ at equivalent vanadium doses (0.6 mmol kg$^{-1}$) is shown in Figure II.ii. The control diabetic animals, given only 3% w/w gum arabic, remained hyperglycemic throughout the time course of the experiment. Initially, both the $\text{V(ma)}_3$ treated animals and $\text{VO(ma)}_2$ treated animals were clearly hyperglycemic (Figure II.ii). Of the animals treated with $\text{V(ma)}_3$ by oral gavage ($n=5$), 40% of the initially hyperglycemic animals achieved a state of euglycemia (Figure II.ii). For the animals which responded to treatment with $\text{V(ma)}_3$ by oral gavage, euglycemia was achieved only briefly, and was followed by a rapid return to hyperglycemia. Of the animals treated with $\text{VO(ma)}_2$ by oral gavage ($n=5$), 60% of the initially hyperglycemic animals achieved a state of euglycemia (Figure II.ii). For the animals which responded to treatment with $\text{VO(ma)}_2$ by oral gavage, euglycemia was sustained for only one rat (33% of the responders) with the other responses followed by a rapid return to hyperglycemia.

Neither $\text{VO(ma)}_2$ nor $\text{V(ma)}_3$ proved lethal at the doses used in these experiments. Hypoglycemia ($\text{[blood glucose]} \leq 5 \text{ mM}$) was not observed in any of
the animals. The only overt sign of acute toxicity was mild gastrointestinal distress with both VO(ma)$_2$ and V(ma)$_3$.

**Figure II.ii** Glucose Normalization of STZ-diabetic Wistar Rats by V(ma)$_3$ (n = 5) or VO(ma)$_2$ (n = 5) Administered by Oral Gavage (Symbols Represent Individual Animals).
II.iv Conclusions

In conclusion, V(ma)$_3$ is an orally active insulin-enhancing agent. Both $V(ma)_3$ and VO(ma)$_2$ resulted in normalization of blood glucose levels at ED$_{50}$ doses of VO(ma)$_2$ when administered as a single dose either by oral gavage or i. p. injection. VO(ma)$_2$ proved superior both in the frequency of response (activity) and in the sustainability of response.

II.v References


Appendix III

X-ray Crystallographic Data for V(dpp)$_3$•12H$_2$O

Table III.i Selected Crystallographic Data for V(dpp)$_3$•12H$_2$O.

<table>
<thead>
<tr>
<th>Formula</th>
<th>C$<em>{21}$H$</em>{48}$N$<em>3$O$</em>{18}$V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fw</td>
<td>681.56</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Trigonal</td>
</tr>
<tr>
<td>Space group</td>
<td>P3 (#147)</td>
</tr>
<tr>
<td>a, Å</td>
<td>16.6167(9)</td>
</tr>
<tr>
<td>c, Å</td>
<td>6.8101(2)</td>
</tr>
<tr>
<td>V, Å$^3$</td>
<td>1628.45(11)</td>
</tr>
<tr>
<td>Z</td>
<td>2</td>
</tr>
<tr>
<td>$\rho$ calc, g/cm$^3$</td>
<td>1.390</td>
</tr>
<tr>
<td>$\mu$(MoK$\alpha$)</td>
<td>3.82 cm$^{-1}$</td>
</tr>
<tr>
<td>Total reflections</td>
<td>15 267</td>
</tr>
<tr>
<td>Unique reflections</td>
<td>3 038</td>
</tr>
<tr>
<td>$R$</td>
<td>0.058</td>
</tr>
<tr>
<td>$R_W$</td>
<td>0.064</td>
</tr>
</tbody>
</table>

Table III.ii Bond Lengths (Å).

<table>
<thead>
<tr>
<th>Bond</th>
<th>Length (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V(1)-O(1)</td>
<td>2.0067(14)</td>
</tr>
<tr>
<td>O(1)-C(2)</td>
<td>1.355(2)</td>
</tr>
<tr>
<td>N(1)-C(1)</td>
<td>1.363(2)</td>
</tr>
<tr>
<td>N(1)-C(6)</td>
<td>1.470(3)</td>
</tr>
<tr>
<td>C(1)-C(7)</td>
<td>1.482(3)</td>
</tr>
<tr>
<td>V(1)-O(2)</td>
<td>2.0354(14)</td>
</tr>
<tr>
<td>O(2)-C(3)</td>
<td>1.294(2)</td>
</tr>
<tr>
<td>N(1)-C(5)</td>
<td>1.370(3)</td>
</tr>
<tr>
<td>C(1)-C(2)</td>
<td>1.390(3)</td>
</tr>
<tr>
<td>C(2)-C(3)</td>
<td>1.405(3)</td>
</tr>
<tr>
<td></td>
<td>C(3)-C(4)</td>
</tr>
<tr>
<td>----------------</td>
<td>------------</td>
</tr>
<tr>
<td><strong>Table III.i</strong></td>
<td><strong>Bond Angles (deg).</strong></td>
</tr>
<tr>
<td>O(1)-V(1)-O(1)'</td>
<td>91.30(6)</td>
</tr>
<tr>
<td>O(1)-V(1)-O(2)'</td>
<td>167.94(6)</td>
</tr>
<tr>
<td>O(2)-V(1)-O(2)'</td>
<td>91.64(6)</td>
</tr>
<tr>
<td>V(1)-O(2)-C(3)</td>
<td>113.00(12)</td>
</tr>
<tr>
<td>C(1)-N(1)-C(6)</td>
<td>121.8(9)</td>
</tr>
<tr>
<td>N(1)-C(1)-C(2)</td>
<td>118.8(2)</td>
</tr>
<tr>
<td>C(2)-C(1)-C(7)</td>
<td>122.5(2)</td>
</tr>
<tr>
<td>O(1)-C(2)-C(3)</td>
<td>116.9(2)</td>
</tr>
<tr>
<td>O(2)-C(3)-C(2)</td>
<td>117.5(2)</td>
</tr>
<tr>
<td>O(5)-V(1)-O(6)</td>
<td>104.4(1)</td>
</tr>
<tr>
<td>N(1)-C(5)-C(4)</td>
<td>121.8(2)</td>
</tr>
<tr>
<td><strong>Table III.iv</strong></td>
<td><strong>Hydrogen bonds and C-H...O Interactions.</strong></td>
</tr>
<tr>
<td>-----</td>
<td>------</td>
</tr>
<tr>
<td>C(7)</td>
<td>H(6)</td>
</tr>
<tr>
<td>O(3)</td>
<td>H(9)</td>
</tr>
<tr>
<td>O(3)</td>
<td>H(10)</td>
</tr>
<tr>
<td>O(4)</td>
<td>H(11)</td>
</tr>
<tr>
<td>O(4)</td>
<td>H(12)</td>
</tr>
<tr>
<td>O(5)</td>
<td>H(13)</td>
</tr>
<tr>
<td>O(6)</td>
<td>H(15)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>O(6)</td>
<td>H(15)</td>
</tr>
<tr>
<td>O(6)</td>
<td>H(16)</td>
</tr>
</tbody>
</table>