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Synthesis, characterization, and biological activity of oxovanadium(IV) complexes with polyalcohols

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Abstract—Oxovanadium(IV) complexes of the polyalcohols sorbitol, galactitol, and mannitol, of stoichiometry $Na_2[VO(L)_2]\cdot H_2O$, were obtained from aqueous alkaline solutions. They were characterized by elemental analysis, infrared and UV–vis spectroscopies, thermoanalytical (thermogravimetric and differential thermal analysis) data, and magnetic susceptibility measurements. The biological activities of the complexes on the proliferation, differentiation, and glucose consumption were tested on osteoblast-like cells (MC3T3E1 osteoblastic mouse calvaria-derived cells and UMR106 rat osteosarcoma-derived cells) in culture. The three complexes exerted a biphasic effect on cell proliferation, being slight stimulating agents at low concentrations and inhibitory in the range of 25–100 μM. All the complexes inhibited cell differentiation in tumor osteoblasts. Their effects on glucose consumption were also discussed. The free ligands did not show any effect on the studied biological parameters.

Keywords: Oxovanadium(IV) complexes; Galactitol; Sorbitol; Mannitol; Osteoblasts; Cells in culture

1. Introduction

Saccharides are considered the third most important bio-macromolecules after proteins and nucleic acids, and they are widely distributed in nature.[1] Because of their functional groups, alditol and low-molecular-weight carbohydrates, as well as oligo- and polysaccharides, are versatile building blocks for the synthesis of metal complexes.[2] Therefore, sugar–metal interactions may be expected to take place in biological fluids, and complexation between sugars and metal ions would certainly affect the activity of biomolecules. Polyols (sugars, cyclitols, and alditols) generate a variety of complexes with metal cations, either in the protonated or deprotonated forms.[3–10] Metal–carbohydrate interactions are of interest because of their fundamental importance in many biochemical processes, such as toxic-metal metabolism, the transport and storage of metals, the function and regulation of metalloenzymes, and the mode of action of metallopharmaceuticals.[11–13]

Vanadium is an important trace element that may be beneficial and possibly essential in humans, but certainly essential for some living organisms.[14] Its physiological effects in many cases stem from the good complexation behavior of VO^{2+} and the chemical similarity between phosphate and vanadate. Vanadium compounds show interesting biological and pharmacological properties. Many of them display insulin-mimetic activities, and others present antitumor effects.[15] Even though a number of simple inorganic vanadium species have beneficial biological properties, the development of new vanadium derivatives with organic ligands to improve their bioavailability and to decrease the toxic side effects is of great interest.[15–20] In this paper, we report the

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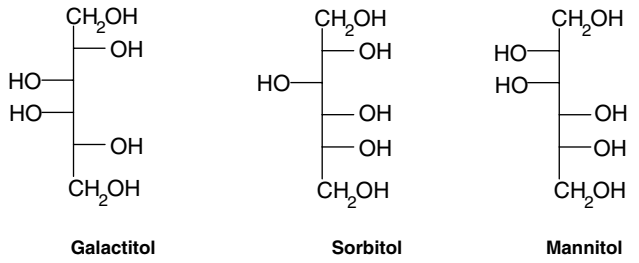

Figure 1. Schematic structure of the three polyalcohols.

interaction of oxovanadium(IV), VO^{2+}, with sorbitol, galactitol and mannitol (Fig. 1). Once absorbed by the organism, vanadium compounds are distributed among the different systems and finally accumulate mainly in hard tissues.[19,21,22] Consequently, the investigation of the biological effects of these vanadium compounds in two osteoblast-like cells (UMR106 and MC3T3E1) are of special interest in relation to the biochemistry of this element.[23]

2. Materials and methods

2.1. Materials

$VOCl_2$ (50% aq solution) was purchased from Carlo Erba. Galactitol (dulcitol), D-mannitol, D-sorbitol, crystal violet, *p*-nitrophenyl phosphate (*p*-NPP), glycine, $MgCl_2$ and all other chemicals used were of analytical grade from Sigma Chemical Co. Tissue culture materials were purchased from Corning (Princeton, NJ, USA), Dulbecco's Modified Eagles Medium (DMEM), DMEM low glucose and trypsin–EDTA from Gibco (Gaithersburg, MD, USA), and fetal bovine serum (FBS) from GibcoBRL (Life Technologies, Germany).

2.2. Synthesis of the complexes

The polyol complexes $Na_2[VO(L)_2]\cdot H_2O$ were prepared by a similar procedure as the one employed for the synthesis of other, previously investigated VO/saccharide complexes.[24–28]

Briefly, the polyols (2 mmol) were dissolved in doubly distilled water (10 mL), and $VOCl_2$ (1 mmol) was added in each case. The pH value of the mixtures was raised to 12 by addition of small amounts of solid NaOH. In order to obtain the solid complexes, abs EtOH was added. The first precipitates were oily, but with successive additions of abs EtOH and discarding the supernatants, microcrystalline green powder products were finally obtained. The complexes were stored at 60 °C, due to their high hygroscopicity. The yield of different preparations ranged from 40% to 60%. The formula of the compounds ($C_{12}H_{26}Na_2O_{14}V$, MW = 491) were confirmed by chemical analysis. Calcd: C, 29.3; H, 5.3; Na, 9.3; V, 10.4. Found: C, 28.9; H, 5.1; Na, 9.5; V, 10.2 for galactitol/VO (**1**); C, 29.5; H, 5.2; Na, 9.4; V, 10.1 for mannitol/VO (**2**); C, 29.0; H, 5.2; Na, 9.5; V, 10.3 for sorbitol/VO (**3**). The water contents were additionally confirmed by the thermogravimetric measurements. The room temperature magnetic moments were 1.65 (**1**), 1.60 (**2**) and 1.58 (**3**) BM, respectively.

2.3. Physicochemical characterization

Electronic absorption spectra were measured with a Hewlett–Packard 8452 diode-array spectrophotometer, using 10-mm quartz cells. Diffuse reflectance spectra were recorded on a Shimadzu UV-300 spectrophotometer, using MgO as a standard. Infrared spectra were obtained with a Bruker IFS 66 FTIR instrument, using the KBr pellet technique. Room temperature magnetic susceptibility was determined with a Cahn-2000 balance, calibrated with $Hg[Co(SCN)_4]$ and at a magnetic field strength of 6 kG. Elemental analyses for C and H were performed using a Carlo Erba 11008 analyzer. Flame photometry and the tungsto-phosphovanadic method[29] were employed to determine sodium and vanadium contents, respectively. Thermogravimetric (TG) and differential thermal analysis (DTA) were performed on a Shimadzu system (models TG-50 and DTA-50, respectively), working in an oxygen flow of 60 mL/min and at a heating rate of 10 °C/min. Sample quantities ranged between 10 and 20 mg. Al_2O_3 was used as a DTA standard.

2.4. Cell culture

MC3T3E1 osteoblastic mouse calvaria-derived cells and UMR106 rat osteosarcoma-derived cells were grown in DMEM supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin and 10% (v/v) fetal bovine serum at 37 °C, 5% CO_2. When 70–80% confluence was reached, cells were sub-cultured using 0.1% trypsin 1 mM EDTA in Ca(II)–Mg(II)-free phosphate buffered saline (PBS) (11 mM KH_2PO_4, 26 mM Na_2HPO_4, 115 mM NaCl, pH 7.4). For experiments, cells were grown in multiwell plates. When cells reached 70% confluence, the monolayers were washed twice with DMEM and were incubated under different conditions according to the experiments.

2.5. Biological assays

Cell proliferation and cell differentiation assays, as well as glucose consumption assays, were performed with the complexes and the free ligands in the same way as described in detail in a previous paper.[27] Briefly, the cell proliferation was assessed by the crystal violet bioassay. The cells in culture were stained with crystal violet after the incubation period. Then, they were washed to

remove the excess of dye. The crystal violet taken up by the osteoblasts was extracted with the appropriate buffer, and the absorbance was measured at 540 nm. Previously, a linear correlation was established for the number of cells and the absorbance.[30] Besides, to study the cell differentiation the specific alkaline phosphatase (ALP) activity was used as a marker of osteoblast differentiation. The extracts of the cells were used to determine ALP specific activity using *p*-nitrophenyl phosphate as a substrate. The production of *p*-nitrophenol was measured at 405 nm. On the other hand, the effects of vanadium compounds on glucose metabolism were estimated through the glucose consumption assay. The glucose content in the conditioned medium was determined after incubation of the osteoblasts with the different doses of vanadium complexes by the glucose oxidase test measured at 505 nm.

At least three independent experiments were performed for each experimental condition in all the biological assays. Results are expressed as mean ± SEM (standard error of the mean). Statistical differences were analyzed using Student's test.

3. Results and discussion

3.1. Characterization of the complexes

3.1.1. Thermal behavior. The thermal behavior of the complexes was investigated by means of TG and DTA measurements under O_2 atmosphere up to 1000 °C.

Galactitol/VO (**1**): the thermogram showed that the water molecule is removed in the range of 25–160 °C with a loss of 3.8%, in excellent agreement with the calculated mass loss, which is 3.7%. After dehydration, a complex series of consecutive events is observed: 5.1% (207 °C, weak, *exo*), 17.0% (264 °C, weak, *exo*), 38.3% (307 °C, very strong, *exo*), 4.6% (no DTA-signal). The weight of the residue at 1000 °C is 31.3% in good agreement with the theoretical value (31.2%).

Mannitol/VO (**2**): the loss of the water molecule of hydration occurs in the range of 25–160 °C ($\Delta\omega_{exp} = 3.9\%$ and $\Delta\omega_{calcd} = 3.7\%$). Five successive degradation steps 4.7% (no DTA-signal), 19.6% (283 °C, very weak, *exo*), 15.7% (305 °C, very strong, *exo*), 18.2% (no DTA-signal) and 6.7% (no DTA-signal), lead to the final residue at 1000 °C (31.2%).

Sorbitol/VO (**3**): the loss of the water molecule of hydration occurs in the range of 25–160 °C ($\Delta\omega_{exp} = 3.9\%$ and $\Delta\omega_{calcd} = 3.7\%$). Four successive degradation steps 4.5% (no DTA-signal), 27.6% (310 °C, very strong, *exo*), 18.6% (no DTA-signal) and 14.2% (no DTA-signal) give the final residue of 31.2% at 1000 °C.

The overall stoichiometry of these processes can be described as follows:

$$2Na_2[VO(L_2)] \cdot H_2O \xrightarrow{\varnothing, O_2} 2NaVO_3 + Na_2O + \text{volatile products}$$

The chemical nature of final solid residues was confirmed by means of IR spectroscopy. In all cases $NaVO_3$ was detected. As can be seen, the three compounds present a similar thermal stability, showing very strong exothermic DTA-peaks in the 305–310 °C range.

On the other hand, and on the basis of the temperature ranges at which the H_2O molecule is lost, it is impossible to establish if it is present as water of crystallization or if it is, eventually, coordinated to the metal center. But, as in all cases, the starting temperature of this event is relatively low. The first possibility may be the most probable one.

Table 1. Assignment of the IR spectra of galactitol, sorbitol and mannitol and their VO^{2+} complexes (band positions in cm^{-1})[a]

Galactitol	Gal/VO	Sorbitol	Sor/VO	Mannitol	Mann/VO	Assignments
1460 s	1458 m	1497 sh	1497 sh	1486 sh	1501 sh	νCC, νCO, τCCCH
1437 sh	1425 m	1454 sh	1454 sh	1460 sh	1460 m	νCC, δCCO, δCCH, τHCCC
	1410 sh	1413 m	1413 m	1419 s	1414 m	νCC, νCO, δCCH, τCCCH
1372 s		1374 sh		1390 sh		νCC, νCO, δCCO, δCCH, δCOH, τCCCO
1352 sh	1344 sh		1356 sh		1352 sh	δOCH, δCCH, δCOH, νCO
		1310 m	1310 sh	1310 sh		
1287 m		1253 m		1285 s		δCOH, νCC, νCO
	1226 w			1249 sh	1233 w	
1208 m		1217 m	1217 m	1213 sh		
1112 vs			1120 sh			νCO, τCCCC, τCCCO, νCC
1082 vs	1082 vs	1094 vs	1078 vs	1084 vs	1069 vs	νCO, νCC
1038 vs	1038 vs	1058 vs	1022 sh	1022 vs	1027 sh	νCO, νCC
		1006 vs				
927 m		939 m	944 sh	924 m		νCC, τCCCO, τOCCH
	903 s		914 m		909 s	νVO
					897 sh	
860 m		880 s	893 sh	868 s		νCC, νCO, τCCCO, τHCCO
	774 w	784 sh	780 m	780 m	787 vw	νCC, νCO, τOCCC, τOCCH,

[a] vs, very strong; s, strong; m, medium w, weak; vw, very weak; sh, shoulder.

3.1.2. IR spectra. The IR spectra of the three oxovanadium(IV) complexes, and those of the free ligands in the most interesting spectral range, between 1500 and 750 cm^{-1}, are presented in Table 1. The assignments have been performed according to general Refs. 31–33 and by comparison with the previously investigated VO^{2+}/sugar complexes.[24–27]

As it can be observed, the deformational modes involving C–O–H bonds showed significant changes. These vibrations are shifted to lower frequencies or disappear upon complex formation and/or deprotonation. In particular, the intensities of the bands due to δCOH, νCC, νCO located in the 1287–1253 cm^{-1} range in the free ligands show an important diminution after coordination to the vanadium center. On the other hand, the V=O stretching frequency is found, as usual for this type of systems, at relatively low frequencies, with values that are similar to those measured in oxovanadium(IV) complexes of mono- and disaccharides.[28,34] As in other cases, this lowering of the ν(V=O) value suggests the presence of an increasing number of deprotonated OH-groups in the coordination sphere, generating a diminution of the V=O bond strength. Notwithstanding, hydrogen-bond interactions of free hydroxyls of the saccharide units with the oxo-group of the cation may also be partially responsible for the observed frequency lowering.[35]

Unfortunately, in these cases and with the spectroscopic data alone, it is not easy to determine which of the OH-groups of the polyalcohols are involved in bonding.

3.1.3. Solution studies

3.1.3.1. UV–vis spectra. The electronic absorption spectra of the three complexes measured in solution at pH 12, and by diffuse reflectance with the solids are shown in Table 2. They display the three-band pattern characteristic of the coordination of the VO^{2+} cation with pairs of deprotonated *cis*-OH-groups of saccharides.[28,34,36]

The diffuse reflectance spectra of the compounds are similar to the electronic spectra in aqueous solutions. Consequently, one may conclude that the coordination sphere is retained upon dissolution.

Table 2. Electron absorption and diffuse reflectance spectra of the three new complexes (band positions in nanometers)

Complex	Band positions (nm)[a]		
$Na_2[VO(galactitol)_2]\cdot H_2O$ (**1**)	702 (22.0)	512 (11.2)	420 (23.6)
	710	*515*	*430*
$Na_2[VO(mannitol)_2]\cdot H_2O$ (**2**)	708 (24.6)	504 (11.4)	422 (24.2)
	710	*510*	*430*
$Na_2[VO(sorbitol)_2]\cdot H_2O$ (**3**)	706 (18.5)	514 (8.9)	420 (19.2)
	710	*520*	*430*

[a] Numbers in parentheses are molar extinction coefficients in M^{-1} cm^{-1}. Italics indicate band positions in the diffuse reflectance spectra.

3.1.3.2. Visible spectral changes upon variation of pH. The different spectral patterns shown by the VO^{2+} cation with sorbitol (1:2 molar ratio) varying the pH from 3.13 to 12.10 by addition of NaOH can be observed in Figure 2. The colors of these solutions turn from light blue to intense blue, and then a gray precipitate is formed (pH 5). This precipitate is redissolved at pH 6 forming a brownish solution that develops into a green one at pH 11.0. The same behavior was observed for the other two polyols.

The two absorption bands observed at acidic pH values can evidently be related to the two lowest energy transitions ($b_2 \rightarrow e$ and $b_2 \rightarrow b_1$) expected from the well-known Ballhausen and Gray M.O. schema.[34,37] The third expected transition ($b_2 \rightarrow a_1$) becomes evident only at the highest pH values.

3.1.3.3. Stoichiometry of the complexes. The biological studies were carried out in culture media at pH 7.4. In order to establish the species present in these solutions spectrophotometric determinations of the complex stoichiometry have been performed. The experiments have been carried out in aqueous solution at pH 7.4 with different ligand-to-metal ratios ranging from 0.5 to 10.0. The absorbance of the lowest energy band of the electronic spectra was monitored in each case. For the three complexes the slope of the absorbance versus L/M ratio curves changes at L/M = 2 as it is illustrated in Figure 3 for the complex mannitol/VO. Even though spectral changes are observed when the pH decreases from 12.5 to 7.0, likely due to the interaction of the hydroxyl groups with the metal center or protonation of the ligands, the stoichiometry of the complexes is retained.

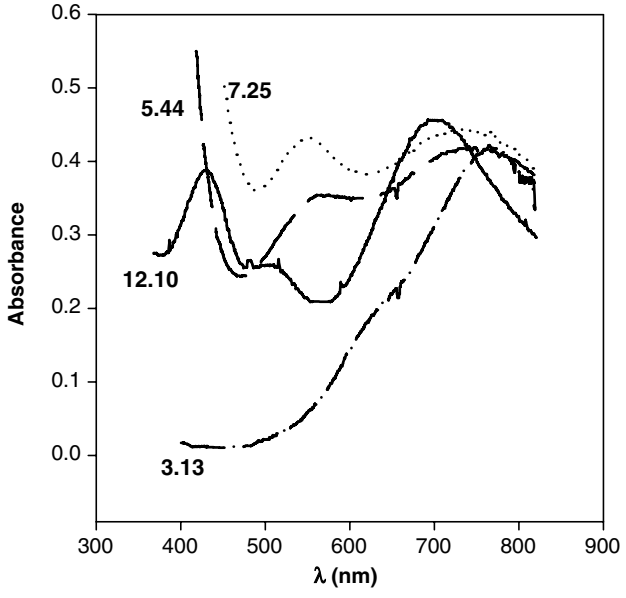

Figure 2. Spectral changes of an aqueous solution of oxovanadium(IV) with sorbitol (1:2 molar ratio) as a function of pH.

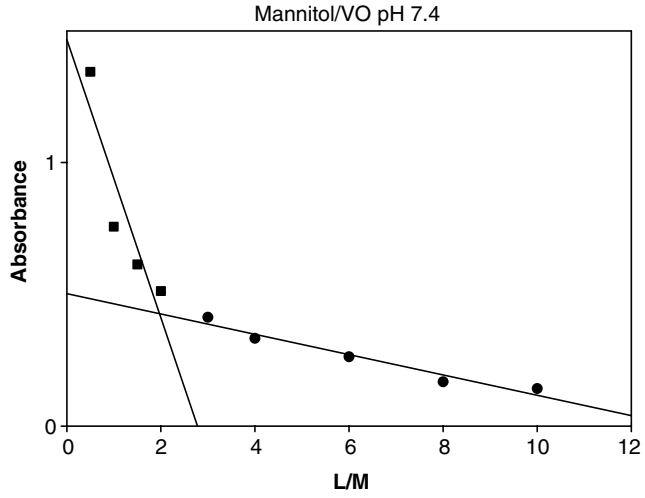

Figure 3. Spectrophotometric titration of VO^{2+} with mannitol at pH 7.4 under nitrogen atmosphere and at 708 nm. L/M = mannitol/VO. Absorbance in arbitrary units.

Table 3. Stability constants (h^{-1}) of $Na_2[VO(galactitol)_2]\cdot H_2O$ (**1**), $Na_2[VO(mannitol)_2]\cdot H_2O$ (**2**), and $Na_2[VO(sorbitol)_2]\cdot H_2O$ (**3**) in culture media at 37 °C

Sorbitol/VO^{2+}	Mannitol/VO^{2+}	Galactitol/VO^{2+}
6.83×10^{-3}	9.05×10^{-3}	10.17×10^{-3}

3.1.3.4. Stability studies. The stabilities of the complexes have been evaluated in culture media at 37 °C, under similar conditions to those of the biological studies. They were determined by measuring the variation of the UV–vis spectra with time. The lowest energy electronic absorption bands ($b_2\rightarrow e$) were monitored at 37 °C. Plots of $\ln A(t)$ versus t were linear at least for a half-reaction period and were first order in the concentration of the complexes. Under these conditions the stability shows the following order: sorbitol/VO^{2+} > mannitol/VO^{2+} > galactitol/VO^{2+} (cf. Table 3).

These results demonstrate that during the manipulation time of the samples before their addition to the culture media, a significant amount of the complexes remains unchanged.

3.2. Biological studies

3.2.1. Effect of the complexes on the osteoblast-like cell proliferation. As it was previously reported, vanadium compounds act as growth factor mimetic compounds in osteoblast-like cells in culture, one derived from a rat osteosarcoma (UMR106) and the other derived from mouse calvaria (MC3T3E1).[30] For this reason, we investigated the effects of the three new oxovandium(IV) complexes on the proliferation of nontransformed and tumor osteoblast-like cells in culture. Figures 4 and 5

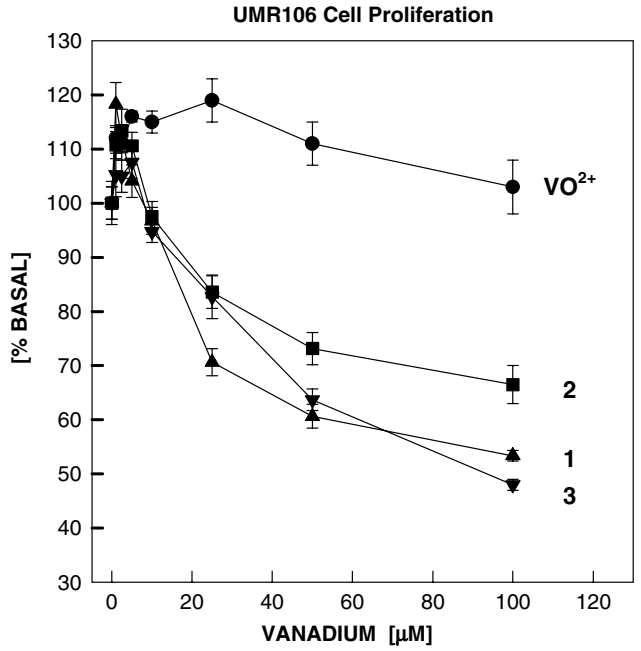

Figure 4. Effect of vanadium compounds on UMR106 cell proliferation: $Na_2[VO(L)_2]\cdot H_2O$ (L = galactitol (**1**), mannitol (**2**), and sorbitol (**3**)), and VO^{2+}. Cells were incubated in serum-free DMEM alone (basal) or with different concentrations of vanadium compounds at 37 °C for 24 h. Basal values are 5.2×10^4 cells/well. Results are expressed as % basal and represent the mean ± SEM, $n = 9$.

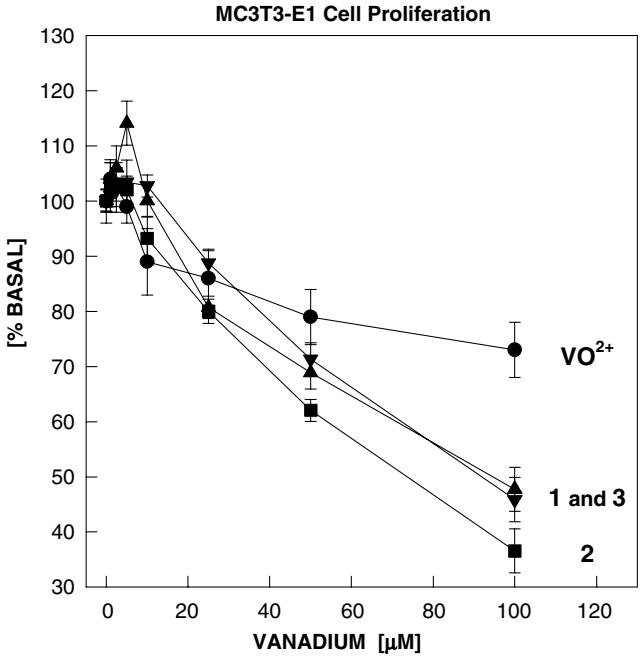

Figure 5. Effect of vanadium compounds on MC3T3E1 cell proliferation: $Na_2[VO(L)_2]\cdot H_2O$ (L = galactitol (**1**), mannitol (**2**), and sorbitol (**3**)), and VO^{2+}. The experimental conditions were similar to those of Figure 4. Basal values are 4.0×10^4 cells/well. Results are expressed as % basal and represent the mean ± SEM, $n = 9$.

show the effect of complexes (**1**–**3**) on the proliferation of UMR106 and MC3T3E1 osteoblast-like cells, determined by the crystal violet bioassay. After a 24-h culture, the complexes caused dose–response inhibition, except at 5 μM where they induced an increase of cell proliferation of ca. 15% over basal ($p < 0.05$). The inhibition produced by complex **2** was more potent in the normal osteoblasts at 100 μM than in the osteosarcoma cell line. On the other hand, complexes **1** and **3** have a similar behavior in both cell lines. On the contrary, two other complexes of the VO^{2+} cation, those with glucose (GluVO) and those with trehalose (TreVO) were more deleterious for the tumor osteoblasts, being good candidates for further investigation as antitumor drugs.[38,39] The same behavior was observed in another VO^{2+} complex with the nonsteroidal anti-inflammatory drug naproxen (NapVO), which was also more cytotoxic for the tumor than for the nontransformed osteoblasts.[39,40]

Moreover, the free ligands did not exert any effect on either cell line in the whole range of tested concentrations. In previous works, we have reported that the oxovanadium(IV) cation stimulated UMR106 proliferation in the same concentration range.[30,41] In MC3T3E1 cells, the cation was an inhibitory agent in the range of 10–100 μM.[42]

3.2.2. Effect of the complexes on osteoblastic differentiation. Alkaline phosphatase (ALP) specific activity is one of the markers of osteoblastic differentiation. Since the MC3T3E1 cells do not express measurable ALP levels after short culture periods (3 days), the effect of the complexes was not assessed in this cell line. This effect was measured only in the UMR106 tumor cells. As can be seen from Figure 6, the complexes (2.5–100 μM) significantly inhibited UMR106 cell differentiation in a dose-dependent manner. Considering the concentration that produced a 50% of inhibition (IC_{50}), it can be seen that complex **1** was the most potent (ca. 40 μM) and complexes **2** and **3** show similar potency (ca. 50 μM). Besides, the VO^{2+} cation is less potent, with $IC_{50} > 100$ μM.

3.2.3. Glucose consumption in UMR106 cells. Some vanadium compounds are of great interest for their insulin mimetic actions. We determined whether the complexes were able to stimulate glucose consumption in the tumor cells in culture. Complex **1** increased glucose consumption at a similar extent as VO^{2+} in this cell line at 10 μM. Figure 7 shows the results of different concentrations of the complexes and VO^{2+} on the glucose consumption in UMR106 cells. Compounds **2** and **3** do not show significant stimulatory effect in this concentration. On the contrary, the related compound, TreVO, a VO^{2+} complex with trehalose, has shown good insulin mimetic activity, increasing the consumption of glucose in a dose-dependent manner, with a maximum effect at a concentration of 25 μM (ca. 145% of basal).[38]

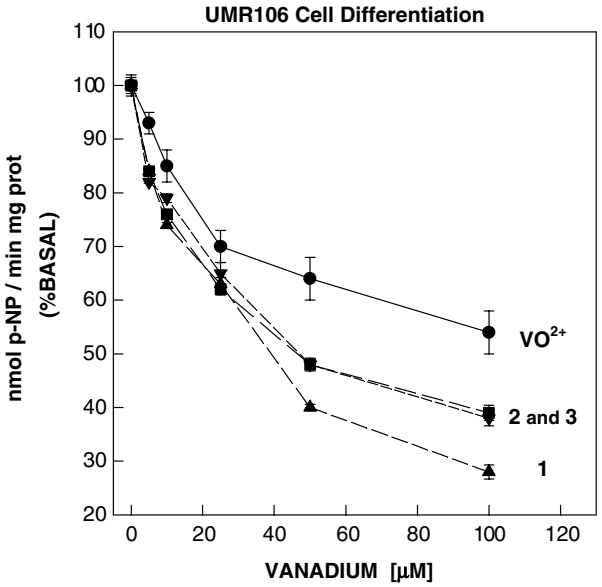

Figure 6. Effect of vanadium compounds on UMR106 osteoblast-like cell differentiation: $Na_2[VO(L)_2].H_2O$ (L = galactitol (**1**), mannitol (**2**), and sorbitol (**3**)), and VO^{2+}. Cells were incubated either in serum-free DMEM alone (basal) or with different concentrations of vanadium compounds at 37 °C for 24 h. Basal activity was 1.8 μmol pNP/min mg protein. Results are expressed as % basal and represent the mean ± SEM, $n = 6$.

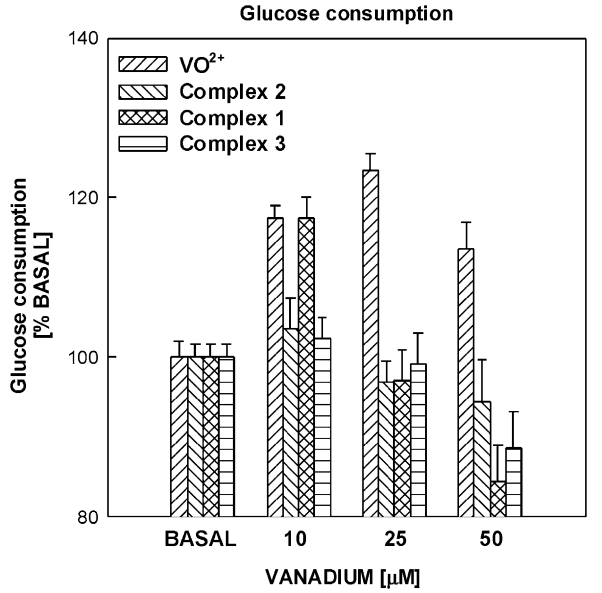

Figure 7. Effect of vanadium compounds on glucose consumption in UMR106 cells: $Na_2[VO(L)_2]\cdot H_2O$ (L = galactitol (**1**), mannitol (**2**), and sorbitol (**3**)), and VO^{2+}. Osteoblast-like cells were incubated either in serum-free DMEM alone (basal) or with different concentrations of vanadium compounds at 37 °C for 10 h. Basal glucose consumption was 2 μg/h/well. Results are expressed as % basal and represent the mean ± SEM, $n = 9$. Differences versus basal: $^{*}p < 0.001$.

4. Conclusions

1. Three new complexes of oxovanadium(IV) with linear polyols were prepared and characterized, both in solution and in the solid state.
2. The complexes are formed at pH $\geqslant$ 12. Then, the interaction of the metal with the ligands occurs through deprotonated OH groups.
3. The effects of the different complexes on cell proliferation are dependent on the nature of the complexes and on the cellular type. All the complexes inhibited cell differentiation in tumor osteoblasts.
4. At 10 μM the galactitol complex (**1**) stimulates glucose consumption to the same extent as the VO^{2+} cation. The advantage of the oxovanadium(IV) complexation with galactitol may be the better solubility of the complex than that of the metal cation at physiological pH values.
5. Complex **1** behaves as a proliferative agent on the normal osteoblasts at low doses. Together these results indicate that among the three complexes of this series $Na_2[VO(galactitol)_2]\cdot H_2O$ deserves a more detailed investigation in future studies.
6. The free polyalcohols did not show any effect on the investigated biological parameters.

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