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VANADIS´CHARMS: FROM THE MITHOLOGY TO THE BIOINORGANIC CHEMISTRY

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Review Article

Abstract

Vanadium is a transition element that presents a rich and varied chemical behaviour. Beyond that, vanadium compounds also show interesting biological effects both *in vivo* and *in vitro* systems. Vanadium promotes glucose transport and metabolism, lipid, DNA and protein synthesis and has also mitogenic effects in different cell types. Moreover, several vanadium compounds show potential pharmacological activity mainly as insulin mimics, antitumoral and osteogenic agents. Nevertheless, in large quantities it can be toxic to humans and other animals. Finally, another interesting line of research on vanadium bioactivity is the understanding of vanadium mechanisms of action. This subject is strongly related with the activation of several intracellular signalling pathways, the generation of free radicals, oxidative stress, and the disruption of cytoskeleton proteins, among others. Even though a great effort has been made by scientists in this area, a lot of work remains to be done in the future to completely elucidate the vanadium mechanisms of action.

In this review, following a brief introduction about the discovery of the element and the general roles of vanadium in biological systems, we place the focus on the interactions of vanadium species with different biological and pharmacological interesting ligands as well as the effects of some vanadium compounds on cells in culture.

Keywords: vanadium, organic ligands, pharmacological applications, osteoblast cultures.

Resumen

El vanadio es un elemento de transición con ricas y variadas propiedades químicas. Además, muchos compuestos de vanadio presentan interesantes efectos biológicos tanto en sistemas *in vivo* como *in vitro*. El vanadio promueve el transporte y el metabolismo de la glucosa, la síntesis de lípidos, DNA y proteínas y presenta efectos mitogénicos en diversos tipos de células. Asimismo, numerosos compuestos de vanadio muestran potencial actividad farmacológica, en especial como agentes insulino-miméticos, antitumorales y osteogénicos. Sin embargo, en cantidades apreciables puede llegar a ser tóxico para el ser humano y otros animales. Finalmente, otra línea de investigación interesante sobre la bioactividad del vanadio es la comprensión de sus mecanismos de acción. Este tema está estrechamente relacionado con la activación de diversas señales intracelulares, la generación de radicales libres, el estrés oxidativo y la alteración de las proteínas del citoesqueleto, entre otros procesos. Aún cuando los científicos han realizado un gran esfuerzo en ese sentido, resta llevar a cabo una ardua tarea en el futuro para elucidar por completo los mecanismos de acción del vanadio. En este trabajo de revisión, a continuación de una breve introducción sobre el descubrimiento de este elemento y sus roles generales en los sistemas biológicos, se centra la atención en las interacciones del vanadio con diferentes ligandos de interés biológico o farmacológico, así como en el efecto de algunos compuestos de vanadio sobre células en cultivo.

Palabras clave: vanadio, ligandos orgánicos, aplicaciones farmacológicas, cultivos de osteoblastos.

Introduction

In the Mithology of North Europe, the Scandinavian goddess Freya was the Vanadis, the ruling goddess of the Vanir or elder gods, who ruled before the arrival of Odin and the Aesir from the east. Her twin brother is the sun god Freir and her parents are Njord and the giantess Skadi.

Vanadis (Figure 1) is contradictory in her essence. In fact, she is known as the goddess of fertility, beauty and love, she is the moon, the seas and the earth, but on the other hand, Freya also represents the goddess of battle, death and underworld. She is virgin and mother. Freya is the leader of Valkyries, the assistants of Odin, and she has inspired all sacred poetry as the "weeping goddess, shedding tears of gold". As the Mistress of Cats, she is always depicted in a chariot drawn by cats and this fact is also under discussion. In fact, most scholars assume that the "cats" are the domestic kind, but some others claim the "cats" could be any small furry animal or even a larger animal like a tiger.[1,2]

Finally, it has been mentioned that Friday, the fifth day of the week, was named after her. But not only Friday derived from Freya. As Vanadis, the chemical element Vanadium was named in this way in honour of Vanadis.

The extraordinary complexity of Freya´s mythology is, in some ways, reflected in the great variability and broad field of research that vanadium Chemistry and Biochemistry offer to the scientists.

Figure 1. "Freja (Vanadis)" by N.Rehder, April 2004, with permission.

The discovery of vanadium happened "twice". It was claimed first by Andrés Manuel del Río (a Spanish mineralogist) at Mexico City in 1801. He prepared a number of salts from a material contained in a "brown lead" (now called vanadite). In this lead from Zimapán (Mexico) he discovered a new metal similar to Chromium and Uranium. A.M. del Río found the colors of the compounds of the new element reminiscent of those displayed by chromium, so he called the element panchromium (meaning "all colors"). Later, he renamed the element erythronium ("red") after noting that most of these salts turned red upon heating. Then, del Río sent the mineral he was studying to colleagues in Europe for confirmation of his discovery. Unfortunately, they concluded that del Río's "new element" was chromium. This news discouraged del Río and he gave up his claim to the new element. It was only 30 years later that it was shown that del Río's work was correct. In fact, in 1831, Nils Gabriel Sefström, a chemist and mineralogist, described a new mineral found in an iron ore from the Taberg mine (Sweden). Sefström and Berzelius named the element Vanadium, in honour of Vanadis because of its beautiful multicoloured compounds. In the same year, Friedrich Wöhler came in to possession of del Río's "brown lead" and confirmed del Río's discovery of vanadium. Both, del Río and Sefström saw vanadium only in the form of vanadium pentoxide (V_2O_5). It was not until 1887 that pure vanadium metal was isolated by the English chemist Sir Henry Enfield Roscoe. He reduced vanadium chloride (VCl_3) with hydrogen gas to give vanadium metal and HCl. Vanadium has the aspect of a white brittle solid that yields rainbow hued compounds. Powdered Vanadium thrown into flame, burns with very brilliant scintillations, which seem to resemble Vanadis glory.

Moreover its splendid and varied chemical behaviour, vanadium compounds also display interesting biological effects both in vivo and in vitro systems. In particular, Vanadium shows insulin-mimetic activity, promotes glucose transport and metabolism, lipid, DNA and protein synthesis and has also mitogenic effects in different cell types. [3-6] Besides, several vanadium compounds also show potential pharmacological activity mainly as insulin mimics, antitumoral and osteogenic agents.[7-13] On the other hand, vanadium in large quantities can be toxic to humans and other animals. However, its effects are not very seRíous.[14, 15]

Finally, another interesting current line of research on vanadium biological and pharmacological actions conveys to the understanding of vanadium mechanisms of action. This subject is strongly related with the activation of several intracellular signalling pathways, the generation of free radicals, the disruption of cytoskeleton proteins, among others.[16-18] Even though a great effort has been made by scientist in this area, a lot of work remains to be done in the future to completely elucidate the vanadium mechanism of action.

The aims of this review article are to summarize the research done by the Bioinorganic group of the Exact Sciences Faculty at the National University of La Plata during more than thirty years. Professor Dr. Enrique J. Baran has been the founder of this research area at the UNLP and many articles, reviews, chapters, congress communications, courses, etc. have been done by him or his group of collaborators to which we have the fortune to belong.

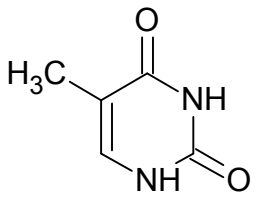
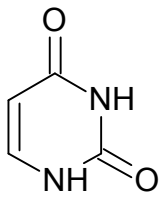
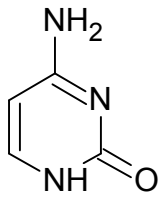
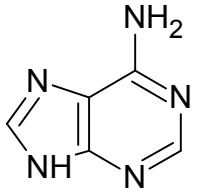
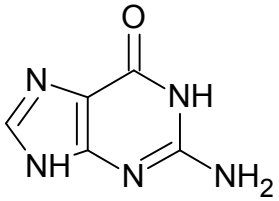
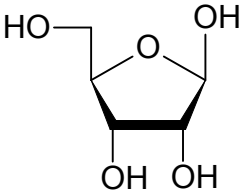
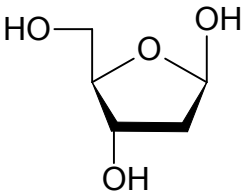
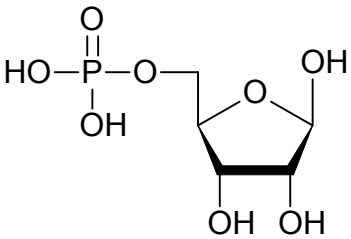
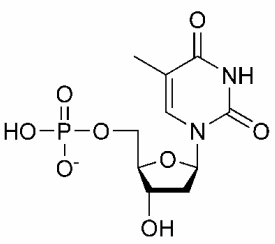
I. Vanadium compounds with biologically interesting ligands

i. Interaction of Vanadium (IV) with Nucleic Acid Components

Nucleic bases, nucleotides y nucleosides. Solution studies.

The nucleic acid, deoxyribonucleic acid (DNA), has the role of storage of information and contains the genetic instructions in order to construct other cell components. It consists of two polymers of simple units, nucleotides (bases linked to a sugar and one or more phosphate groups, see Table 1) in which sugars and phosphate groups are joined by ester bonds. The sugar in DNA is 2-deoxyribose. Attached to each sugars are the bases, and its sequence encodes information. These bases interact by pairs through hydrogen bonds between the different strands of the DNA helix (base pairs). The four bases found in DNA are adenine (A), cytosine (C), guanine (G) and thymine (T). Adenine forms a base pair with thymine via two hydrogen bonds and guanine with cytosine form three hydrogen bonds. Another biological important base, uracil (U) occurs in RNA (ribonucleic acid). In order to obtain a wider insight on the interaction of VO^{2+} cation with nucleobases, nucleosides, nucleotides, ribose and ribose phosphate, electronic absorption spectrophotometric studies were performed.

Table 1. Nucleic bases derived from pyrimidine and purine, ribose, ribose-5-phosphate and the deoxyribonucleotide thymidine monophosphate.

Pyrimidines		
 **Thymine**	 **Uracil**	 **Cytosine**
Purines		
 **Adenine**	 **Guanine**	 **Ribose**
 **Deoxyribose**	 **Ribose-5-phosphate**	 **Thymidine monophosphate**

Nucleic bases

The nucleic bases adenine, guanine, cytosine, thymine and uracil interact with vanadyl(IV) in different ways, at different metal-to-base ratios (from 1:1 to 1:10) and a wide range of pH values. Adenine, guanine and uracil react with the cation at pH > 5. The three bases coordinate in different modes. The interactions occur through the nitrogen atoms of adenine and the oxygen atoms of guanine and uracil.[19]

Nucleosides

The interaction of vanadyl(IV) cation with the nucleosides (units formed by nucleic bases and sugars) was determined at different pH values and metal-to-ligand ratios. The selected nucleosides were: adenosine, guanosine, inosine, cytidine, thymidine and uridine. All of them contain the sugar ribose except thymidine that includes 2'-deoxyribose. Interactions start at pH values > 9 and take place with the two adjacent deprotonated hydroxyl groups of the ribose moiety. Obviously, the metal did not interact with thymidine, due to the absence of pair of OH groups in cis- position in the 2'-deoxyribose.[20]

Nucleotides

Adenine nucleotides (formed by adenosine and phosphate), adenosine-triphosphate (ATP), and -diphosphate (ADP) interact with the metal ion at pH > 3 through the adenine ring. At pH ≤ 3 the metal-to-ligand interaction occurs only with the phosphate moieties of the nucleotides and adenosine-monophosphate (AMP) behaves in the same manner even at higher pH values.[21] In the case of cytidine-monophosphate, -diphosphate and -triphosphate, the interactions occur through the cytosine ring at pH between 3 and 5. The interaction with the phosphate oxygen at neutral pH values was also demonstrated by comparison with the data obtained using the pyrophosphate anion. The behavior is different from ATP and ADP (interaction at pH values between 4.9 and 6.5) and no interaction with the base in AMP could be determined.[22]

Monophosphate nucleotides as well as D-ribose and D-ribose-5-phosphate showed different coordination spheres around vanadyl(IV) with raising pH. At pH = 11.0 the interaction with the ribose moieties of the nucleotides takes place through the two cis- OH-groups. The obtained compounds displayed a stoichiometry characterized by a 2:1 ligand-to-metal ratio. Thymidine monophosphate, the only investigated deoxyribonucleotide, shows a different behavior, in comparison to the other systems.[23] In another series of experiments the interaction between oxovanadium(IV) cation with the nucleotides (ATP, ADP, AMP, GMP) was investigated by means of ^{31}P-NMR and FTIR spectroscopies in solutions.[24] The ^{31}P-NMR results obtained at pH=4.5 showed that in all cases the phosphate groups are affected by the interaction with the VO^{2+} cation. These observations were in agreement with the solution FTIR results.

ii. Interaction of Vanadium (IV) with Carbohydrates

Carbohydrates or saccharides are the most abundant of the four major classes of biomolecules, which also include proteins, lipids and nucleic acids. They fill numerous roles in living organisms, such as the storage and transport of energy and structural components in plants and animals. Additionally, carbohydrates and their derivatives play relevant roles in the working process of the immune system, fertilization, pathogenesis, blood clotting, and development.[25]

Different solid complexes of oxovanadium(IV) cation were then obtained with different saccharides. Elemental and thermal analysis, diffuse reflectance and infrared spectra allow their characterization. A unique compound can be obtained with ribose and VO^{2+} at pH 12 while with D-ribose-5-phosphate, three different complexes were synthesized at different pH values. At pH 12, both sugars interact through the two pairs of adjacent OH groups and at pH 3 and 6, the interaction

occurs through the phosphate moiety of the latter compound.[26] Besides, three new vanadyl(IV) compounds were obtained with glucose and glucose-1-phosphate. At pH 12 the typical interaction through cis- OH groups was detected for glucose. The same interaction turned hindered in glucose-1-phosphate because of the lack of a pair of hydroxyl groups in cis position. For this ligand, solid complexes were obtained at pH 4 and 6 and the bond of the vanadium center with the phosphate groups was established.[27]

After those investigations, studies of similar kind were undertaken with other carbohydrates in order to obtain a better insight in the biological interaction of vanadyl(IV) cation with sugars. This aim was accomplished by the synthesis of the solid sodium salts at high pH values, in order to reach the deprotonation of *cis* OH groups in the saccharide. This event is the key to obtain complex formation. The characterization of the compounds was done by elemental analysis, ultraviolet-visible, diffuse reflectance and infrared spectroscopies, magnetic susceptibilities and thermal analysis Oxovanadium(IV) complexes of turanose, sucrose and their constituents, D-glucose and D-fructose were obtained as sodium salts at pH 12. All of them were mononuclear compounds except D-fructose that generated a dinuclear species bridged by a sugar molecule. The values found for the characteristic stretching V=O vibrations (ca. 930 cm^{-1}) suggest that the coordination sphere of the vanadyl cation consists of four deprotonated sugar oxygen atoms with the possible presence of another OH group in the sixth coordination position. Moreover, the conformation of the coordinated sugars was determined by infrared spectroscopy.[28] Subsequently, a new series of saccharides was selected to perform further research studies. New sodium salts of vanadyl(IV) complexes with D- and L-arabinose, D-galactose, D-mannose, D-lyxose, D-xylose and the disaccharides maltose [29] and lactose [30] were obtained in aqueous solutions at pH 13.[31] All the complexes were found to be mononuclear, possessing the VO^{2+} moiety. For most of these complexes the identity of the OH groups that bind the metal center, together with the conformation of each saccharide moiety and the type of the glycosidic linkage, could be determined by infrared spectroscopy. In all cases the V=O stretching frequency remained at ca. 930 cm^{-1} indicating the participation of deprotonated cis-OH groups in the chelation and coordination. The biological effect of the disaccharides lactose, maltose and sucrose, the monosaccharides glucose, fructose and galactose, the metal cation and their complexes were then tested measuring their inhibitory effect on alkaline phosphatase (ALP) activity. The enzyme catalyzes phosphoryl groups transference and the inhibitory effect found for vanayl(IV) cation can be attributed to the formation of trigonal bipyramidal transition state analogs, exhibiting a similar structure than the phosphate group. It has been previously shown that carbohydrates can interact with the enzyme through electrostatic binding. In this study it was demonstrated that the sugars can inhibit ALP activity. The effect of the vanadyl(IV) complexes was stronger than the sugars and the metal independently, being the complexes with monosaccharides the most potent inhibitory agents, and among the disaccharides the sucrose-VO complex showed the weakest inhibitory effect. These phosphorous-mimicking vanadium compounds likely fit more tightly in the active site of the enzyme, causing inhibition of the enzymatic reaction. The greater inhibitory effect caused by monsaccharide-VO in comparison with disaccharide-VO complexes may be related to steric factors.

In relation to the studies of the interaction of vanadyl(IV) cation with carbohydrates and their biological behavior, linear and cyclic polyalcohols were also investigated.[32,33] The selected linear polyalcohols were sorbitol, galactitol and mannitol On the other hand, conduritol C and myo-inositol were chosen as cyclic polyalcohols. Sodium salts of the vanadyl(IV) complexes with ligand-to-metal ratios 2:1 were obtained and characterized. Afterwards, their biological properties were investigated (see below).

iii. Interactions of Vanadium (V) and (IV) with Bovine Serum Albumin

Serum albumin is the most abundant protein in the serum of higher animals and it functions as a transporter of different substances. The study of bovine serum albumin (BSA) interactions with vanadium might provide significant insights into structural features, critical to biological functions. Most studies of metals binding to proteins focus on the metal coordination and not metal-induced perturbations of the protein structure. In a recent investigation, in order to understand the complex metal-protein relationship, complementary data were obtained with the systems vanadate and vanadyl ions-BSA. The ability of vanadate and vanadyl ions to produce conformational changes in native BSA, in buffer solutions at physiological pH was investigated.[34] For this purpose, FT-IR/ATR, FT-Raman and UV-vis spectroscopic techniques were used. A quantitative analysis was provided for each conformational component such as α-helix, β-sheet, turns, and random coil structures of BSA, and the corresponding vanadium compounds. The native conformation of BSA was affected when it was incubated with both VO^{2+} and VO_3^- species. Regarding BSA/VO^{2+} complexes, a substantial increase in random coil conformation, a higher exposure of tyrosine groups, a significant disruption of salt bridging sites and conformational variations of disulfide bonds were the main structural changes observed in comparison with BSA structure. In contrast, BSA/VO_3^- complexes showed changes pointing to a more ordered-secondary structure conformation with a substantial increase of α-helix structure. Additionally, though in a minor proportion than oxovanadium(IV) complexes, salt bridging sites were disrupted with a higher exposition of tyrosine residues. Finally, conformational variations of disulfide bonds were detected for BSA-VO_3^- complexes in comparison with BSA structure. The relevance of the role of the vanadate anion could be also related to its ability to bind BSA protein, which is mainly controlled by its size. The results obtained for vanadyl were consistent with those for the interaction of positive ions, though oxovanadium species seemed to cause stronger perturbation on BSA than the observed for human serum albumin (HSA).

II. Model systems for vanadium haloperoxidases

Haloperoxidases are enzymes that catalyze two-electron oxidation of a halide (X^- Br^-, I^-, and Cl^-) with hydrogen peroxide, leading to the formation of halogenated organic substrates. The hypohalous acid formed reacts with a broad range of nucleophilic acceptors to perform the peroxidative synthesis of carbon-halogen bond.

Three classes of haloperoxidases (containing heme, non-heme or vanadium prosthetic group) have been identified. Vanadium peroxidases were discovered in seaweed, fungi and lichen.[35-37] The central vanadium(V) ion is coordinated with a histidine (peptidyl ligand), three nonprotein oxygens and an apical oxygen atom belonging to hydroxyl ligand, conforming a trigonal bipyramidal structure.

Bromoperoxidases

Vanadium peroxocompounds were tested as functional models of these enzymes. The biomimetic activity has been related to the simple structure of the active vanadium site in V-bromoperoxidase (V-BrPO). The studies of phenol red (HPhR) bromination to yield bromophenol blue have been performed using species arising from monomeric and dimeric oxodiperoxovanadium(V) complexes, $[VO(O_2)_2(NH_3)]^-$ and $[O\{VO(O_2)_2\}_2]^{4-}$, at pH 6.5.[38] Kinetic measurements were performed by the stopped-flow technique. The rate law is $R= k[V]_T[Br^-][HPhR]$ and this mechanism suggests that phenol red bromination is mediated by halide coordination, forming $[VO(O_2)Br]$ ($k= 2.49x10^5$ dm^6 mol^{-2} s^{-1}). This intermediate has also been postulated by other authors who suggest that bromination is mediated by a previous internal reorganization, leading to the formation of a coordinated hypobromite.[39] In our studies, previous generation of

$[VO(O_2)]^+$ in strongly acidic media is required in order to make the complexes active, so the initial site of attack of bromide is supposed to be the vanadium atom. The difference with native enzymes is that vanadium bromoperoxidases do not require any previous transformation to develop catalytic activity under physiological conditions.

Simple Inorganic Model Complexes with Organic Ligands Containing N,O Donor Atoms

In the study of inorganic complexes of interest for a better understanding of structural properties of vanadium in biological systems, ligands with N,O donor atoms had special relevance to "model" the environment of the metal in the active center. Among other biological systems, this is the case of vanadium-dependent haloperoxidases. Available information about these systems suggests a characteristic feature for the active site, containing simultaneously N and O donor atoms and involving V=O and V-OH groups.[40,41]

a) 8-hydroxiquinoline and related ligands

8-hydroxyquinoline, quinolin-8-ol or "oxine" (Figure 2, QH from now on) has the particular behavior of forming stable complexes with many metal cations that has given rise to broad applications in analytical chemistry.

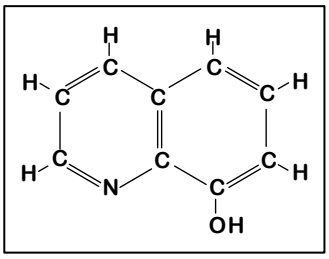


Figure 2. 8-hydroxyquinoline (QH)

In particular, this ligand is capable to bind vanadium in various (III, IV and V) oxidation states, acting as a bidentate chelating agent. It coordinates the metal center through the N atom of the heterocyclic ring and the O atom of the deprotonated phenolic group.

Complexes of QH with vanadium had been prepared since middle of last century, but their characterization was incomplete and the information was somehow controversial. The contradictory results mainly arose from the uncontrolled oxidation of vanadium and hydrolysis reactions due to the exposure to air. With the aim of completing the characterization and extending the study to vanadium compounds containing different derivatives of QH, a series of complexes were synthesized and their detailed characterization was performed.

Initially, the known compounds with non substituted QH were prepared and studied as the "parent" members of the series. The VQ_3 complex, containing V(III), was prepared and a complete spectroscopic study, including IR, Raman and pre-resonance Raman spectra of the solid compound and electronic absorption spectra of methanolic solution were complemented with semi-empirical MO calculations to help in the interpretation of the spectroscopic behavior. The metal center resulted in a strongly distorted octahedral N_3O_3 environment [42], depicted in Figure 3.

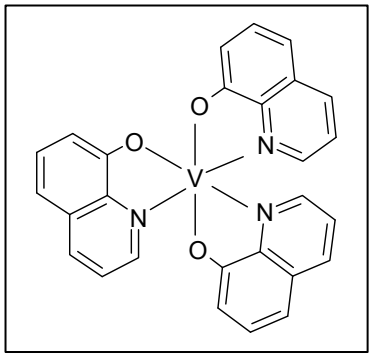


Figure 3. Tris(8-hydroxiquinolinate)vanadium(III)

The vanadium(IV) VOQ_2 complex was found to be stable only under strict absence of air, otherwise the greenish solid oxidized immediately to the black vanadium(V) compound. As in all vanadium(IV) complexes of the series, the "vanadyl" VO^{2+} moiety is present and, in the present case, the square based pyramidal coordination of the metal was completed with two ligands in trans position.[43]

The interaction of QH with vanadium(V) gave rise to the VOQ_2OH complex, that is considered as an inorganic analog of a carboxylic acid. In consequence, it is possible to obtain the respective salts, esters and anhydride (Figure 4).

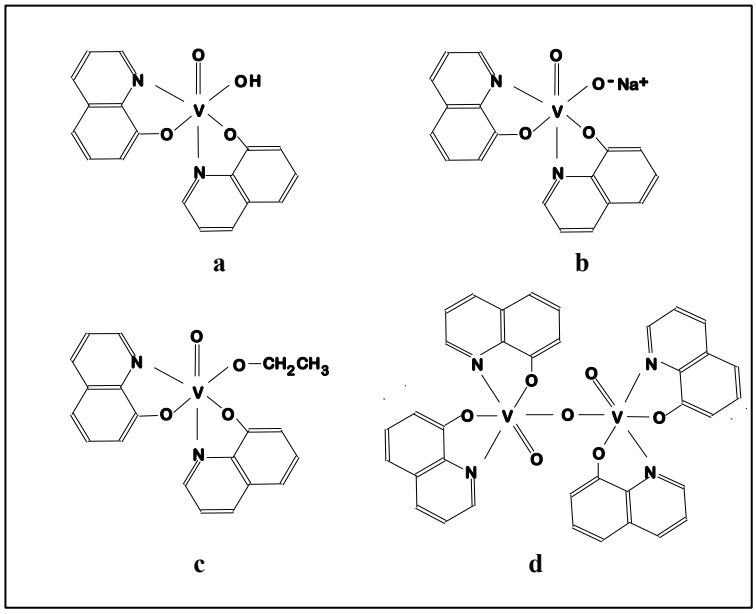


Figure 4. Vanadium(V) complexes with QH. a) acid; b) salt; c) ester; d) anhydride.

This behavior was, in part, responsible of old controversial results, because the authors did not distinguish properly between the monomeric "acid" and the dimeric "anhydride", and the spectroscopic methods did not allow an unambiguous discrimination. This dilemma was finally elucidated based on differences in the voltamperometric response of both complexes.[44] The sodium salt $NaVO_2Q_2$ was obtained and characterized. The Raman, pre-resonance Raman and electronic absorption spectroscopic study of the $[VO_2Q_2]^-$ anion was performed, and the experimental results were complemented with MO calculations in order to assign the

transitions.[45] The vanadium atom is hexa-coordinated by two oxygen atoms, in the characteristic VO_2^+ moiety and by two ligands in *cis* posititon.

Concerning to the substituted quinoline ligands, an important group consisting in some mono and dihalogenated derivatives with the general formula depicted in Figure 5 (5-chloro, 5,7-dichloro, 5,7-dibromo, 5,7-diiodo and 5-chloro, 7-iodo-8-hydroxiquinoline) were used to obtained oxovanadium (IV) complexes.[43]

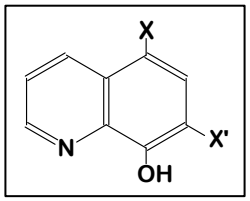


Figure 5. General scheme for halogenated oxines (QCl: X = Cl and X´= H, QCl_2: X = X´= Cl; QBr_2: X = X´= Cl; QI_2: X = X´= I; QICl: X = Cl and X´= I).

The vanadyl complexes appeared as polymeric species, interacting through V=O···V=O linkages. The magnetic moments at room temperature indicated completely quenched orbital contribution. The analysis of the electronic spectra in chloroform revealed a complex behavior, including oxidation of vanadium, ligand loss and solvent interaction. The complex with the 5-chloro substituted quinoline $VO(QCl)_2$ seemed to be the more sensitive to oxidation. It had to be prepared in absence of air, otherwise the V(V) complex $VO(QCl)_2OH$ was obtained. This was also the final species after heating the vanadyl (IV) complex in air or after reaction with traces of alcohol present in chloroform, with formation of the respective ester $VO(QCl)_2OR$ in solution.

The investigation of the magnetic behavior of the complex containing 5,7-dichloroquinoline, in a wide temperature range, clearly supports the existence of ferromagnetic interactions, below 40K. The results constitute the first direct evidence of the formation of ferromagnetic chains along the V=O bonds in some oxovanadium(IV) compounds.[46]

On the other hand, TG and DTA measurements of this compound showed that the presence of the halogen atoms in the ligand does not significantly affect the thermal stability of the complexes.[47]

The complex of vanadium(V) with this ligand $VO(QCl_2)_2OH$ was also prepared, and from its reaction with ethanol, a new example of ester-like inorganic complex $VO(QCl_2)_2OCH_2CH_3$ was obtained. A complete spectroscopic analysis was also performed and the crystal structure of the last complex was determined, supporting the proposed structures for “acids” and “esters” of oxovanadium(V) with quinoline and its derivatives.[48]

The electrochemical behavior of a series of esters with general formula $VO(L)_2OCH_2CH_3$, being L= Q, QCl, QCl_2 and Quin (2-methyl,8-hydroxiquinoline or quinaldine) was studied in organic solvents by means of cyclic voltammetry.[49]

The results, complemented with the electronic spectra of the solutions, denoted the presence of different species in solution and a dependence of the behavior with the presence of water, as a consequence of the formation of a mix-valence dimer complex. The quinaldine complex differs in its behavior from the other members of the series since it acts as a monodentate ligand, coordinating the metal only through its oxygen atom. This characteristic is in agreement with the well-known poor tendency of the quinaldine complexes to form dimer species, due to the steric hindrance imposed by the methyl group. This fact also explains the differences in the geometry of the

complexes of this ligand with vanadium(IV) and (V) in comparison with characteristic environment of the metal when is coordinated by other Q-type ligands.

Other related complexes investigated in detail were those containing 7-iodo-8-hydroyquinoline-sulfonic acid, the well-known analytical reagent "ferron". A complete vibrational and electronic spectroscopic study of the vanadium(V) VOL_2OH "acid", VOL_2OCH_3 "ester", and the $K[VOL_2O]$ "salt", together with the oxovanadium(IV) complex, was performed including pre-resonance Raman measurements for the last two compounds.[50]

Complexes with 8-Hydroxyquinoline-N-oxide (QNO) were included in this group even when the ligand is attached to the metal through two oxygen atoms. Even tough, the proposed structures for the vanadium(IV) and (V) complexes, shown in Figure 6, are analogue to the other members of the series.[51]

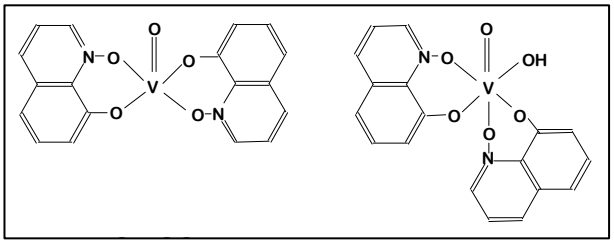


Figure 6. Complexes of vanadium(IV) and vanadium(V) with QNO

b) Dipicolinic acid (pyridine-2,6-dicarboxylic acid)

Dipicolinic acid (H_2dipic - Figure 7) is a versatile N-O chelating agent that can act as bidentate, tridentate, meridian or bridging ligand with different metal ions as well as with oxometal cations. It is present in many natural compounds as an oxidative degradation product of vitamins, coenzymes and alkaloids and is also a component of fulvic acids. It shows vaRíous biological functions including activation-inactivation of some metalloenzymes and inhibition of electron transport and of LDL oxidation.[52] It is a suitable ligand for modeling potential pharmacological active compounds because of its low toxicity and amphophilic nature. It was determined that vanadium complexes of this bio-ligand are more effective than the ligand alone in the treatment of diabetes.[53,54]

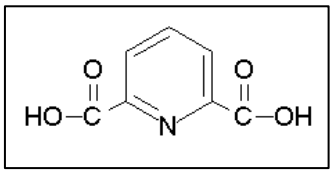


Figure 7. Dipicolinic acid (H_2-dipic)

New vanadium(V) complexes, containing this ligand were synthesized.[55,56] The physicochemical characterization of the compounds were performed by chemical analysis, infrared, Raman and electronic spectroscopy. Their crystal structures were determined by X-ray diffraction methods.

The binuclear complex $[CH_3NHC(NH_2)_2]_2[V_2O_4(dipic)_2]$ is one of the few examples of bis-oxo-bridged compounds reported in the literature.[55] The dimeric anion sits at a crystallographic inversion center with the pair of V(V) atoms in an edge-sharing distorted octahedral environment

with the dipicolinate group acting as a tridentate planar ligand. The two methyl guanidinium counterions stabilized the crystal structure by an extended H-bonds network. It was found that the H-bond pattern gives rise to an eight-member ring, with irregular hexagonal geometry, similar to that described for the interaction between the guanidyl moiety of arginine and different oxoanion groups.[57, 58] Specifically, the double hydrogen bond with the carboxylate moiety of aspartic acid is a determinant step in the interaction between the HIV virus and the cell primary receptor.[59] Methylguanidine is a metabolic product of creatinine oxidation in living organisms. However, it is uncertain which enzymes are involved in its biosynthesis.[60]

Additionally, the novel mononuclear complex, $C(NH_2)_3[VO_2(dipic)].2H_2O$, and its analogous, $NH_4[VO_2(dipic)]$, were also synthesized.[56] The guanidinium and ammonium salts of the $[VO_2(dipic)]^-$ anion crystallize in different space groups. Both salts show a similar penta-coordinated conformation with the planar $dipic^{2-}$ ligand coordinating the VO_2^+ moiety in an analogous manner than in the dimeric complex and the crystal structures are also stabilized by hydrogen bridging interactions. The optimized geometry of the $[VO_2(dipic)]^-$ anion and its corresponding harmonic vibrational frequencies were calculated using methods of the density functional theory.

III. Vanadium detoxification

Vanadium can be an environmental pollutant in industrial and busy urban areas so further elucidation of the detoxification mechanisms of this metal ion is required.[61,62] In coal and petroleum deposits vanadium appears mainly in the form of vanadyl(IV) porphyrinic compounds.[63] The metal is generally liberated as V_2O_5 by combustion of fossil fuels. Many of the toxic effects of vanadate have been attributed to a mechanism of "ionic mimicry". In fact, as it was already mentioned, vanadate has structural and metabolic similarities with phosphate and, by competing with endogenous phosphate, it affects enzymatic and receptor-mediated processes.[64-66] *In vitro* studies have shown an inhibitory effect of vanadate on Na^+/K^+ and P-type ATPases.[67-70]

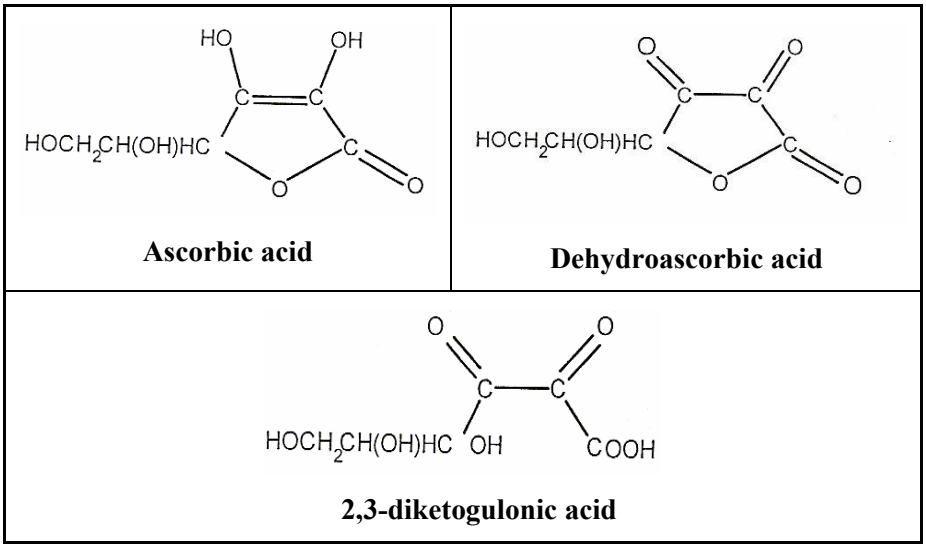
Vanadyl(IV) *meso*-tetraphenylporphyrin (VOTPP) has been prepared and characterized. Electron and photoelectron studies were performed.[71] The electron absorption spectrum of VOTPP could be assigned on the basis of the four-orbital model. The effect of different solvents on the electronic transitions was investigated. Apparently the axial binding and also electrostatic interactions (mainly hydrogen bonding) of the solvent plays an important role. The most important change in the Soret band was observed for DMSO, pyridine and DMF giving a splitting of this band for the last solvent. From the photoelectron spectrum of VOTPP it was established that Vp3/2 and O1s values were the higher energy values in accordance with the metal coordination sphere dominated by N atoms. According to Infrared and Raman spectra [72] the V-N modes were assigned (IR band, 454 cm^{-1} and Raman lines, 400 and 286 cm^{-1}, respectively). Besides, by pre-resonance Raman measurements it was determined that the band at 400 nm exhibited the most important enhancement effects in both the γ and the $(\alpha+\beta)$ electronic transitions.

Vitamin C (Ascorbic Acid), a water-soluble vitamin, is a potent antioxidant and studies suggest that this nutrient may prevent premature death from heart disease and cancer. Vitamin C was originally revealed for its anti-scurvy properties but the focus nowadays is more on its potential cell protective properties. An interesting fact is that most animals synthesize their own vitamin C from glucose, but the human beings depend on dietary sources. From the chemical point of view, the oxidation properties have been the principal objective of many investigations specially because it is one of the possible natural reducing agents of vanadate(V) to oxovanadium(IV) in biological systems.[73]

The reductor effect of ascorbic acid on vanadium(V) and the chelate formation between the reaction products makes it a promising candidate for biological detoxification processes. Among the

different detoxification ligands which presented good interactions with vanadium species, ascorbic, dehydroascorbic and 2,3-diketogulonic acids (see Table 2) were investigated at different ligand-to-metal ratios and pH values both in solution and in the solid state.

Table 2: Detoxyfication ligands.



Ascorbic acid

Dehydroascorbic acid

2,3-diketogulonic acid

The interaction with vanadyl (IV) cation and ascorbic acid took place at pH 12 and at different molar ratios. The electronic UV-vis spectra showed the typical three band pattern similar to those observed for the interaction with carbohydrates (see above). The interaction with dehydroascorbic acid occurred at pH between 4 and 6 via the oxygen moieties and in the range of pH 6 and 10 through the deprotonated 2,3-diketogulonic acid which is a product of the oxidation of dehydroascorbic acid. Two new solid complexes were prepared and characterized for VO(IV) and L-Ascorbic acid. For the dehydroascorbic acid it was not possible to isolate solid complexes but for its derivative, the 2,3-diketogluconic acid, a solid vanadyl (IV) complex was obtained.[74]

Glutathione (GSH, γ-L-glutamyl-L-cysteinil-L-glycine) is regarded as the most abundant low-molecular weight thiol in a variety of cell types. By EPR studies it was demonstrated that when vanadate(V) ions enter into rat adipocytes GSH is involved in their intracellular reduction including the binding to the generated vanadyl(IV) ion.[75,76]

The oxidation product of GSH was the disulfide GSSG (oxidized glutathione). This detoxification mechanism was also studied in aqueous solution evidencing the formation of VO^{2+}/GSH and VO^{2+}/GSSG complexes. Besides, a solid complex with oxidized glutathione could be isolated and characterized. At physiological pH values a blue 1:2 VO^{2+}:GSH complex was formed with coordination through the two carboxylate groups of the ligand.[77] Higher GSH concentrations produced a violet complex, which is the most stable specie of this system. The same specie was obtained in the interaction of VO_3^- with GSH. The coordination sphere of the violet complex involves the S and N atoms of the ligand.

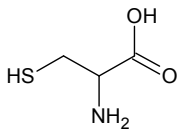
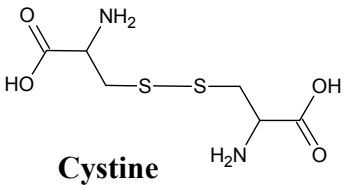
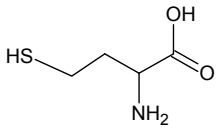
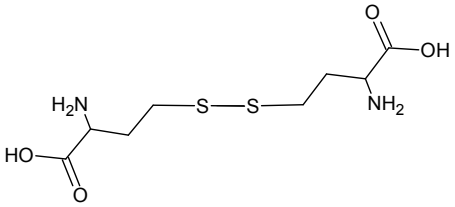
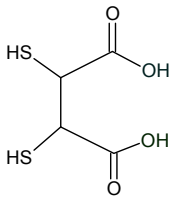
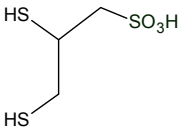
In VO^{2+}/GSSG system two different compounds were formed at ligand-to-metal molar ratios 10:1 and 100:1. By the UV-visible spectral changes in the first case carboxylate group coordination can be established while at higher GSSG concentrations the participation of N donors was clearly defined.[78] The complexes can be transformed into each other by simply changing the metal-to-ligand ratio. These results suggest that GSSG might participate in the stabilization and transport of the vanadium(IV) generated after the reduction of vanadium(V) by GSH inside the cells. A solid

GSSG/VO^{2+} 2:1 complex was obtained and characterized by elemental analysis, magnetic measurements, diffuse reflectance and FTIR spectroscopies. The obtained results suggested the presence of oxygen ligands in the coordination sphere of the vanadyl cation.

In addition, glycine, glutamic acid and cysteine (the three components of GSH) were also studied by electronic absorption spectroscopy to complete the characterization of the models.[79]

The interaction of the VO^{2+} cation with cystine, homocysteine, *meso*-2,3-dimercapto succinic acid (DMSA) and the sodium salt of 2,3-dimercapto-1-propanesulfonic acid (DMPS) (Table 3) was also investigated by the same techniques in aqueous solution at different metal-to-ligand ratios and/or pH values.[80-83]

Table 3. Cysteine, cystine, homocysteine, homocystine, *meso*-2,3-dimercapto succinic acid (DMSA) and the sodium salt of 2,3-dimercapto-1-propanesulfonic acid (DMPS).

 **Cysteine**	 **Cystine**
 **Homocysteine**	 **Homocystine**
 **DMSA**	 **DMPS**

It was shown that the interaction with homocysteine is different from that found in the case of cysteine (other detoxifying agent)[84] and occurs through pairs of amino and carboxylate groups of the amino acid. The same coordination is assumed for the interaction with cystine. VO^{2+} is also competent to interact with homocystine, the oxidation product of homocysteine.

On the other hand, with DMSA, a complex formulated as $[VO(DMSA)_2]^{2-}$ was generated and it was shown that the oxocation interacts with two pairs of deprotonated –SH groups of the acid. With DMPS, the spectral behavior pointed to the generation of a $[VO(DMPS)_2]^{4-}$ complex in which the vanadyl(IV) also interacts with two pairs of deprotonated –SH groups of the ligand. The direct reduction of vanadate(V) (VO_3^-) to vanadyl(IV) (VO^{2+}) solutions by cysteine, homocysteine, DMSA and DMPS was also investigated. It was found the rapid reduction of the metal with a

simultaneous chelation by an excess of the ligands. In the case of cysteine and homocysteine, the vanadyl(IV) cation generated by reduction of vanadate(V) can be complexed by an excess of the reducing agent and is also able to interact with their oxidized forms. The capacity to produce the partial reduction of a V_2O_5 suspension by DMSA (pH = 5.2) and DMPS (pH 7.1) make them possible candidates as potentially useful detoxifying agents for vanadium.

The results with homocysteine are of interest in relation to the metabolism and detoxification of vanadium as they showed that also this compound can eventually act as a natural reducing agent of vanadium(V), and that the VO^{2+} cation generated as the reduction product may be complexed by both, homocysteine and its oxidation product, homocystine. This means that the homocysteine/homocystine system behaves in a similar manner as cysteine/cystine and the previously investigated pairs related to reduced and oxidized glutathione and ascorbic acid. According to our results DMSA and DMPS would be considered new very promising and interesting detoxification agents for vanadium(V), including V_2O_5.

IV. VANADIUM EFFECTS ON BONE

i. Interaction of Vanadyl(IV) with Hydroxyapatite

Bone is a highly organized, metabolically active tissue consisting of a mineral phase of hydroxyapatite and amorphous calcium phosphate crystals deposited in an extra cellular organic matrix (ECM).[85] Hydroxyapatite is the main mineral component of bones and hard tissues in mammals. It is thermodynamically more stable and is formed via intermediate precursors such as amorphous calcium phosphate (ACP), octacalcium phosphate, or dicalcium phosphate dehydrate.[86-87] The chemical nature and the open lattice of hydroxyapatite (Figure 8) lend itself to substitution, meaning that it is usual to find different types of substitutions in biological hydroxyapatite.

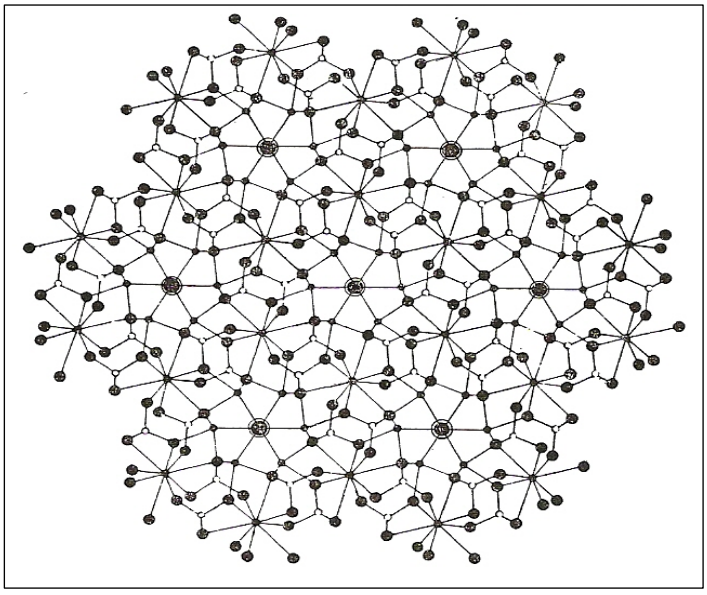


Figure 8. Hydroxyapatite lattice. Taken from reference [88]

The most common ones involve carbonate, fluoride and chloride substitutions for hydroxyl groups, while defects can also exist resulting in deficient hydroxyapatite. Physiologically, the mineralization process takes place with a non random distribution. It occurs inside matrix vesicles

(MVs) which are extracellular, membrane-invested particles selectively located at sites of initial calcification in cartilage, bone, and predentin. Matrix vesicle biogenesis occurs by polarized budding and pinching-off of vesicles from specific regions of the outer plasma membranes of differentiating growth plate chondrocytes. The first crystals of apatite bone mineral are formed within the MVs.[89]

On the other hand, it is known that when vanadium is absorbed in vertebrates, it distributes among different tissues and is storage mainly in bone.[90] The skeletal retention and bone effects of vanadium are of particular research interest.[91] Bone is an active vanadium and other metals accumulator. It seems likely that vanadium and strontium participate in the first stage of the mineralization process.[92] Vanadate ions can be incorporated into hydroxyapatite lattice possibly for its analogy to phosphate.[93-95] For these reasons, special attention was focused on the interaction of vanadium species with hydroxyapatite and with some components of the ECM such as chondroitin sulfate A, N-acetyl-D-galactosamine and D-Glucuronic acid. In a first stage, the structural and spectroscopic effects of the incorporation of VO_4^{3-} in the hydroxyapatite lattice were investigated [88] as a model for the process of incorporation of vanadium in bone.[96] The substitution of PO_4^{3-} by VO_4^{3-} is facilitated for the structural behavior of the hydroxyapatite lattice which allows substitutions. Nevertheless, it is required that the apatite would be in an amorphous form to allow the incorporation of vanadate since it could not be observed when the apatitic lattice is in the crystalline state. The incorporation of small quantities of VO_4^{3-} in the PO_4^{3-}sites produces only slight distortions in the macroscopic and microscopic structure of the mineral phase of bones. This fact could be determined by X-ray diffraction studies of substituted hydroxyapatite powder samples. Based on these results, it can be assumed that bone has an active role in the detoxification process when the organisms are exposed to high vanadium concentrations, comparable to that known for other toxic trace elements that can be also incorporated in bone. Besides, spectroscopic studies revealed that the incorporation of small amounts of vanadate into the apatitic lattice cause little or no effect in the strength of P-O bonds, as it was previously reported for mixed phosphate/vanadate fluoroapatites in which IR and Raman spectra show a negligible effect of the incorporation of VO_4^{3-} on the strength of P=O bonds.[97] On the contrary, the substitution of calcium by other cations in the hydroxyapatite or fluoroapatite lattices affects these bonds more strongly.[98,99] Besides, it was also demonstrated that VO^{2+} could not be incorporated into the apatite lattice although it is strongly adsorbed on its surface. The ESR spectra of the samples indicate an inhomogeneous distribution of the adsorbed species and a generation of $O=V(O)_4$ units which display a higher stability towards oxidation.[100] All together these studies showed that both, hydroxyapatite and fluoroapatite are good models for the study of the incorporation of vanadium in bone. Moreover, $Sn_3PO_4F_3$, a compound derived from hydroxyapatite interactions with SnF_2, displayed interesting hydrolytic properties relevant to dental research.[101,102] $Sn_3PO_4F_3$ showed a very high hydrolytic stability under different experimental conditions, as it was established by X-ray diffractometry, infrared spectroscopy and ^{119}Sn-Mösbauer spectroscopy.[103] $Sn_3PO_4F_3$ generates on the dental surface after topic applications of SnF_2 and, at least in part, it owes the protective capacity to its high hydrolytic stability. In addition, its hydrolytic stability increases at low pH and this would be another reason to explain its protective role against caries formation.

ii. Interactions of Vanadyl(IV) with Components of the ECM

To continue with the understanding of the interactions of vanadium species with hard tissues and related materials, we investigated the interactions of vanadium with chondroitin sulfate A (CSA), an acid muchopolysaccharide present in connective tissue and other mineralized systems.[104] Results from electronic absorption and IR spectroscopies in aqueous solutions demonstrated the formation of $VO(CSA)_2$. Coordination of oxovanadium(IV) to the carboxylate group and the glycosidic oxygen of the D-glucuronate moieties could be suggested. These

interactions were also detected in the solid state complex. It could be seen that the interactions are highly dependent on the VO/CSA molar ratio. As this parameter and pH values increased, results suggested a participation of N-acetyl groups in the bonding. These results are relevant for the interaction of VO^{2+} with collagen, a system in which interactions of oxovanadium(IV) with nitrogen has been clearly established.[105] Next, to have an overwiew, it was studied the behavior of vanadyl(IV) cation in the presence of N-acetyl-D-Galactosamine and D-Glucuronic acid at different molar ratios and pH values.[106] The cation interacts with the ligands only at high pH values. The complexes obtained at a 2:1 ligand-to-metal ratio showed that coordination occurs through two pairs of deprotonated OH groups of the rings. In the case of the complex with D-glucuronic acid, the involvement of the glycosidic oxygen could be suggested at pH=3. Besides, the complex of vanadyl cation with D-Glucuronic acid could be obtained and characterized.[107] The spectroscopic characterization suggested that the OH groups involved in coordination are those placed at the C(1) and C(2) atoms of D-Glucuronic acid, similarly to the interaction of vanadyl cation with the ligand in alkaline solutions.

iii. Effects of Vanadium Compounds on Osteoblasts in Culture

Osteoblasts, which are specialized mineral phase-forming cells, synthesize the major constituents of the ECM, mainly type I collagen and noncollagenous proteins. These cells also regulate their content of mineral ions through the activity of different enzymes such as alkaline phosphatase which is anchored in the osteoblastic membranes. Besides, the organic matrix exerts a great degree of crystallographic control over the nucleation and growth of mineral particles. In the different steps of mineralization, type I collagen, the predominant protein in the ECM of bone and teeth, plays a crucial role.[89]

The importance of vanadium in bone tissue is not only related to its interaction with the mineral phase of hydroxyapatite and other amorphous precursors since vanadium compounds also exert important biological and pharmacological actions in hard tissues constituents in vertebrates.

The relevance of vanadium in bone tissue arises from the studies performed to establish the essentiality of this element in animals and human beings.[90,91]

As bone is quantitatively the main tissue for vanadium storage (bone accumulation is twice that kidney and ten fold liver accumulation)[108], the research was extended to the study of the effects of vanadium compounds in bone related cells. Cells in culture are an appropriate system to investigate different biological events such as cell proliferation, cellular differentiation and the intracellular mechanisms by which vanadium derivatives exert their biological actions.

In particular, for the biochemical aspects of this project devoted to the study of bioinorganic and pharmacology of vanadium compounds, two osteoblast-cell lines of murine origin were used in cell culture experiments.[6,109-111]

Figure 9 shows the morphology of the non-transformed osteoblasts MC3T3E1 derived from mouse calvaria. These cells display the features of typical fibroblasts. They are stellate in shape and exhibit slender lamellar expansions that connect each cell with its neighbours.[111] MC3T3E1 cell line is a model of preosteoblasts that can differentiate to mature osteoblasts in culture. The different maturation stages of this cell line *in vitro* resemble that of the physiological process *in vivo* (proliferation, differentiation and mineralization), providing an adequate system for biological studies referred to bone tissues. MC3T3E1 cell line grows in monolayer and presents a fibroblastic phenotype in the proliferative stage. Then, the cells differentiate to osteoblasts and express different specific markers. Finally, they mineralize the ECM due to the synthesis and deposition of hydroxyapatite.[112] In the proliferative stage, MC3T3E1 cells show a fusiform morphology and do not express a great level of ALP specific activity. After 10 days of culture the cells reach confluence, display cuboid shape and arrest their growth. At this time they expressed specific

markers of mature osteoblast phenotype. The differentiation step correlates with the expression of different proteins such as alkaline phosphatase (ALP), procollagen processing and collagen accumulation in the ECM. The mineralization of ECM begins approximately at 16 days of culture. Finally, the mineralization of ECM takes place near 30 days of culture. After that the cells programme their death by apoptosis.[113]

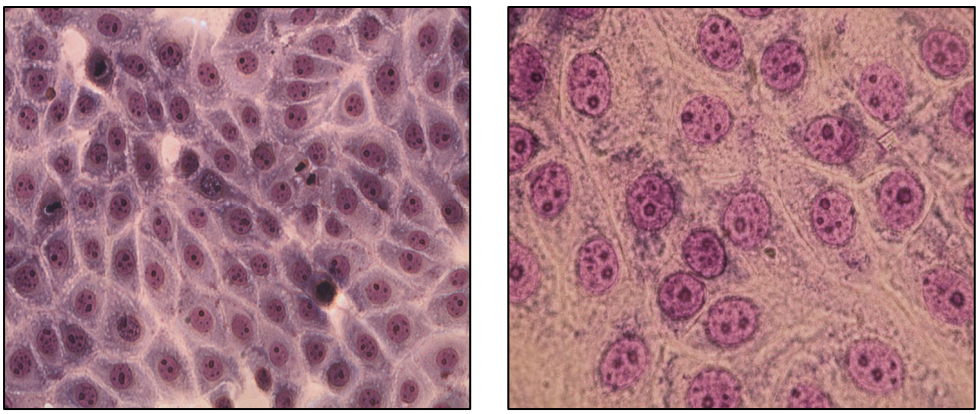


Figure 9 MC3T3E1 osteoblast-like cells. The osteoblasts were cultured in DMEM at 37°C for 24 h, fixed and stained with Giemsa for microscopy observation as described in reference 132 (Magnification: 40X (left), 100X (right)).

UMR106 osteoblast-like cells are derived from a rat osteosarcoma induced by ^{32}P. This immortalized cell line exhibits the characteristic osteoblast phenotype but is unable to mineralize the ECM. These cells express high levels of specific ALP activity and produce type I collagen but they are unable to synthesize bone mineral phase in culture. [110] Figure 10 shows the morphology of the osteosarcoma cells stained with Giemsa and examined under light microscopy.

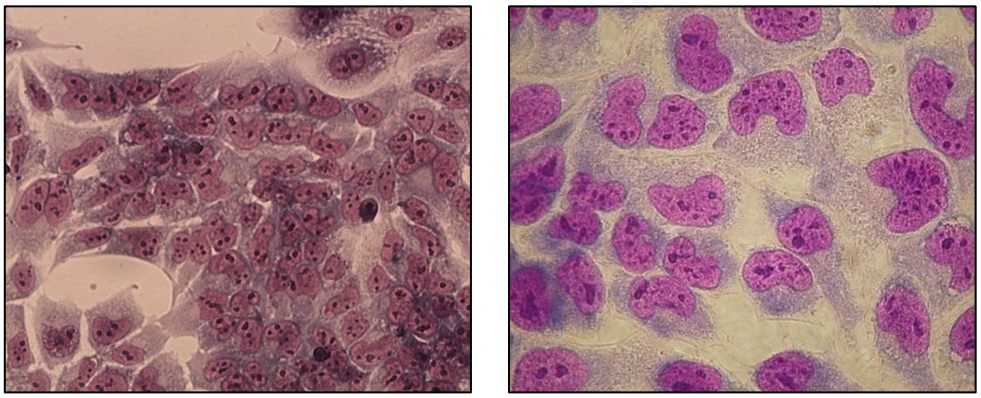


Figure 10. UMR106 osteoblast-like cells. The osteoblasts were cultured in DMEM at 37°C for 24 h, fixed and stained with Giemsa for microscopy observation as described in reference 132 (Magnification: 40X (left), 100X (right)).

These cells show a polygonal morphology with well stained nuclei many of them with a kidney-like shape. The cytoplasms present processes that connect the cells in the monolayer.[114]

This simple model of osteoblast-like cells in culture allows the investigation of biological and pharmacological effects of many vanadium compounds. Specially, complexes of vanadyl(IV)

cation with simple sugars (mono- and disaccharides, and related compounds such as polyhols and acids) were investigated. Moreover, the bioactivity of vanadyl(IV) derivatives with non steroidal antiinflammatory drugs (NSAID) and with flavonoids and related compounds as ligands were investigated with the aid of this cellular *in vitro* model.

Complexes of VO^{2+} with the most common simple sugars were synthesized and physicochemically characterized.[26,28-30,32,33,115,116] Practically all these vanadyl-sugar complexes were evaluated for their bioactivity on osteoblast cell lines in culture. This approach has allowed us to find an outstanding compound among this series: the complex of vanadyl(IV) cation with the disaccharide trehalose (TreVO) which displayed insulin mimetic activity by promoting glucose consumption, and, besides, showed antitumoral action since it caused a greater inhibition of cell proliferation on the tumoral than in the normal osteoblasts. Besides, in long term cultures carried out with the MC3T3E1 normal cell line, this complex revealed as a good osteogenic compound since it promoted type I collagen production and the mineralization of the ECM in the cultures.[115] Moreover, it could also be established the mechanism of action of this compound which exerted its mitogenic effect, at low doses, through the activation of the extracellular regulated kinase (ERK) pathway. On the other hand, it was demonstrated the participation of oxidative stress in the cytotoxic actions of this complex at high concentrations.[110,117]

Besides, other vanadium-sugar complexes investigated showed some of the potential pharmacological activities mentioned above, but any of them presented all these activities together as TreVO did.[118] NSAID is a group of compounds with very well established pharmacological properties.[119-121] As part of this project devoted to the investigation of new vanadium derivatives with potential therapeutic applications, complexes of aspirin, ibuprofen, naproxen, tolmetin, diclofenac and indometacin with oxovanadium(IV) were synthesized and characterized.[122-125] Two of these complexes, the aspirin vanadium derivative (VOAspi) and the complex with naproxen (NapVO) demonstrated interesting antitumoral properties in the osteoblast-cell line in culture.[114,126,127] As bone homeostasis is the result of the balance between bone resorption and bone formation, we have studied the activation of macrophages because these cells are related with bone resorption. We investigated the effect of VOAspi on a culture of murine macrophage RAW 264.7. VOAspi caused the activation of macrophages by a mechanism dependent on L-type calcium channel and the generation of nitric oxide. On the contrary, free vanadyl(IV) cation exerted cytoxic effects by a mechanism independent of calcium channel and nitric oxide generation.[128]

Other interesting group of ligands is the flavonoids. They are polyphenolic compounds obtained from plants. Recently they have aroused a great scientist interest due to their broad pharmacological activity. They present antioxidant, antitumoral and antibacterial properties.[129,130] In this context, a new complex of Quercetin and vanadyl(IV) cation, QuerVO, was synthesized and characterized by means of UV-vis, FT-IR and EPR spectroscopies. Then, its biological activity was studied in cultures of MC3T3E1 and UMR106 cells. Cell proliferation was evaluated by the crystal violet bioassay. For studying cellular differentiation, two markers of osteoblast phenotype (ALP and collagen production) were analyzed. Finally, to get an insight into the putative mechanisms of action, studies on the effect of this complex on the activation of ERK pathway were performed and reported.[131] QuerVO displayed interesting osteogenic actions such as induction of the synthesis of collagen type I. Moreover, antitumoral actions on the osteoblasts in culture could also been established. The antitumoral effect of the free ligand, Quercetin, was also demonstrated as it inhibited the proliferation of the tumoral osteoblasts and did not exert any effect on the normal osteoblasts. The complex caused stimulation of ERK phosphorylation and this activation seems to be one of the possible mechanisms used by the complex to exert its biological effects.

Another flavonoid complex with vanadyl cation which showed interesting biological activity

was the complex of hesperidin with VO^{2+}.[132] This compound behaved as an antioxidant agent. In fact, VO-hesp improved the SOD-like activity of the hesperidin. In this case, another tumoral cell line was also included in the study to evaluate the antitumoral activity. Caco-2 cells are derived from a human colon adenocarcinoma. The complex enhanced the antiproliferative behavior of the free ligand and this effect correlated with the morphological changes towards apoptosis that could be observed under microscopy.

Conclusions

The integration of the chemical and biochemical knowledge of vanadium, a versatile element with notorious and fascinating aspects both from the chemistry and biochemistry, have been worthy to find some new vanadium compounds with organic ligands that present potential pharmacological properties. These compounds are good candidates on behalf of further *in vivo* studies for pharmacological purposes.

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References

[1] P.M. Lafayllve, *Freyja, Lady, Vanadis: An Introduction to the Goddess,* Books Express - Publisher, Outskirts Press**,** 2006, pp 1-124.

[2] J. Carrington Sellars, *Chemistianity*, **1873**, 92

[3] Y. Shechter, *Diabetes* **1990,** *39*, 1.

[4] J. Meyerovitch, P. Rothenberg, Y. Shechter, A. Weir, C.R. Kahn, *J. Clin. Invest.* **1991**, *87*, 1286.

[5] E. Canalis, *Endocrinology* **1985**, *116*, 855

[6] Etcheverry, S.B.; Cortizo, A.M., Bioactivity of Vanadium Compounds on Cells in Culture, in: Nriagu, J.O. (Ed.), Vanadium in the Environment. Advances in Environmental Science and Technology; Part A, Chap. 15, John Wiley & Sons, New York, 1998, pp. 359-394.

[7] A. Sheela, S.M. Roopan, R. Vijayaraghavan, *Eur. J. Med. Chem.* **2008**, *43*, 2206.

[8] M. Hiromura, A. Nakayama, Y. Adachi, M. Doi, H. Sakurai, *J. Biol. Inorg. Chem.* **2007,** *12*, 1275.

[9] Y.Adachi, J.Yoshida, Y. Kodera, A. Katoh, J. Takada, H. Sakurai, *J. Inorg. Biochem.* **1999**, *76*, 251.

[10] Djordjevic, C. in: Sigel, H.; Sigel,A. (Eds), *Metal Ions in Biological Systems*, *vol 31,* Marcel Dekker, New York, 1995, pp 595-616.

[11] A.M. Evangelou, *Crit. Rev. Oncol. Hematol.* **2002**, *42,* 249.

[12] R.S. Ray, B. Ghosh, A. Rana, M.Chatterjee, *Int. J. Cancer* **2007,** *120*, 13.

[13] A.M. Cortizo, M.S. Molinuevo, D.A. Barrio, L. Bruzzone, *Int. J. Biochem. Cell Biol.* **2006**, *38*, 1171.

[14] J.L. Domingo, *Reprod. Toxicol.* **1996**, *10*, 175.

[15] B. Gummow, C.J. Botha, J.P. Noordhuizen, J.A. Heesterbeek, *Prev. Vet. Med*. **2005,** *72*, 281

[16] Y. Shechter, S.D.J. Karlish, *Nature* **1980,** *284*, 556.

[17] W.C Duckworth, S.S. Solomon, J. Liepnicks, F.G. Hamel, S. Hand, D.E. Peavy, *Endocrinology* **1988,** *122*, 2285.

[18] S. Tamura, T.A. Brown, J.H. Whipple, Y. Fujita-Yamaguchi, R.E. Dubler, K. Cheng, J. Larner, *J. Biol. Chem.* **1984**, *259*, 6650.

[19] P.A.M. Williams, S.B. Etcheverry, E.J. Baran, *Z. Naturforsch.* **1993**, *48b*, 1845.

[20] P.A.M. Williams, S.B. Etcheverry, E.J. Baran, *Anales Asoc. Qca. Argent.* **1994**, *82,* 13.

[21] P.A.M. Williams, E.J. Baran, *J. Inorg. Biochem.* **1992**, *48,* 15.

[22] P.A.M. Williams, S.B. Etcheverry, E.J. Baran, *J. Inorg. Biochem.* **1996***, 61,* 285.

[23] P.A.M. Williams, E.J. Baran, *J. Inorg. Biochem.* **1993**, *50*, 101.

[24] S.B. Etcheverry, E.G. Ferrer, E.J. Baran, *Z. Naturforsch.* **1989**, *44b*, 1355.

[25] Anthe M.; Hopkins J.; McLaughlin C. W.; Johnson S.; Warner M.Q.; LaHart D.; Wright J.D, *Human Biology and Health*, Prentice Hall, Englewood Cliffs, New Jersey, USA, 1993, pp 52-59.

[26] P.A.M. Williams, S.B. Etcheverry, E.J. Baran, *J. Inorg. Biochem.* **1997,** *65*, 133.

[27] L. Gagliardi, P.A.M. Williams, *Anales Asoc. Qca. Argent*. **1999**, *87*, 165.

[28] S.B. Etcheverry, P.A.M. Williams, E.J. Baran, *Carbohydr. Res.* **1997**, *302,* 131

[29] P.A.M. Williams, S.B. Etcheverry, E.J. Baran, *Carbohydr. Res.* **2000,** *329,* 41.

[30] S.B Etcheverry, D.A. BarRío, P.A.M. Williams, E.J Baran, *Biol. Trace Element. Res.* **2001***, 84,* 227.

[31] E.J. Baran, *J. Carbohydr. Chem.* **2001**, *20*, 769.

[32] S.B. Etcheverry, D.A. Barrio, J. Zinczuk, P.A.M. Williams, E.J. Baran, *J. Inorg. Biochem.* **2005**, *12*, 2322.

[33] P.A.M. Williams, S.B. Etcheverry, D.A. Barrio, E.J. Baran, *Carbohydr. Res.* **2006,** *341*, 717.

[34] E.G.Ferrer, A.Bosch. O.Yantorno, E.J.Baran, *Bioorg. Med. Chem*. **2008,** *16*, 3878.

[35] H. Vilter, *Phytochemistry* **1984,** *23*, 1387.

[36] J.W.P.M. Van Schijndel, E.G.M. Vollenbroek, R. Waver, *Biochim. Biophys. Acta* **1993,** *1161,* 249.

[37] H. Plat, B.E. Krenn, R. Wever, *Biochem. J.* **1987**, *248*, 277.

[38] R.M. Tótaro, P.A.M. Williams, M.C. Apella, M.A. Blesa, E.J. Baran, *J. Chem. Soc. Dalton Trans.* **2000**, 4403.

[39] A. Buttler, *Coord. Chem. Rev.* **1999**, *187*, 17.

[40] D. Rehder, *Coord. Chem. Rev.* **1999**, *182*, 297.

[41] A.G.J. Ligtenbarg, R.Hage, B.L.Feringa, *Coord. Chem. Rev.* **2003**, *237*, 89.

[42] A.H. Jubert, A.C. González-Baró, E.J. Baran, O. Sala, *J. Raman Spectrosc*. **1989**, *20*, 555.

[43] A.C. González Baró, E.J. Baran, *Monatsh. Chem*. **1997**, *128*, 323.

[44] B.S. Parajón Costa, A.C. González Baró, E.J. Baran, *J.Coord. Chem.* **1999**, *47***,** 417.

[45] A. Jubert, A.C. González Baró, R. Pis-Diez, E.J. Baran, *J. Raman Spectrosc*. **1992**, *23,* 273.

[46] R. Sáez Puche, J. Romero, A.C. González Baró, E.J. Baran, *Chem. Phys. Lett.* **1998,** *282***,** 273.

[47] E.G. Ferrer, A.C. González Baró, E.J. Baran, *J.Therm. Anal. Cal.* **1999**, *57,* 595.

[48] A.C. González Baró, O.E. Piro, B.S. Parajón Costa, E.E. Castellano, E.J. Baran, *Monatsh. Chem.* **1998,** *129,* 31.

[49] B.S. Parajón Costa, A.C. González Baró, E.J. Baran, *J.Coord.Chem.* **1999**, *49,* 17.

[50] A.C. González Baró, E.J. Baran, *J. Braz.Chem. Soc.* **2001,** *12(2),* 2008.

[51] A.C. González Baró, E.J.Baran, *J.Coord.Chem.* **1998,** *43*, 335.

[52] K. Murakami, Y. Tanemura, M. Yoshino, *J. Nutr. Biochem*. **2003**, *14*, 99 (and references therein).

[53] D.C. Crans, M. Mahroof-Tahir, M.D Johnson, P.C. Wilkins, L. Yang, K. Robbins, A. Johnson, J.A. Alfano, M.E. Godzala, L.T. Austin, G.R Willsky, *Inorg. Chim. Acta* **2003,** *356*, 365.

[54] D.C. Crans, L. Yang, T. Jakusch, T. Kiss, *Inorg. Chem*. **2000**, *39*, 4409.

[55] A.C. González Baró, E.E. Castellano, O.E. Piro, B.S. Parajón Costa, *Polyhedron* **2005,** *24,* 49.

[56] B.S. Parajón-Costa, O.E. Piro, R. Pis-Diez, E.E. Castellano, A.C. González-Baró, *Polyhedron* **2006**, *25*, 2920.

[57] K. A. Schug, W. Lindner, *Chem. Rev*. **2005**, *105,* 67.

[58] A. Melo, M.J. Ramos, *Chem.Phys. Lett*. **1995,** *245,* 498.

[59] P.D. Kwong, R. Wyatt, J. Robinson, R.W. Sweet, J. Sodroski, W.A. Hendrickson, *Nature* **1998**, *393,* 648.

[60] K. Takamura, K. Aoyagi, S. Nagase, M. Gotoh, A. Hirayama, A. Ueda, C. Tomida, A. Koyama, *Nephron,* **1998**, *7*.

[61] E.J. Baran, *Vanadium detoxification,* in: Nriagu, J.O. (Ed):*Vanadium in the Environment* Part II, Wiley, New York, 1998b, pp. 317-345.

[62] E.J. Baran, *Chem. Biodiv.* **2008**, *5*, 1475.

[63] Baker, E.W.; Palmer, S.D. in: Dolphin (Ed.), *The porphyrins*, *vol IA*, Academic Press, New York, 1978, pp. 485- 488.

[64] R.N. Lindquist, J.L. Lynn, G.E. Lienhard, *J. Am. Chem. Soc.* **1973**, *95*, 8762.

[65] D.W. Boyd, K. Kustin, *Adv. Inorg. Biochem.* **1984**, *6*, 312.

[66] T.W. Clarkson, *Annu. Rev. Pharmacol. Toxicol.* **1993**, *32*, 545.

[67] L.C. Cantley, M.D. Resh, G. Guidotti, *Nature* **1978,** *272*, 552.

[68] S.J.D. Karlish, L.A. Beaugé, I.M. Glynn, *Nature* **1979**, *282,* 333.

[69] A. Wach, P.Graber, *Eur. J. Biochem.* **1991***, 201*, 91.

[70] J.G. Foulkes, E. Erikson, R. Erikson, J. Biol. Chem. **1983**, *258*, 431.

[71] E.G. Ferrer, E.J. Baran, *J. Electron. Spectrosc. Relat. Phenom.* **1991**, *57*, 189.

[72] E.J. Baran, A.H. Jubert, E.G. Ferrer, *J. Raman Spectrosc.* **1992***, 23*, 489.

[73] K. Kustin, D. L. Toppen, *Inorg. Chem.* **1973**, *12*, 1404.

[74] M.B. Davies, *Polyhedron* **1992**, *11*, 285.

[75] Rabenau, D.L.; Guevremont, R.; Evans, Ch.A. in: Sigel, H. (Ed), *Metal Ions in Biological Systems*, *vol 9*, M. Dekker, New York, , 1979, pp. 103-118.

[76] H. Degani, M. Gochin, S.J. D. Karlish, Y. Shechter, *Biochemistry* **1981**, *20*, 5795.

[77] E.G. Ferrer, P.A.M. Williams, E.J. Baran, *Biol. Trace Elem. Res.* **1991***, 30,* 175.

[78] E.G. Ferrer, P.A.M. Williams, E.J. Baran, *J. Inorg. Biochem.* **1993***, 50,* 253.

[79] E.G. Ferrer, P.A.M. Williams, E.J.Baran, *Biol. Trace Elem. Res.* **1996**, *55*, 79.

[80] E.G. Ferrer, P.A.M. Williams, E.J. Baran, *J. Trace Elements Med. Biol.* **1998**, *12*, 56.

[81] E.G. Ferrer, P.A.M. Williams, E.J. Baran, *Biol. Trace Elem. Res* .**2005***,105*, 53.

[82] P.A.M. Williams, E.J. Baran, *Biol. Trace Elem. Res.* **2006***, 109*, 189.

[83] P.A.M. Williams, E.J. Baran, *J. Inorg. Biochem.* **2008**, *102*, 1195.

[84] H. Sakurai, S. Shimomura, K.Ishizu, *Inorg. Chim. Acta* **1981**, *55*, L67.

[85] V. Kartsogiannis , K.W. Ng, *Mol. Cell. Endocrinol.* **2004,** *228*, 79.

[86] E.D. Eanes, *Monogr. Oral Sci.* **2001**, *18*, 130.

[87] E.D. Eanes, I. H. Gillessen, A. Posner, *Nature* **1965**, *208,* 365.

[88] M. Massuyes, J.C. Trombe, G. Bonel, G. Montel, *Bull. Soc. Chim. Fr.* **1969,** *7,* 2308.

[89] H.C Anderson, *Curr. Rheumatol. Rep.* **2003**, *5*, 222.

[90] Nielsen F.H., *Vanadium and Its Role in Life*, in: Sigel H.; Sigel A. (Eds.), *Metal Ions in Biological Systems*, *vol 31*, Marcel Dekker, New York, 1995, pp 543-573.

[91] Anke M.; Groppel B.; Krause U., in: Momcilovic B. (Ed.), *Trace Elements in Man and Animals*, vol 7, IMI, Zagreb, 1991, pp 11.9-11.10.

[92] O. Righ, *Bull. Soc. Chim. Biol*. **1949**, *31*, 1403.

[93] Gresser M.J.; Tracey A.S.; *Vanadates as Phosphate Analogs in Biochemistry*, in: Chasteen N.D. (Ed), *Vanadium in Biological Systems*, Dordrecht, Kluwer Academic, 1990, pp 63–79.

[94] D.C. Crans, *Comm. Inorg. Chem.*, **1994**, *16*, 35.

[95] S. Bhattacharyya, A. S. Tracey, *J. Inorg. Biochem.* **2001**, *85*, 9.

[96] S.B. Etcheverry, M.C. Apella, E.J. Baran, *J. Inorg. Biochem.* **1994**, *20*, 269.

[97] A. Lavat, S.B. Etcheverry, E.J. Baran, *Z. Naturforsch.* **1986,** *41b*, 987.

[98] M.C. Apella, S.B. Etcheverry, E.J. Baran, *Z. Naturforsch*. **1981**, *36b*, 1190.

[99] G.E. Narda, C. Pedregosa, S.B. Etcheverry, E.J. Baran, *Z. Naturforsch*. **1990**, *45b*, 1133.

[100] G.E. Narda, D.E. Vega, J.C. Pedregosa, S.B. Etcheverry, E.J. Baran, *Z. Naturforsch.* **1992**, *47b*, 395.

[101] G.E. Narda, M.C. Apella, S.B. Etcheverry, E.J. Baran, *Z. Anorg. All. Chem.* **1984**, *515*, 207.

[102] S.B. Etcheverry, G.E. Narda, M.C. Apella, E.J. Baran, *Caries Res*. **1986**, *20*, 120.

[103] M.C. Apella, E.J. Baran, S.B. Etcheverry, R.C. Mercader, *Monatsh. Chem*. **1983**, *114*, 1149.

[104] S.B. Etcheverry, P.A.M. Williams, E.J. Baran, *Biol. Trace Elem. Res.* **1994***, 42*, 43.

[105] R.P. Ferrari, *Inorg. Chim. Acta,* **1990**, *176*, 83.

[106] S.B. Etcheverry, P.A.M. Williams, E.J. Baran, *Biol. Trace Element Res*. **1996**, *51*, 169.

[107] S.B. Etcheverry, P.A.M. Williams, E.J. Baran, *J. Inorg. Biochem.* **1996**, *63*, 285.

[108] I.A. Setyawatti, K.H. Thomson, V. G. Yuen, Y. Sun, M. Battell, D.M Lyster, *J. Applied Physiol.* **1998,** *84*, 569.

[109] A.M. Cortizo, S.B. Etcheverry, *Mol. Cell.Biochem.* **1995,** *145*, 97.

[110] D.A. Barrio, S.B. Etcheverry, *Can. J. Physiol. Pharmacol*. **2006**, *84*, 677.

[111] V.C. Sálice, A.M. Cortizo, C.L. Gómez Dumm, S.B. Etcheverry, *Mol. Cell Biochem*. **1998**, *198,* 119.

[112] H. Sudo, H.A. Jodama, Y. Amagai, S. Yamamoto, S. Kasai, *J. Cell Biol.* **1983**, *96*, 191.

[113] L.D. Quarles, D.A. Yohay, L.W. Lever, R. Catton, R.J. Wenshup, *J. Bone Miner. Res.* **1992**, *7*, 683.

[114] M.S. Molinuevo, D.A. Barrio, A.M. Cortizo, S.B. Etcheverry, *Cancer Chemother. Pharmacol.* **2004,** *53*, 163.

[115] D.A. Barrio, P.A.M. Williams, A.M. Cortizo, S.B. Etcheverry, *J. Biol. Inorg. Chem*. **2003**, *8*, 459.

[116] P.A.M. Williams, D.A. Barrio, S.B. Etcheverry, E.J. Baran, *J. Inorg. Biochem.* **2004**, *98*, 333.

[117] Etcheverry S.B.; Barrio D.A., in: Kustin K.; Costa Pesoa J.; Crans D.C (Eds), *Vanadium: The Versatile Element*, American Chemical Society Series 974. vol 15, 2007, pp. 204-216.

[118] D.A. Barrio, E.R. Cattáneo, M.C. Apezteguía, S.B. Etcheverry, *Can J Physiol Pharmacol* **2006**, *84,* 765.

[119] Goodman Gilman A. (Ed), 9th edition. *Las Bases Farmacológicas de la Terapéutica. vol II.* Mc Graw- Hill Interamericana, Mexico, 1996, pp 661-706.

[120] J.P. Pelletier, *Osteoarthritis Cartilage* **1999**, *7*, 374-378.

[121] Williams P.A.M.; Etcheverry S.B., Oxovanadium(IV) Complexes with Non Steroidal Antiinflammatory Drugs (NSAIDs): Pharmacological Relevance, in Aureliano Alves M. (Ed.), Vanadium Biochemistry, Signpost. India, 2008, pp.205-234.

[122] S.B. Etcheverry, P.A.M. Williams, D.A. Barrio, V.C. Sálice, E.G. Ferrer, A.M. Cortizo, *J. Inorg. Biochem.* **2000**, *80*, 169.

[123] S.B. Etcheverry, P.A.M. Williams, V.C. Sálice, D.A. Barrio, E.G. Ferrer, A.M. Cortizo. *Biometals* **2002**, *15*, 37.

[124] S.B. Etcheverry, D.A. Barrio, A.M. Cortizo, P.A.M. Williams, J.Inorg. Biochem. **2002**, 88, 94.

[125] P.A.M. Williams, M.S. Molinuevo, N. Okulik, A.H. Jubert, S.B. Etcheverry, Appl. Organomet. Chem. **2005**, *19*, 711.

[126] M.S. Cortizo, J.L. Alessandrini, S.B. Etcheverry, A.M. Cortizo, J. Biomater. Sci. Polymer Edn, **2001**, *12*, 945.

[127] M.S. Molinuevo, A.M. Cortizo, S.B. Etcheverry, *Cancer Chemother. Pharmacol.* 2008, *61*, 767.

[128] M.S. Molinuevo, S.B. Etcheverry, A.M. Cortizo, *Toxicology* **2005**, *210*, 205.

[129] C.A. Rice-Evans; L. Packer, *Flavonoids in Health and Disease*. Marcel Dekker, New York, 1998.

[130] J. Khang, L. Zhuo, X. Lu, M. Zhang, H. Wu, *J. Inorg. Biochem*. **2004**, *98*, 79.

[131] E.G. Ferrer, M.V. Salinas, M.J. Correa, L. Naso, D.A. BarRío, S.B. Etcheverry, L. Lezama, T. Rojo, P.A.M Williams, J. Biol. Inorg. Chem. **2006**, *11*, 791.

[132] S.B. Etcheverry, E.G. Ferrer, L. Naso, J. Rivadeneira, V. Salinas, P.A.M. Williams, *J. Biol. Inorg. Chem.* **2008**, *13*, 435.