New Lanthanide Complexes with Antioxidant Activity

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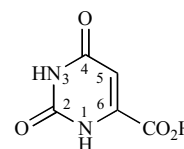
Abstract: The cerium (III), lanthanum (III) and neodymium (III) complexes of 5-aminoorotic acid were synthesized and their structures were determined by means of theoretical study, spectral and physicochemical analysis. Significant differences in the spectra of the complexes were observed as compared to the spectra of the ligand. The ligand and the complexes were tested for the antioxidant activity. The results obtained indicate that the tested compounds exerted a considerable antioxidant effect upon the evaluated radicals' formation in the blood plasma of Wistar rat.

Key Words: Lanthanide (III) complexes, 5-Aminoorotic acid, antioxidant activity.

INTRODUCTION

Orotic acid (2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylic acid, vitamin B_{13}, H_3L') and its coordination complexes have been an area of great activity, research in this area ranging from bioinorganic to pharmaceutical and materials chemistry. Orotic acid (vitamin B_{13}), Fig. (**1**) and its salts play an important role in biological systems as precursors of pyrimidine nucleosides [1] and are found in cells and body fluids of many living organisms [2-4]. These compounds are applied in medicine as biostimulators of the ionic exchange processes in organisms, and different metal complexes of orotic acid were studied [5-9]. There is also a great interest in orotic acid in relation to food protection and nourishment research [10-12]. The crystal and molecular structure of orotic acid was determined by Takusawaga and Shimada [13], and Mutikainen [14] studied the crystal structure of metal complexes of orotic acid. Because of the importance of orotic acid and its metal complexes in living systems, a reliable assignment of their vibrational spectra is a useful basis in the study of their interactions with other chemical species present in the biological milieu. The orotic acid molecule is related to the molecules of uracil or thymine. Different studies on these type of molecules were done by vibrational spectroscopy [15, 16], as well as normal coordinate analysis (NCA) and *ab initio* calculations [16-27]. The results of these studies may help in the assignment of the spectra of orotic acid.

Metal-ion complexes of orotic acid, Fig. (**1**) and its substituted derivatives continue to attract attention because of its multidentate functionality and its pivotal role in bioinorganic chemistry. It is an interesting multidentate ligand capable of coordinating to metal ions through the nitrogen atoms, the two carbonyl oxygens and the carboxylate oxygens. Existing studies of its coordination complexes demonstrate that it occurs as a dianion coordinating often *via* the N1 atom and

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Fig. (1). The structure of orotic acid (H_3L').

the carboxylic acid group so forming a five membered chelate ring. Despite its polydentate nature only a few polymeric complexes of orotic acid have been observed.

The multifunctionality of the hydroorotate, $H_2L'^-$, and orotate, HL'^{2-}, anions offer interesting possibilities in crystal engineering as a versatile ligand for supramolecular assemblies. Metal ion coordination may occur through the two N atoms of the pyrimidine ring as well as the two carbonyl oxygen atoms or the carboxylic group, which results in a multi-faceted coordination chemistry. The coordinated orotate anions exhibit a ligand surface with double or triple hydrogen-bonding capabilities, depending on the metal coordination mode, and has thus a potential to adopt several modes of interligand hydrogen bonding to allow the formation of extended, self-assembled structures.

Thus, besides the biological relevance, the orotic acid and its anions $H_2L'^-$, HL'^{2-} and L'^{3-} are interesting multidentate ligands as they can coordinate through the two pyrimidine nitrogen atoms, the two carbonyl oxygens and the oxygens from the carboxyl group. The equilibrium composition of the reactant mixture and thus the solution pH are critical factors which determine the mode of coordination. Between pH 3 and 9, orotic acid exists mainly as readily-coordinating mono-deprotonated $H_2L'^-$ (the carboxylic group has pK_a 2.07). The literature lists many reports on the coordinating preferences of the orotate moiety in metal complexes. It was found that in solutions with neutral or slightly acidic pH, Cu(II), Zn(II), Co(II), Mn(II), Fe(III), Cr(III), VO(II), Cd(II), Hg(II) and Ag(I) are coordinated through the carboxylate group, while Ni(II), Co(II) and Cu(II) are coordinated through the carboxylate end and the adjacent N1 [28-31]. Bidentate binding through N1 and the carboxylate group was observed by sev-

eral crystal structure determinations [32-36]. In the complexes [Co(HL')(OH)(H$_2$O)(NH$_3$)]n and [Ni(HL')(OH)(H$_2$O)$_2$ (NH$_3$)]n the orotate anion bridges the metal ions through the carboxylate and the N1 and O2 atoms, forming one-dimensional polymeric chains [37]. A recent reinvestigation of nickel(II) orotate pentahydrate [Ni(HL')(H$_2$O)$_4$]·H$_2$O by modern diffraction, spectroscopic and theoretical methods revealed novel structural features [38].

Despite the interest in orotate metal complexes, the coordination chemistry of the derivatives of orotic acid has received rather scant attention. One of these derivatives is 5-aminoorotic acid (5-amino-2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylic acid, H$_4$L) which has relatively unknown coordination chemistry [6].

We have recently synthesized lanthanide complexes with a number of biologically active ligands, and we reported their significant cytotoxic acitivity in different human cell lines [39-48]. These promising results prompted us to search for new lanthanide complexes with 5-aminoorotic acid, see Fig. (**2**). Thus, the aim of this work was to synthesize and characterize complexes of cerium (III), lanthanum (III) and neodymium (III) with 5-aminoorotic acid and to determine the antioxidant properties of these complexes.
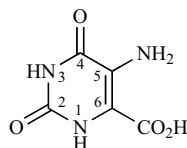

Fig. (2). The structure of the ligand 5-aminoorotic acid (H$_4$L).

RESULTS AND DISCUSSION

Chemistry

The newly synthesized complexes were characterized by elemental analysis. The metal ions were determined after mineralization. The water content in the complexes was determined by Karl Fisher analysis. The nature of the complexes was confirmed by theoretical and spectroscopic studies.

The data of the elemental analysis of the compounds obtained serving as a basis for the determination of their em-pirical formulas and the results of the Karl Fisher analysis and thermal analysis are presented in Table **1**.

IR Spectra of the Compounds

The mode of bonding of the ligand to Ce(III), La(III) and Nd(III) ions was elucidated by recording the IR spectra of the complexes as compared with those of the free ligand (Table **2**).

The monoanions of orotic acid and its derivatives show a preference for monodentate O$_{carboxylate}$ coordination to Zn(II) [49]. In the new three lanthanide complexes with the anion of 5-aminoorotic acid as ligand, monodentate coordination *via* one of the carboxylate oxygen atoms is suggested, which is in accordance with the literature data [49].

Tentative assignments of selected IR bands of the complexes are given in the Table **2**. The assignments have been given by studying literature reports [49] and comparing the spectra of the ligand and the metal complexes. As a general remark we must emphasize that some stretching and deformation modes are coupled, so that the proposed assignments should be regarded as approximate descriptions of the vibrations.

In the $\nu(OH)_{water}$ region the spectra of Ce(III), La(III) and Nd(III) complexes show one medium band at 3442, 3448 and 3449 cm^{-1}, respectively, attributed to the presence of coordinated water. This band overlaps with the $\nu_{asym}(NH_2)$ band [49].

In the spectrum of Ce(III) complex the bands due to the $\nu_{asym}(NH_2)$, $\nu_{sym}(NH_2)$, $\nu(C^2O)$ and $\nu(C^4O)$ vibrations appear [49] at 3442, 3340, 1718 and 1684 cm^{-1}, respectively; in the spectrum of La(III) complex the bands due to the $\nu_{asym}(NH_2)$, $\nu_{sym}(NH_2)$, $\nu(C^2O)$ and $\nu(C^4O)$ vibrations appear at 3448, 3340, 1717 and 1684 cm^{-1}, respectively and in the spectrum of Nd(III) complex the bands due to the $\nu_{asym}(NH_2)$, $\nu_{sym}(NH_2)$, $\nu(C^2O)$ and $\nu(C^4O)$ vibrations appear at 3449, 3338, 1719 and 1687 cm^{-1}, respectively. The absence of large systematic shifts of these bands in the spectra of the complexes implies that there is no interaction between the amino nitrogen or the carbonyl oxygens and the lanthanide (III) ions. It was not possible to differentiate clearly the spectroscopic behaviour of the different carbonyl groups of these complexes [49].

Table 1. Elemental Analysis of Ln(III) Complexes of 5-Aminoorotic Acid

Compound Formulae	Calculated/Found C	H	N	H$_2$O	Ln
Ce(AOA)$_3$.3H$_2$O	25,57/ 25,38	2,56/ 2,78	17,90/ 17,52	7,67/ 7,34	19,89/ 20,10
La(AOA)$_3$.3H$_2$O	25,60/ 26,14	2,56/ 3,24	17,92/ 17,48	7,68/ 7,25	19,77/ 20,06
Nd(AOA)$_3$.3H$_2$O	25,42/ 26,62	2,54/ 3,15	17,80/ 17,37	7,63/ 7,28	20,34/ 20,15

HAOA = C$_5$N$_3$O$_4$H$_5$ AOA= C$_5$N$_3$O$_4$H$_4^-$

Table 2. Results of the IR-Spectra of Ln(III) Complexes of 5-Aminoorotic Acid

ν, cm^{-1}	AOA	Ce(AOA)$_3$.3H$_2$O	La(AOA)$_3$.3H$_2$O	Nd(AOA)$_3$.3H$_2$O
νOH	-	3442	3448	3449
$\nu_{asym}NH_2$	3457	3442	3448	3449
$\nu_{sym}NH_2$	3322	3340	3340	3338
νNH	3196	3258	3263	3251
		3166	3172	3169
$\nu C^2=0$	1680	1718	1717	1719
$\nu C^4=0$	1667	1684	1684	1687
$\nu_{asym}COO^-$	-	1636	1637	1644
νC=C	1566	1555	1556	1556
	1604	1672	1672	1673
νC-N	1436	1499	1499	1500
	1457			
$\nu_{sym}COO^-$	-	1424	1424	1424
δNH	1405	1390	1389	1391

The $\nu_{asym}(CO_2)$ and $\nu_{sym}(CO_2)$ bands are at 1636 and 1424 cm^{-1} for Ce(III) complex, at 1637 and 1424 cm^{-1} for La(III) complex, at 1644 and 1424 cm^{-1} for Nd(III) complex, respectively; the participation of the monodentate carboxylate group of 5-aminoorotic acid in hydrogen bonds or/and the anionic character of the aminoorotate ligand may be responsible for the observed low Δ value (210-220 cm^{-1}) [49].

The data of the IR analysis are presented in Table **2** and they are in agreement with the compositions of the complexes.

Theoretical Investigations

The chemical composition of a complex involved three water molecules associated in a Me(AOA)$_3$.3H$_2$O. The FT-IR data indicated a disappearance of the characteristic signal for -NH$_2$ group in the spectrum of the complex. Molecular weights indicated a strong possibility for participation of a deprotonated HAOA in the complex, along with three H$_2$O molecules. Therefore, it was proposed that the Me(III) complex might contain three anions AOA$^-$ and 3 water molecules, coordinatively bonded with the central cation. The exact formula of an anion was predicted from the calculations of the ionization pathways of the free HAOA. Other basic requirements for the complex were to have minimum standard heat of formation, and minimum steric energy.

As some of the parameters of the Ce(III), La(III) and Nd(III) ions are unknown, the Fe(III) ion was selected as a model for the Me(III). The coordination of three anions AOA$^-$ (formed by dissociation of the O(1)-H bond), and three H$_2$O in the first coordination sphere of Fe(III) with symmetry O_h^6, was examined, as a possibility for the formation of the Me-AOA complex.

Ionization of the HAOA Molecule

The partial atomic charges of the HAOA molecule were calculated using the MM2-subrutine of the Chem3D program package. Because the positive partial atomic charges of the N(1)-H group, Fig. (**3**), it was proposed that the ionization of HAOA molecule might proceed by braking the O-H bond in the CO(OH) group, followed by formation either of a Zwitter-ion, or of anion HAOA$^-$, Fig. (**4**).
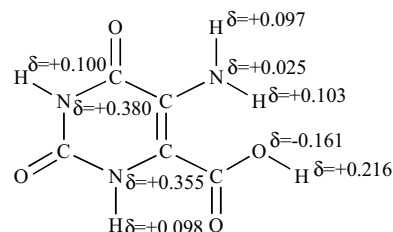
Fig. (3). Geometry and partial atomic charges (δ) of atoms in the free neutral HAOA molecule.

As seen in Table **3**, the formation of the anion by dissociation of the O(1)-H bond is an energetically favorable transformation of the free HAOA molecule. This would be expected at physiological pH (7.4 – 7.5). The steric energy minimization indicated that some Zwitter-ions might be expected too (Table **3**), along with HAOA molecules, in a solution.

On the basis of data from the MOPAC and MM2 calculations, it was proposed that in a solution at pH>7, the HAOA molecule might form either a Zwitter-ion, or dissociate into an anion, by braking the O(1)-H bond. It was proposed, that
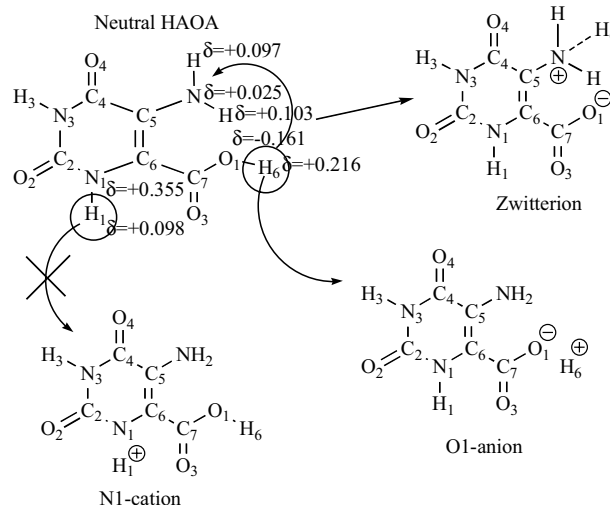
Fig. (4). Ionization pathways of the free HAOA molecule.

at conditions stabilizing the HAOA molecule, the formation of a Zwitter-ion might be not neglected.

Construction and Optimization of the Me(III)-AOA-Complex

The complex was constructed from three basic elements, Fig. (**5**): octahedrally coordinated metal cation Me^{3+}, Fig. (**5-a**); three anions AOA^-, Fig. (**5-b**); and three water molecules, Fig. (**5-c**).

The initial topology of the complex was as illustrated in Fig. (**6**). The first coordination sphere of the cation Me(III) was an octahedron. Molecules and anions were positioned in the centers of the walls of this octahedron, water molecule and anion opposing each – other. This topology resulted in a stable and symmetrical construction. Then, the Standard heat of formation of this complex was calculated using the AM2 version (open shell restriction) of the MOPAC subroutine within the Chem3D software package. The geometry and topology of the model complex were in agreement with the absence of the well characteristic signal for the –NH_2 group in the IR spectra, Fig. (**7**). The application of the MOPAC subroutine suggested another topology of the Me(AOA)$_3$.3H$_2$O complex, Fig. (**6-b**). This complex, Fig. (**8**), fulfilled all requirements of the model, giving standard heat of formation ΔH^0_{298}=-539.94 kcal/mole and minimum steric energy E_{min}=-628.93 kcal/mole.
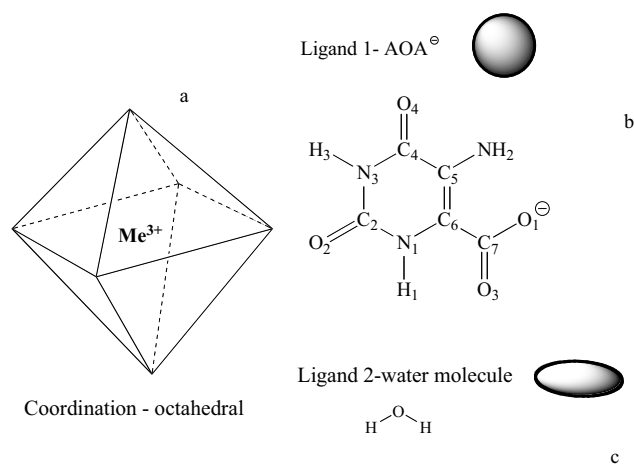

Fig. (5). Basic building blocks of the [Me(AOA)$^-_3$.3H$_2$O] complex: octahedrally coordinated Me(III) cation (a), anion AOA$^-$ (b), and H$_2$O molecule (c).

Pharmacology

The screening performed revealed that all of the evaluated lanthanide complexes of 5-aminoorotic acid exerted antioxidant effects, as depicted on Figs. (**9-10**).

The *in vitro* free radicals formation in a rat's blood plasma was monitored by using the ability of the light yellow

Table 3. Standard Heat of Formation (ΔH^0_{298}, kcal/mole) and Minimum Steric Energy (E, kcal/mole) of the Free HAOA Molecule, the AOA(O1)$^-$ Anion and the Zvitter-Ion

Specimen:	ΔH^0_{298}, kcal/mole	E_{min}, kcal/mole
Free HAOA molecule	-113.65	-0.22
AOA (O1) anion	-181.93	-2.25
Zwittwer-ion	-113.65	-83.94
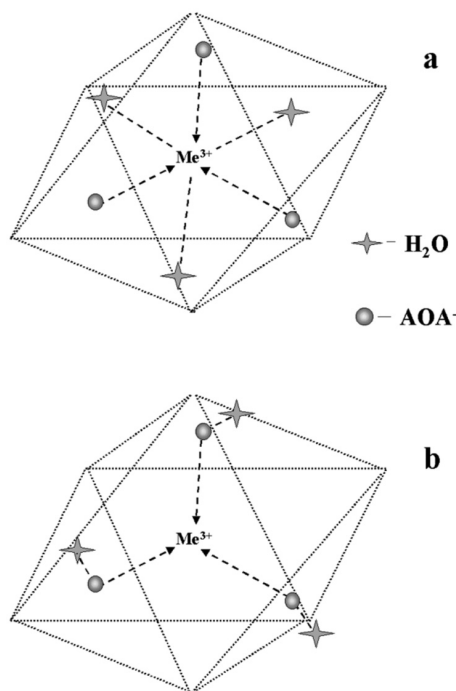
Fig. (6). Input (a) and output (b) topology of the complex of cation Me(III), three anions AOA⁻, and three water molecules.

salt 3-(2,5-dimethyl-2-thiazolyl(-2,5-diphenyl)-2H-tetrazolium bromide (named also MTT) to transform itself into an intensively purple formazan, if interacting with free radicals and Reactive Oxygen Species (ROS). The intensity of this purple coloration is proportional to the amount of free radicals and ROS formed at given amount of time, in the solution. If a substance (salt, ion, molecule or complex) can exchange electrons or protons with free radicals and/or ROS this way transforming them to chemically stable species, then it acts as antioxidant. In presence of antioxidant, a diminished overall free radical formation in a tissue will be detected, compared with the same tissue if the substance is being omitted. In Fig. (**9**) it was seen, that all lanthanide complexes possessed antioxidant effects. If comparing data for the effects of the 10 minutes incubation, the antioxidant activities of the lanthanide complexes was lower than this of the free ligand HAOA. Among the lanthanide complexes, the antioxidant properties decreased in the order: HAOA (the free ligand)>>CeAOA>LaAOA>NeAOA. The relative difference between antioxidant activities of the strongest (HAOA) and weakest (NdAOA) antioxidant in the group after 10 minutes incubation was almost 45%. After one hour on incubation, the antioxidant effects of all samples dramatically increased compared to the 10 minutes incubation, these of HAOA and CeAOA being almost identical (HAOA≥CeAOA>LaAOA≥ NdAOA). The relative difference between antioxidant activity of the strongest and the weakest antioxidant in the group after 1 hour of incubation was at about 15%. These differences might related with the ability of the complex to decompose (slower or faster) in the biological environment, and further antioxidant action of the ligand alone in the solution. This proposition was checked by screening of the rela-

tive change of the MTT-formazan with time, in presence of Xanthine, in blood plasma, Fig. (**10**), with no incubation period. When plotting the formazan formation versus time, a linear relationship between these two parameters was observed for the plasma alone, and in presence of HAOA and NdAOA as well. This linearity was lost if the formazan formation was monitored in the blood plasma contacting with LaAOA and CeAOA. Moreover, in the first two minutes these complexes showed some pro-oxidant activity. This kind of behavior can be related with the fast decomposition of the complex and further pro-oxidant action of the free lanthanide cations, probably due to changes in their positive charge. After exhausting their pro-oxidant activity, the antioxidant activity of the ligand was predominant. Evidently, more complicated mechanisms of antioxidant activity than a simple scavenging of free-radicals might be present, especially in the case of La and Ce. It is difficult to explain these observations on the basis of the experiment shown here alone.

Taken together the results from the antioxidant screening give us reason to conclude that the lanthanide (III) complexes with 5-aminoorotic acid, being very active antioxidant agents necessitate further more detailed pharmacological evaluation.

In conclusion, the complexes described above demonstrate once more the versatility of the 5-aminoorotate ligand, which adopts different coordination modes. The different charge and co-ordination mode of the ligand have a major effect on the supramolecular structures adopted by the complexes. From previous results [49], and this work, it is clear that the nature of 5-aminoorotic acid makes its various anionic forms versatile ligands for use with a variety of metals and for a variety of objectives/advantages, including variable coordination modes, high-nuclearity aggregate formation and/or linking of aggregates into polymeric arrays. Thus, we believe that H_4L has great potential as a generally useful new polyfunctional ligand in metal chemistry and it will prove attractive to a variety of coordination chemists.

CONCLUSIONS

The coordination ability of the biologically active ligand 5-aminoorotic acid has been proved in complexation reaction with cerium (III), lanthanum (III) and neodymium (III) ions. The elemental analysis confirmed the compositions of the compounds. Theoretical and IR- spectral analysis of the ligand and its lanthanide (III) complexes confirmed the suggested coordination of the ligand through oxygen atom of the the carboxyl ion.

In our hands the new lanthanide complexes under investigation exhibited antioxidant effects in tested concentrations. The screening performed revealed that all of the lanthanide complexes evaluated exerted pronounced effects. The antioxidant activity of the complexes investigated seems to depend on two major factors:

1) The strength of the bond between the ion and the ligand in the complex, especially if the ligand is the carrier of the antioxidant activity detected. As strong the bond is, as less the decomposition of the complex will be, as weak the antioxidant effect.
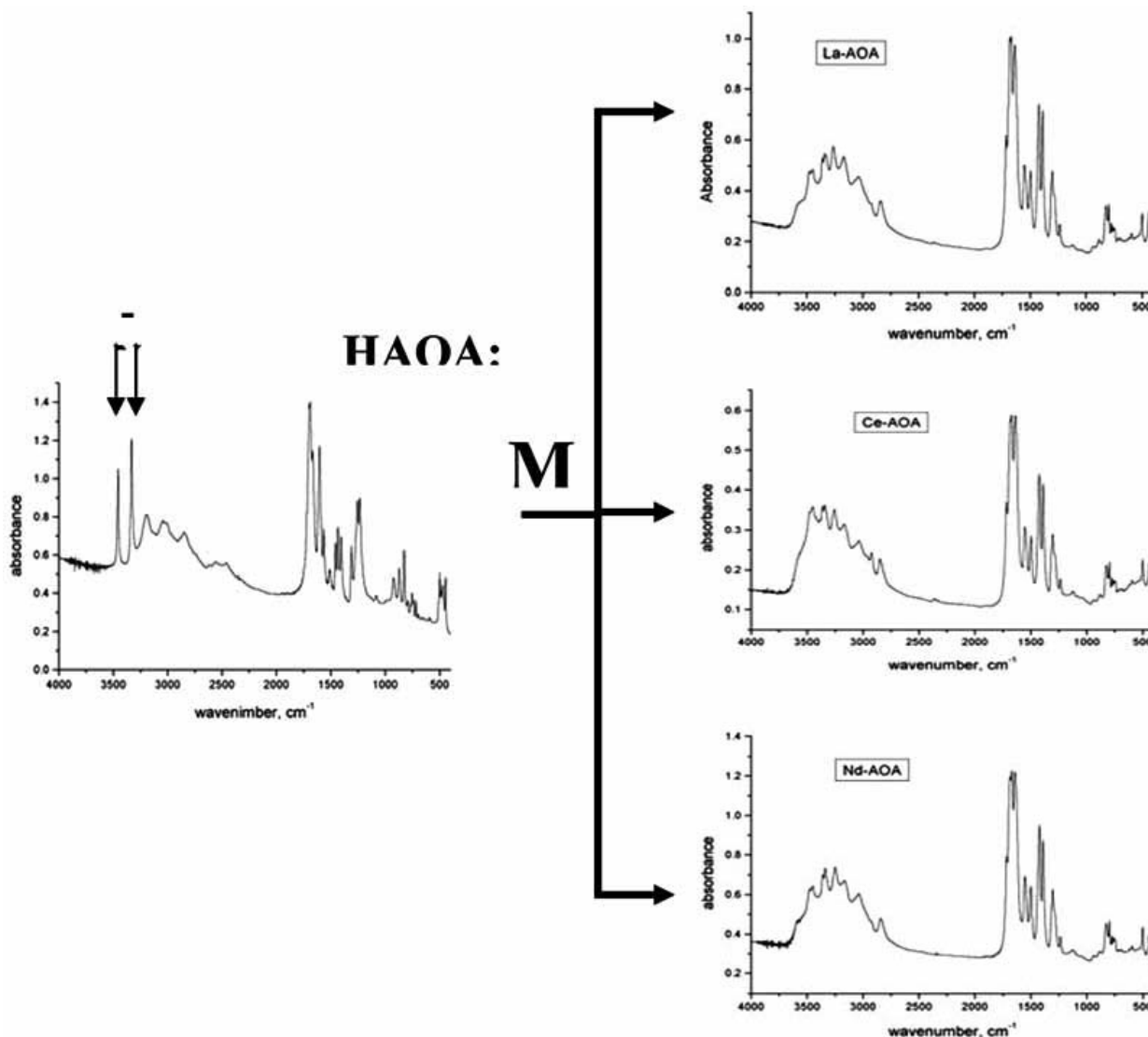
Fig. (7). IR spectra of the ligand and lanthanide complexes.

2) The capability of the cation to change its positive charge. It is well known, that cations which can vary their positive charge in a biological environment initiate free radical chain reactions, and show pro-oxidant properties. As easy a cation is capable to vary its charge, as strong pro-oxidant it is. In our experiment we investigated cations with different abilities to exchange electrons with the environment, and with different abilities to decompose their complexes with 5-aminoorotic acid, in a biological solution (blood plasma). The exact explanation of the antioxidant properties observed, is very complex. This is one of the assumptions we made from out preliminary investigations [50].

Taken together the results from the performed screening give us reason to conclude that the lanthanide (III) complexes with 5-aminoorotic acid, being active antioxidant agents necessitate further more detailed pharmacological evaluation. According to our expectations, these results confirmed once again our previous observations on the antioxidant properties of cerium (III), lanthanum (III) and neodymium (III) complexes with other biologically active ligands [50].

EXPERIMENTAL SECTION

Reagents

The compounds used for preparing the solutions were Merck products, p.a. grade: $Ce(NO_3)_3.6H_2O$, $La(NO_3)_3.6H_2O$ and $Nd(NO_3)_3.6H_2O$. 5-Aminoorotic acid (H_4L, Fig. (**2**) was used for the preparation of the metal complexes as a ligand.

Chemistry

The complexes were synthesized by reaction of cerium (III), lanthanum (III) and neodymium (III) salts and the ligand, in amounts equal to metal: ligand molar ratio of 1: 3. The complexes were prepared by adding an aqueous solution of cerium (III), lanthanum (III) and neodymium (III) salt to an aqueous solution of the ligand subsequently raising the
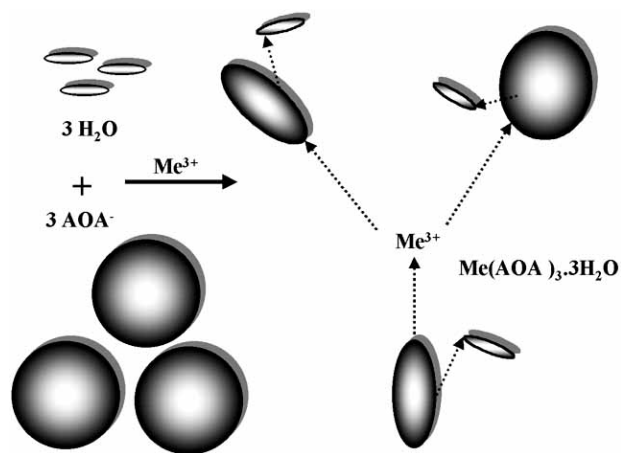
Fig. (8). The geometry and topology of the model complex.

pH of the mixture gradually to ca. 5.0 by adding dilute solution of sodium hydroxide. The reaction mixture was stirred with an electromagnetic stirrer at 25 °C for one hour. At the moment of mixing of the solutions, precipitates were obtained. The precipitates were filtered (pH of the filtrate was 5.0), washed several times with water and dried in a desicator to constant weight. The complexes were insoluble in water, methanol and ethanol and well soluble in DMSO.
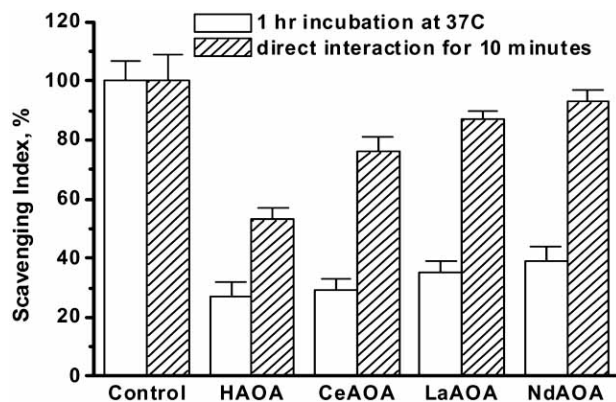

Fig. (9). Scavenging Indices of the solutions of AOA-complexes in blood plasma, using procedures 1 and 2. The control sample was not in contact with any Me-AOA solution.

The carbon, hydrogen and nitrogen contents of the compounds were determined by elemental analysis. The water content was determined by Metrohn Herizall E55 Karl Fisher Titrator.

The solid-state infrared spectra of the ligand and its Ln(III) complexes were recorded in KBr in the 4000-400 cm^{-1} frequency range by FT-IR 113V Bruker spectrometer and in Nujol by IR-spectrometer FTIR-8101M Shimadzu.

Theoretical Investigations

The Chem3D program package was used to determine the exact topology and geomety of the HAOA molecule, its most evident ionization pathways, and the formation of the [Me(AOA)$_3$.3H$_2$O] complex. The ionization pathways of the

HAOA molecule were examined after calculating the partial atomic charges in the HAOA molecule, and considering which bonds might brake in a solution with pH≥7 (7.4-7.8). Then, the most polar bonds were broken, and the energies of the remaining species were calculated again.
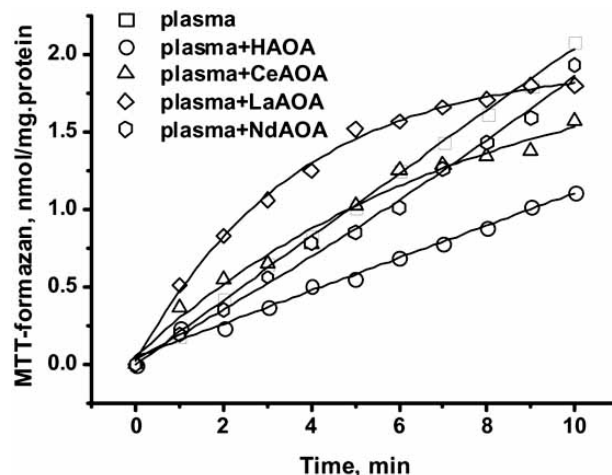

Fig. (10). Relative change of the formazan concentration with time in presence of blood plasma alone, and in contact with AOA-solutions.

Pharmacology

The effect of the tested lanthanide complexes of 5-aminoorotic acid on the radicals' formation in the blood plasma of Wistar rat were assessed using the next two procedures:

Procedure 1

Blood plasma containing proteins of 1 mg/ml was incubated for 1 hr at 37ºC, with 10^{-4} (100 µM) solution of the corresponding complex. The same amount of the complex was incubated at the same conditions, for the same time. Then 0.1 mg MTT (3-(4,5-dimethylthyazolyl-2)-2,5-diphenyltetrazolium bromide) solution (1 mg/ml in PBS pH 7.4) and 0.1 ml 0.3 mM solution of Xanthine (deionized water), were introduced in each vial. The samples were incubated for 30 minutes at the same temperature, and after being cooled to a room temperature, the absorbance at 576 nm was measured spectrophotometrically. The MTT-formazan produced in the samples was determined in nmol per mg protein, by using the extinction coefficient of 13 mM^{-1}cm^{-1}. The reduction of MTT to formazan due to interactions with the complexes themselves was excluded from data basis in presence of blood plasma. Then, data for each complex were presented as percentage of these for the blood plasma alone, this value named Scavenging Index. The value of the Scavenging index was as high as less the antioxidant effect observed was. Scavenging index of 100% corresponded to no antioxidant effect, while this above 100% indicated prooxidant properties.

Procedure 2

The same amounts of blood plasma and lanthanide complexes of 5-aminoorotic acid solutions, as in procedure 1, were introduced in vials, left in contact for 10 minutes, and

MTT was added. After further incubation for 30 minutes at 37ºC MTT-formazan was measured, using the absorption of the signal at 576 nm. The MTT-formazan produced in the samples was determined in nmol per mg protein, by using the extinction coefficient of 13 mM^{-1}cm^{-1}. The reduction of MTT to formazan due to interactions with the complexes themselves was excluded from data basis in presence of blood plasma. Then, data for each complex were presented as percentage of these for the blood plasma alone, this value named Scavenging Index. The value of the Scavenging index was as high as less the antioxidant effect observed was. Scavenging index of 100% corresponded to no antioxidant effect, while this above 100% indicated pro-oxidant properties.

Procedure 3

The same amount of blood plasma and lanthanide complexes of 5-aminoorotic acid solutions, as in procedure 1, were introduced in quartz cuvette at 25°C (293K), and same amount of MTT (50 μl, 1mg/ml in PBS with pH 7.4) was added. The free radicals formation was then initiated by adding 0.1 ml of Xanthine into the cuvette. The relative change of the intensity of the band at 576 nm was measured for 10 minutes. The MTT-formazan produced in the samples was determined in nmol per mg protein, by using the extinction coefficient of 13 mM^{-1}cm^{-1}. The reduction of MTT to formazan due to interactions with the complexes themselves was excluded from data basis in presence of blood plasma.

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