



Synthesis, spectroscopic study and anticancer activity of a water-soluble Nb(V) peroxo complex

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ABSTRACT

We synthesized, characterized and studied the anticancer properties of a new water-soluble peroxo niobium complex ($K_3[Nb(Asc)(O_2)_3] \cdot 4H_2O$ ($Asc = \text{ascorbate anion } C_6H_6O_6^{2-}$)), as well as that of ascorbic acid, in human leukemic cells. The complex was synthesized and characterized by elemental, IR, Raman, thermogravimetric analysis, detailed NMR and mass spectra analysis. The cytotoxic activity of the complex on HL-60 and K562 human leukemia cell lines has been investigated by assessing vital cellular mechanisms, such as the metabolic activity (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MTT) and the proliferation capacity (growth curves) of leukemia cells, as well as the structural integrity of cell membrane (trypan blue assay). The complex exerts an increased antiproliferative effect primarily on HL60 human leukemia cells, compared to ascorbic acid alone, as well as an inhibitory effect on necrosis caused by ascorbic acid. Its effect on K562 cells concerns mainly its inhibitory effect upon cell necrosis induced by ascorbic acid alone. Our results support a concentration- and time-dependent enhanced antileukemic effect of the complex, suggesting its significance as a promising tool in the confrontation of leukemia.

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1. Introduction

Metal complexes coordinated to ascorbate ligands have received great attention because of their biological activity and applications [1–4]. Ascorbate plays a crucial role as an antioxidant molecule in hydroxylation reactions that determine interactions and functioning of thousands of cellular proteins and in redox reactions that are involved in protection against oxidative damage arising from the treatment of many diseases, including cancer [5,6]. There is increasing evidence that ascorbic acid exhibits cytotoxic effect and anticancer activity against melanoma, human leukemia, neuroblastoma and tumor ascite cells [7,8]. *In vitro* studies using ascorbate at pharmacologic concentrations only achievable by intravenous administration show that ascorbate is selectively toxic for some types of cancer but not for normal cells [9]. In addition, it has been proposed that combining ascorbate with other vitamins or metal complexes exhibits a synergistic anticancer activity [10].

It is also known that the most important chemical characteristics of ascorbic acid are its redox properties (Schemes 1 and 2). The routes of

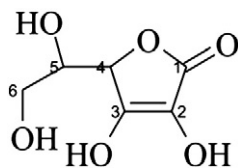
degradation of ascorbic acid depends both on pH and on the presence of metal ions and involve its oxidation to dehydroascorbic acid, followed by hydrolysis that leads to the opening of the lactone ring to produce 2,3-diketogulonic acid (DKG). Further decomposition, in alkaline solutions, produces oxalic acid and trihydroxybutyric acid (L-threonic acid) in both aerobic and anaerobic conditions [11–14]. Particularly, when AscA is exposed to H_2O_2 the products formed are proposed to be 4,5,6-tetrahydroxy-2,3-diketohexanoic acid (THDH) and threonic acid [15]. These data imply that the instability of the ascorbate anion to oxidation reduces the possibility of isolating metal–ascorbate complexes in the solid state [11–13,16]. However, metal chelate formation can occur and ascorbate complexes can be synthesized and characterized [17–22].

The chemistry of the early transition metal peroxo complexes has received special attention due to their importance in a variety of biological, pharmaceutical and industrial processes [23–31]. In previous studies, peroxomolybdate and peroxotungstate were found to mimic the biological actions of insulin [32]. General patterns of structure and reactivity are evident for the group 5 in the highest oxidation state and the group 6 hexavalent metal peroxo complexes. These complexes take part in the basic biochemical processes in cell systems [33]. Oxovanadium(IV) complexes of 1,10-phenanthroline (phen) have been examined for their cytotoxic activity against human ovarian cancer cell lineal and for the apoptosis inducing [34] and peroxovanadium complexes containing various heteroligands represent antitumor activity using L1210 murine leukemia test systems quite different from the vanadocene complexes [35].

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Scheme 1. Structure of the ascorbic acid.

We have recently synthesized and reported the crystal structure of the triperoxo niobate complex with 2-quinaldic acid ($(\text{gu})_2[\text{Nb}(\text{O}_2)_3(\text{quin-2-c})]\cdot\text{H}_2\text{O}$ (where $\text{gu}^+ = \text{guanidinium ion}, \text{CN}_3\text{H}_6^+$) [36]. The present work describes the results of a comparative study of the reaction of ascorbic acid with niobium pentachloride and proposes a novel triperoxo niobate complex containing ascorbic acid. The isolated complex was studied via elemental analysis, TGA and DTA (thermogravimetric and differential thermal analysis), IR, Raman, ESR, NMR spectroscopies and mass spectrometry. The mechanism of the reaction of the peroxo ascorbate complex in aqueous solution and the kind of the degradation products were also investigated (via ^1H and ^{13}C -NMR, ESI-MS). The anticancer activity of the title compound was estimated against HL-60 and K562 human leukemia cells.

2. Experimental

2.1. Chemistry

All chemicals were used as received. Ascorbic acid ($\text{AscA } \text{C}_6\text{H}_8\text{O}_6$), NbCl_5 , KOH and all solvents were purchased from Aldrich Chemical Co. ^1H -, ^{13}C - and 2D-COSY (two dimensional correlation spectroscopy) NMR spectra were recorded on a Varian UNITY plus operating at 300 MHz spectrometer or a Bruker Avance operating at 500 MHz. Cyclic voltammograms were recorded on an AFCBP1 Biopotentiostat (Pine Instrument Company) and a standard three-electrode assembly (working: glassy carbon disk, auxiliary: platinum wire, reference: Ag/AgCl , supporting electrolyte: LiCl (1 M), scan rate: 100 mV s^{-1}). UV-Vis spectra were recorded with 1-cm cuvette on a Hitachi U-2000 spectrophotometer. IR spectra were recorded in a KBr pellet on a Perkin-Elmer 880 IR spectrophotometer. High-frequency Raman spectra were recorded with a Perkin-Elmer GX Fourier transform spectrometer with a diode pumped Nd:YAG laser at 1064 nm as the excitation source. Elemental analyses were performed using a Perkin-Elmer 240B elemental analyzer. Potassium determination was carried out with atomic absorption spectrometry. Thermogravimetric and differential thermal analysis were carried out on a Mettler Toledo S R apparatus in N_2 atmosphere (temperature range: $40\text{--}1200^\circ\text{C}$; heating rate: $20^\circ\text{C}/\text{min}$). ESI-MS spectra were recorded with the Fisons Instruments VG BIO-Q quadrupole mass spectrometer, with the solution injected via a Rheodyne injector valve directly through the 10 μL loop in the OCP-V4.06 QUATT 4000 spectrometer. Nitrogen was

the nebulizing and drying gas. Focus voltage was set to 10 V, the capillary voltage at 3.00 kV and HV lens voltage at 0.46 kV. 20–50 spectra combination was required for acceptable S/N ratio. Sample flow rate was 3 $\mu\text{L}/\text{min}$, with CH_3CN or CH_3OH used as mobile phase while the electrostatic ion source operated at atmospheric pressure. The fast-atom bombardment (FAB) mass spectrometric measurements were performed on a Micromass 70-SE magnetic sector in negative ion mode with the dynamic ion source running in static mode at -8 kV using a MNOBA matrix and calibrated to 2000 mass using caesium iodide. EPR spectra in solid state (298 and 10 K) were recorded on a Bruker ER-200D-SRC X-band spectrometer interfaced to a personal computer and equipped with an Oxford ESR 900 cryostat, an Anritsu MF76A frequency counter and a Bruker 035 N NMR gaussmeter. Electric conductance measurements were carried out with a WTW model LF 530 conductivity outfit and a type C cell that had a cell constant of 0.996. This represents a mean value calibrated at 25°C with potassium chloride.

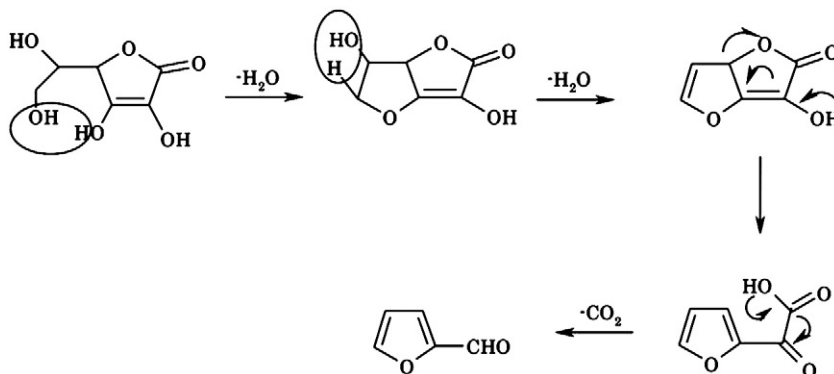
2.2. Synthesis of the complex $\text{K}_3[\text{Nb}(\text{Asc})(\text{O}_2)_3]\cdot 4\text{H}_2\text{O}$

A methanol solution of ascorbic acid (0.175 g, 1 mM in 10 mL) was added slowly to a methanol solution containing 0.27 g, 1 mM of NbCl_5 (in 10 mL) under continuous stirring. To the resulting yellow reaction mixture a methanol solution of base KOH 20 M (40 mL) was added in sufficient excess to provide the necessary counterions and to ensure the formation of the peroxo groups. The addition of base yielded immediately a yellow precipitate that was isolated by filtration and air-dried. The synthesis was carried out in the presence of air. Yield: 70%. Anal. Calcd for the complex $\text{C}_6\text{H}_{14}\text{K}_3\text{NbO}_{16}$ ($\text{Mw} = 552.37$): C, 13.05; H, 2.55; K, 21.23; Nb, 16.82. Found: C, 12.91; H, 2.90; K, 20.18. Potassium was determined by atomic absorption spectroscopy. According to the method, the analysis value for K is acceptable.

The complex was found to be stable in air. It is soluble in water, slightly soluble in DMSO and insoluble in alcohols, acetonitrile and dichloromethane. The molar conductance measurements carried out in water being in agreement with the charge of the complex. Various attempts made towards crystallization of the complex were unsuccessful. The electronic spectrum of the complex shows a band at 257 nm which indicates the formation of the peroxo niobium complex. The yellow color of the solution and the UV absorption peak are in accordance with other peroxo niobium complexes of the literature [37].

2.3. Cell culture conditions

HL-60 (peripheral blood human promyelocytic leukemia), U937 (human histiolytic lymphoma) and K562 (human chronic myelogenous leukemia) cell lines were maintained in RPMI 1640, supplemented with 10% fetal bovine serum (FBS), 200 U/mL penicillin, 100 $\mu\text{g}/\text{mL}$ streptomycin, 0.3 g/mL L-glutamine and 2 mM NaHCO_3 in



Scheme 2. Possible reaction process for formation of furfural.

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