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Research Article

Vibrational Characterization and Antioxidant Activity of Newly Synthesized Gallium(III) Complex

Abstract

The gallium(III) complex of orotic acid (HOA) was synthesized and its structure was determined by means of analytical and spectral analyses. Detailed vibrational analysis of HOA, sodium salt of HOA (NaOA) and Ga(III)-OA systems based on both the calculated and experimental spectra confirmed the suggested metal-ligand binding mode. Significant differences in the IR and Raman spectra of the complex were observed as compared to the spectra of the ligand and confirmed the suggested metal-ligand binding mode. The calculated vibrational wavenumbers including IR and Raman scattering activities for the ligand and its Ga(III) complex were in good agreement with the experimental data. The vibrational analysis performed for the studied species, orotic acid, sodium salt of orotic acid and its Ga(III) complex, helped to explain the vibrational behaviour of the ligand vibrational modes, sensitive to interaction with Ga(III). The compounds HOA, NaOA and GaOA were investigated for possible antioxidant activity in a model of non-enzyme-induced lipid peroxidation on isolated rat microsomes. On isolated rat microsomes, administered alone, the compounds didn't revealed pro-oxidant effects. In conditions of non-enzyme-induced lipid peroxidation, only the complex GaOA showed antioxidant activity. HOA and NaOA didn't reveal antioxidant activity. We suggest that the antioxidant activity of the complex GaOA, might be due to the presence of gallium in the structure of GaOA.

Introduction

Orotic acid (2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylic acid, HOA, Figure 1) is a natural substance classified more than 40 years ago as vitamin B13 and mainly used in the past for the treatment of pernicious anaemia. Its major metabolic role within the human body consists of that it is the first fully formed intermediate in the manufacture of the pyrimidine bases required for the RNA/DNA synthesis. Coordination chemistry of orotic acid and its derivatives has attracted great interest [1-5] in the fields of bioinorganic and pharmaceutical chemistry. Metal orotates are widely applied in medicine [6,7].

The structure of sodium salt of orotic acid NaOA is presented in Figure 2. Orotic acid acts as a diacid in aqueous solution [8,9]. The coordinated orotate anions exhibit a ligand surface with double or triple hydrogen-bonding capabilities, depending on the metal coordination mode, and have thus a potential to adopt several modes of interligand hydrogen bonding. Orotic acid has demonstrated versatile coordination modes during the formation of coordination frameworks, that is why it was a challenge for us to obtain new metal coordination complexes with orotic acid, especially in view of

their application as anticancer and antioxidant agents. We have recently synthesized lanthanide complexes with a number of biologically active ligands, and we reported their significant antioxidant and cytotoxic activity in different human cell lines [10-16]. These promising results prompted us to search for new metal complexes with orotic acid. Thus, the aim of this work was to synthesize and characterize a complex of gallium(III) with orotic acid in view of determination of its antioxidant activity.

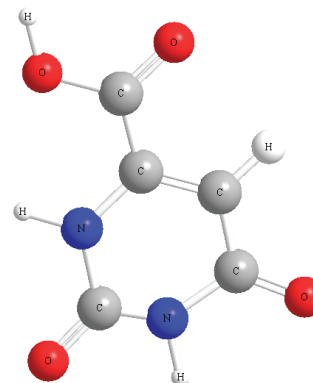


Figure 1: The structure of the ligand orotic acid.

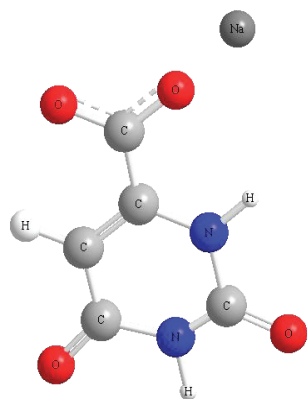


Figure2: The structure of sodium salt of orotic acid.

Gallium is the second metal ion, after platinum, to be used in cancer treatment. Its activities are numerous and various. It modifies three-dimensional structure of DNA and inhibits its synthesis, modulates protein synthesis, inhibits the activity of a number of enzymes, such as ATPases, DNA polymerases, ribonucleotide reductase and tyrosine-specific protein phosphatase. Gallium alters plasma membrane permeability and mitochondrial functions [17,18]. New Ga(III) compounds with a better bioavailability are now under clinical investigations and could improve the anticancer and antioxidant activity first demonstrated with Ga nitrate or Ga chloride [18].

In this paper we report analytical and spectroscopic results about the new Ga(III) complex of orotic acid (HOA). The Ga(III)-OA binding mode was identified and characterized by elemental analysis, IR and Raman spectroscopies. For estimation of the most preferred reactive sites of HOA for electrophilic attack and metal binding, DFT calculations of the vibrational structure of HOA have been performed. The compounds HOA, NaOA and GaOA have been investigated for possible antioxidant activity in a model of non-enzyme-induced lipid peroxidation on isolated rat liver microsomes, model of lipid membrane.

Materials and Methods

Synthesis of the coordination complex

The compounds used for preparing the solutions were Sigma-Aldrich products, p.a. grade: Ga(NO₃)₃. The sodium salt of orotic acid was used for the preparation of the metal complex as a ligand.

The complex was synthesized by reaction of gallium(III) nitrate and the sodium salt of orotic acid in aqueous solution, in amounts equal to metal: ligand molar ratio of 1: 3. The complex was prepared by adding an aqueous solution of gallium(III) salt to an aqueous solution of the sodium salt of orotic acid. The reaction mixture was stirred with an electromagnetic stirrer at 25 °C for one hour. At the moment of mixing of the solutions, precipitate was obtained. The precipitate was filtered (pH of the filtrate was 5.0), washed several times with water and dried in a desiccator to constant weight.

The complex was insoluble in water, methanol and ethanol and well soluble in DMSO.

Chemistry device descriptions

The carbon, hydrogen and nitrogen contents of the compound were determined by elemental analysis. The water content was determined by thermogravimetric analysis.

The solid-state infrared spectra of the ligand and its Ga(III) complex were recorded in KBr in the 4000–400 cm⁻¹ frequency range by FT-IR 113V Bruker spectrometer.

The Raman spectra of orotic acid and its new Ga(III) complex were recorded with a Dilor Labram spectrometer (Horiba-Jobin-Yvon, model LabRam) using the 784.8 nm excitation line from a near infrared Diode laser. The Labram integrated system is coupled through an Olympus LMPlanFL 50x objective to the optical microscope. The spectra were collected in the backscattering geometry with a resolution of 2 cm⁻¹. The detection of Raman signal was carried out with a Peltier-cooled CCD camera. The laser power of 35 mW was used in our measurements.

Computational details

The geometry of orotic acid was optimized using the Gaussian 03 program [19]. Becke's three-parameter exchange functional (B3) [20] with Perdew and Wang's gradient-corrected correlation functional (PW91) [21,22] and Becke's three-parameter hybrid exchange functional (B3) [23,24] using the LYP correlational functional of Lee, Yang and Parr (LYP) [25,26] were employed in the DFT calculations. The 6-311++G** Pople split valence basis sets along with the LANL2DZ basis set implemented in the Gaussian 03 program [19] were chosen in the geometry optimization and normal modes calculations.

Using the fully optimized molecular geometry we performed the density functional theory (DFT) calculations on harmonic vibrational modes. Harmonic vibrational wavenumbers including IR and Raman intensities were calculated analytically for the fully optimized molecular geometry. Only real harmonic vibrational wavenumbers were obtained for all structures, confirming the localization of global minima on the potential energy surfaces.

Pharmacology

In our experiments, KH₂PO₄, K₂HPO₄, KCl, (Scharlau Chemie SA, Spain), 2-thiobarbituric acid (4,6-dihydroxypyrimidine-2-thiol; TBA), Fenol reagent, FeSO₄, Ascorbinic acid (Sigma Aldrich), trichloroacetic acid (TCA) and Glycerol (Valerus, Bulgaria) were used.

Animals

Male Wistar rats (body weight 200–250 g) were used. The rats were housed in plexiglass cages (3 per cage) in a 12/12 light/dark cycle, under standard laboratory conditions (ambient temperature 20 ± 2 °C and humidity 72 ± 4 %) with free access to water and standard pelleted rat food 53-3, produced according to ISO 9001:2008.

Animals were purchased from the National Breeding Centre, Sofia, Bulgaria. At least 7 days of acclimatization was allowed before the commencement of the study. The health was monitored regularly by a veterinary physician. The vivarium (certificate of registration of farm № 0072/01.08.2007) was inspected by the Bulgarian Drug Agency in order to check the husbandry conditions (№ A-11-1081/03.11.2011). All performed procedures were approved by the Institutional Animal Care Committee and made according Ordinance № 15/2006 for humaneness behavior to experimental animals. The principles stated in the European Convention for the Protection of Vertebrate Animals used for Experiments and other Scientific Purposes (ETS 123) (Council of Europe, 1991) were strictly followed throughout the experiment.

Isolation of liver microsomes

Liver is perfused with 1.15 % KCl and homogenized with four volumes of ice-cold 0.1 M potassium phosphate buffer, pH=7.4. The liver homogenate was centrifuged at 9 000 x g for 30 min at 4°C and the resulting post-mitochondrial fraction (S9) was centrifuged again at 105 000 x g for 60 min at 4°C. The microsomal pellets were re-suspended in 0.1 M potassium phosphate buffer, pH=7.4, containing 20 % Glycerol. Aliquots of liver microsomes were stored at -70°C until use [27].

The content of microsomal protein was determined according to the method of Lowry using bovine serum albumin as a standard [28].

FeSO₄/Ascorbic acid-induced lipid peroxidation in vitro

As a system, in which metabolic activation may not be required in the production of lipid peroxide, 20 µM FeSO₄ and 500 µM Ascorbic acid were added directly into rat liver microsomes and incubated for 20 min at 37 °C [29].

Microsomes' incubation with HOA, NaOA and GaOA

Liver microsomes were incubated with concentration 100 µM of the investigated compounds [30].

Lipid peroxidation in microsomes

After incubation of microsomes (1 mg/ml) with the compounds, we added to the microsomes 1 ml 25 % (w/v) trichloroacetic acid (TCA) and 1 ml 0.67 % 2-Thiobarbituric acid (TBA). The mixture was heated at 100 °C for 20 min. The absorbance was measured at 535 nm, and the amount of MDA was calculated using a molar extinction coefficient of 1.56 x 10⁵ M⁻¹cm⁻¹ [29].

Statistical analysis

Statistical analysis was performed using statistical programme 'MEDCALC'. Results are expressed as mean ± SEM for 5 experiments. The significance of the data was assessed using the nonparametric Mann-Whitney test. A level of P < 0.05 was considered significant. Three parallel samples were used.

Results and Discussion

Chemistry

The new complex was characterized by elemental analysis. The content of the metal ion was determined after mineralization. The water content in the complex was determined thermogravimetrically. IR and Raman spectra confirmed the nature of the complex.

The data of the elemental analysis of the new gallium(III) complex obtained serving as a basis for the determination of its empirical formula are presented below.

Elemental analysis of Ga(III) complex of orotic acid: (% calculated/found): Ga(OA)₃·3H₂O: C: 30,56/31,01; H: 2,55/2,98; N: 14,26/14,23; H₂O: 9.17/9,28; Ga: 11,83/12.15, where HOA = C₅N₂O₄H₄ and OA⁻ = C₅N₂O₄H₃⁻.

The mode of bonding of the ligand to Ga(III) ions was elucidated by recording the IR and Raman spectra of the complex as compared with those of the free ligand and the theoretical predictions. The vibrational fundamentals from the IR and Raman spectra were analysed by comparing these modes with those from the literature [31-35] in combination with the results of our DFT calculations (i.e., harmonic vibrational wavenumbers and their Raman scattering activities) for the ligand [34] and for the Ga(III) complex.

Vibrational spectroscopy

In Table 1 the selected calculated and experimental IR and Raman data together with their tentative assignments are given. The vibrational IR and Raman spectra of HOA, sodium salt of orotic acid NaOA and Ga(III)-OA are presented in Figures 3,4. The vibrational fundamentals from the IR and Raman spectra were analyzed by comparing these modes with those from the literature [34] in combination with the results of our DFT calculations (i.e., harmonic vibrational wavenumbers and their Raman scattering activities).

In the 3600-2000 cm⁻¹ spectral region from the IR spectrum the O-H and N-H stretches give rise to medium IR bands (Figure 3). The O-H and N-H bands appear overlapped in the same spectral region, and the involvement of these groups in hydrogen bonds affects their wavenumbers and produces a relevant band broadening [32-35]. In the IR spectrum of orotic acid the medium band at 3520 cm⁻¹ was assigned to the N-H stretching modes, while the shoulder at 3232 cm⁻¹ was attributed to the O-H stretching modes (Table 1). The wavenumber region 2700-2500 cm⁻¹ in the IR spectra of orotic acid and its complex is typical of strongly hydrogen bonded intermolecular complexes with overtones and combinations of lower frequency modes of the bonded molecules [36-43].

When the carbonyl is hydrogen bonded but not dimerized, a bond active in both IR and Raman spectra appears at 1730-1705 cm⁻¹. In our IR spectra, one very strong band can be observed in this region (at 1712 cm⁻¹ for orotic acid), which was assigned to the symmetrical stretching mode of C2=O2 and to the N-H stretching mode. In this region were observed one medium

Table 1: Selected theoretical and experimental IR and Raman wavenumbers (cm^{-1}) of orotic acid (HOA) and its Ga(III) complex (GaOA) and their tentative assignment.

Freq. scaled	Exp. Raman HOA	Exp. Raman GaOA	Exp. IR HOA	Exp. IR GaOA	Assignments
253		306			$\nu(\text{O1-Ga})$
269					$\nu(\text{O1'-Ga}), \nu(\text{O3'-Ga})$
337		345			$\nu(\text{C6-C7})$
380	395	373			$\nu(\text{O1'-Ga}), \nu(\text{C7'-Ga}), \nu(\text{O3'-Ga}), \delta(\text{C5'-C4'-O4'}), \delta(\text{N3'-C2'-O2'}), \delta(\text{N1'-C2'-O2'}), \delta(\text{N3'-C4'-O4'})$
409					$\delta(\text{C5-C4-O4}), \gamma(\text{O5-H6}), \delta(\text{C2-N3-C4}), \delta(\text{N1-C2-O2})$
434		440		434	$\nu(\text{O1'-Ga}), \nu(\text{O3'-Ga}), \nu(\text{C7'-Ga})$
464	460	460		451	$\delta(\text{C6-C7-O1}), \nu(\text{O1-Ga}), \delta(\text{C7-O1-Ga}), \delta(\text{N1-C2-O2})$
480					$\gamma(\text{O5-H6}), \gamma(\text{H1-O5})$
506	513	519		507	$\gamma(\text{C7-O1}), \gamma(\text{N1-C6}), \gamma(\text{C6-C7})$
510					$\gamma(\text{C6'-C7'}), \delta(\text{N1'-C6'-C5'})$
529					$\delta(\text{C5'-C4'-N3'}), \delta(\text{N3'-C4'-O4'}), \delta(\text{C6'-C5'-C4'}), \delta(\text{C2'-N1'-C6'}), \delta(\text{N3'-C2'-O2'}), \delta(\text{N1'-C2'-N3'}), \delta(\text{C2'-N3'-H3'})$
533	546	553		549	$\gamma(\text{H1-O5}), \delta(\text{N3-C4-O4}), \delta(\text{C6-C5-C4}), \delta(\text{C5-C4-N3}), \delta(\text{C2-N1-C6})$
579					$\delta(\text{C2-N3-C4}), \gamma(\text{C4-N3}), \delta(\text{N1-C2-N3})$
582					$\delta(\text{C2'-N3'-C4'}), \gamma(\text{C4'-N3'})$
596	608	608		603	$\nu(\text{Ga-O5})$
615					$\gamma(\text{N1'-H1'})$
659					$\delta(\text{N3-C2-N1}), \gamma(\text{N3-H3}), \gamma(\text{H1-N1})$
691	653	643		645	$\gamma(\text{N3'-H3'}), \gamma(\text{N1'-H1'})$
696					$\gamma(\text{N3-H3}), \delta(\text{N1-C2-N2}), \gamma(\text{N1-H1})$
758					$\gamma(\text{O2-C2}), \gamma(\text{H5-C5})$
765	753	757		758	$\gamma(\text{C2'O}), \gamma(\text{C2'N})$
769					$\gamma(\text{C2-O2}), \gamma(\text{C2N})$
782					$\delta(\text{C7O}_2), \nu(\text{O1-Ga})$
795	792	787		786	$\delta(\text{C7'O}_2), \nu(\text{C7'-Ga})$
800					$\gamma(\text{O1-Ga}), \omega(\text{CO}_2), \gamma(\text{C-H, N-H})$
810					$\omega(\text{CO}_2), \gamma(\text{C-H})$
885	887	874		892	$\gamma(\text{C5'-H5'}), \gamma(\text{C5'-C4'}), \gamma(\text{C6'-C5'}), \gamma(\text{C4'-N3'})$
891					$\gamma(\text{C5-H5})$
930	933	939		930	$\nu(\text{C6'-C7'})$
931					$\nu(\text{C6-C7}), \delta(\text{C2-N3-C4})$
973	1011	1016		1015	$\delta(\text{C2-N3-H3}), \delta(\text{C2-N3-H3}), \delta(\text{C6-C5-H5}), \delta(\text{C2-N1-C6})$
992	1047	1047		1042	$\delta(\text{C6'-C5'-C4'}), \delta(\text{N1'-C6'-C5'}), \nu(\text{C6'-C7'}), \delta(\text{C2'-N1'-C6'})$
1071		1119			$\delta(\text{C4'-C5'-H5'}), \delta(\text{C6'-C5'-H5'})$
1075	1134	1145		1125	$\delta(\text{C6-C5-H5}), \delta(\text{C4-C5-H5})$
1165		1205			$\nu(\text{C4-N3})$
1169	1254	1244		1241	$\nu(\text{C4'-N3'})$
1247	1282	1300		1284	$\delta(\text{N1'-H1'}), \delta(\text{C5'-H5'})$
1281	1326	1318		1345	$\delta(\text{N1-H1}), \delta(\text{C5-H5})$
1383	1414	1392		1407	$\nu_{\text{as}}(\text{C7'O}_2), \nu(\text{C6'-C7'})$
1464	1522			1522	$\nu(\text{N1'-C6'}), \nu(\text{C7'-O3'}), \nu(\text{C6'-C7'})$
1512					$\nu_{\text{as}}(\text{C7'-O}_2)$
1612	1615	1619		1617	$\nu(\text{C6-C5})$

1623					$\nu(\text{C6'-C5'})$
1721	1657	1676	1684	1682	$\nu(\text{C4-O4})$
1725					$\nu(\text{C4'-O4'})$
1727		1748			$\nu(\text{C7-O3})$
1736	1715	1714	1712		$\nu(\text{C2-O2})$
1766					$\nu(\text{C2'-O2'})$
3138					$\nu(\text{C5'-H5'})$
3354			3520	3437	$\nu(\text{N1-H1}), \nu(\text{H1-O5})$
3642	3144		3232	3199	$\nu(\text{O5-H6})$

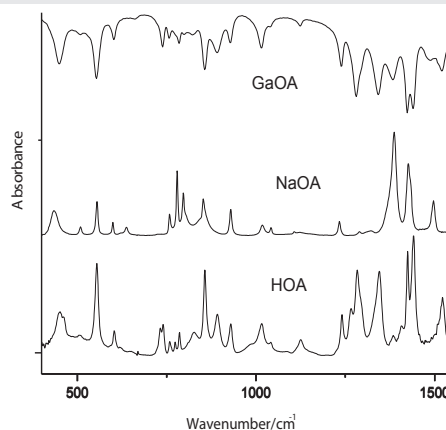


Figure 3: IR spectra of orotic acid (HOA), sodium salt of orotic acid (NaOA) and its Ga(III) complex (400-2000 cm^{-1}).

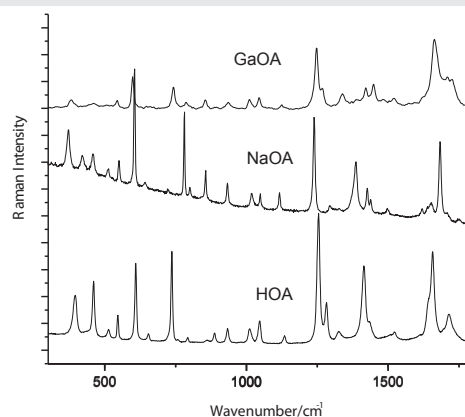


Figure 4: Raman spectra of the solid state of orotic acid (HOA), sodium salt of orotic acid (NaOA) and its Ga(III) complex. Excitation: 784.8 nm, 35 mW.

band at 1715 cm^{-1} in the Raman spectrum of the free ligand, and two shoulders in the Raman spectrum of the complex (Figure 3) [36–40]. The very strong bands at 1684 cm^{-1} were assigned to the symmetrical stretching modes of $\text{C4}=\text{O4}$ and to the $\text{C6}=\text{C5}$ stretching modes. In the Raman spectra, these vibrations can be observed as very strong bands at 1657 and 1676 cm^{-1} for orotic acid and its Ga(III) complex, respectively.

The asymmetrical COO^- stretching mode was observed as a medium band at 1522 cm^{-1} (in the IR spectrum of orotic acid) and as a shifted shoulder (in the IR spectra of the complex). The symmetrical COO^- stretching mode was observed in the IR spectra at 1407 and 1380 cm^{-1} for the ligand and its Ga(III) complex, respectively, while in the Raman spectra this vibration appears as a strong peak at 1414 cm^{-1} for the free ligand and as

a medium band for the complex (Figure 4). In the 1800–900 cm^{-1} spectral region of the IR spectra, the bands at 1424 and 1410 cm^{-1} for the free ligand and its complex (Figure 3) can be due to the stretching modes of N–H. These vibrations are rather different and shifted in the Raman spectra for the free ligand and for the complex.

The C5–H5, N1–H1, and C–O–H bending modes are present in the IR spectra as well as in the Raman spectra. In the IR spectra they are observed at 1284 cm^{-1} for the free ligand, while in the Raman spectra they are detected at 1282 cm^{-1} . The weak band at 1266 cm^{-1} , which can be observed only in the IR spectrum of the orotic acid can be due to the C=O bending mode and to the symmetrical stretching mode of N1–C2–N3. The weak peak at 1241 cm^{-1} that appear only in the IR spectrum of the free ligand, was assigned to the stretching modes of C2–N3–C4 and C6=C5–C4, while in the Raman spectra these vibrations appear also for the complex. The bands around 1015 cm^{-1} , weak in IR and medium in Raman spectra, can be due to the symmetrical C=O stretching mode, whereas bands around 930 cm^{-1} almost weak in IR and medium in Raman spectra, were attributed to the symmetrical C(ring)–C(carboxyl) bridge bond stretching mode. The uracilate ring bending vibration and the skeletal deformation bands of the free orotic acid, mainly in the 900–300 cm^{-1} wavenumber region, show considerable changes on complex formation (Figures 3,4; Table 1). These changes may be attributed to distortion of the uracilate rings upon coordination.

The new bands at 430–440 cm^{-1} in the IR and Raman spectra, which appear only for the Ga(III) complex, can be due to the gallium–oxygen interactions [35]. In the low wavenumbers region of the Raman spectrum of orotic acid (Figure 4), the medium strong band at 395 cm^{-1} is importantly shifted to the shorter wavenumbers in the Raman spectrum of the Ga(III) complex and became weaker. This one and the new neighbouring band at 345 cm^{-1} (in the Raman spectrum of the complex) can be due to the gallium–oxygen vibration modes [44–48]. The metal affects the carboxylate anion as well as the ring structure. The ionic potential of the metal is the most important parameter responsible for the influence of the metal on the rest of the molecule [49–51]. The carboxylic acids interact with the metals as symmetric [52,53], bidentate carboxylate anions and both oxygen atoms of the carboxylate are symmetrically bonded to the metal [54]. In this sense, we can observe in the Raman spectra of the Ga(III) complex a very weak peak at 306 cm^{-1} , which can be due to the O–Ga–O vibration modes (Table 1) [48,55–57].

From our previous results regarding the newly synthesised lanthanide complexes and this work, it is clear that the nature of orotic 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. Thus, the ligand orotic acid has great potential as a generally useful polyfunctional ligand in metal coordination chemistry and it will prove attractive to a variety of coordination chemists.

On the basis of the detailed vibrational analysis the most probable structure of the obtained Ga(III) complex was suggested (Figure 5).

Pharmacology

Effects of HOA, NaOA and GaOA on isolated rat liver microsomes

One of the most suitable sub-cellular *in vitro* systems for investigation of drug metabolism is isolated microsomes.

Administered alone, HOA, NaOA and GaOA, didn't reveal statistically significant toxic effects on isolated rat microsomes. The level of malondialdehyde (MDA), marker for lipid peroxidation, was not increased statistically significant from all compounds, compared to the control (non-treated microsomes) (Figure 6).

In conditions of non-enzyme-induced lipid peroxidation, only the complex GaOA revealed statistically significant antioxidant activity, compared to toxic agent – Fe^{2+}/AA (iron/ascorbate). HOA and NaOA didn't show antioxidant activity at this toxicity model (Figure 7).

Microsomes incubation with Fe^{2+}/AA , resulted in statistically significant increase of the amount of MDA with 191 % vs control (non-treated microsomes). In non-enzyme-induced lipid peroxidation model, the pre-treatment only with the complex GaOA, at concentration 100 μM , significantly reduced lipid damage by 42 %, as compared to the toxic agent (Fe^{2+}/AA). HOA and NaOA didn't show antioxidant activity in this toxicity model (Figure 7).

The microsomal fraction, which is prepared by differential

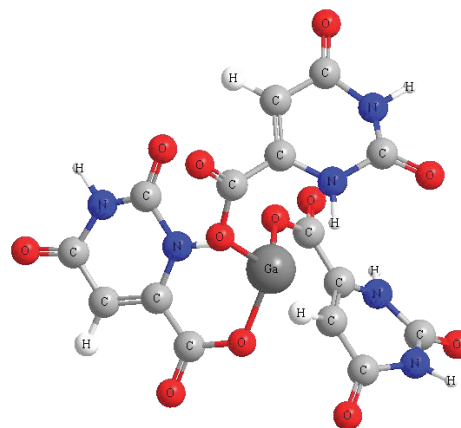


Figure 5: The suggested structure of Ga(III) complex of orotic acid.

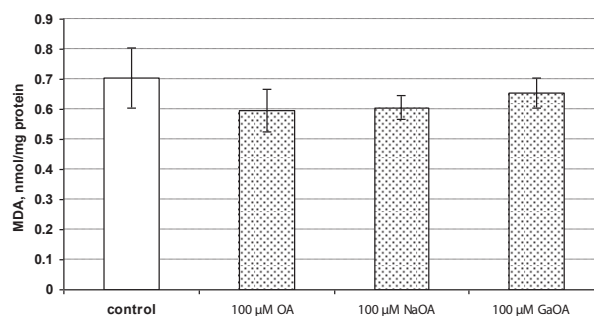


Figure 6: Effects of HOA, NaOA and GaOA (at concentration 100 μM), administered alone, on isolated rat microsomes.

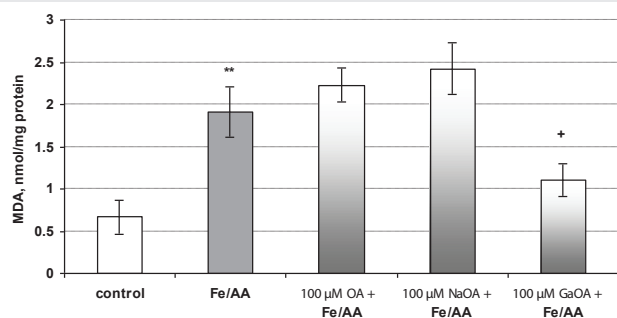


Figure 7: Effects of HOA, NaOA and GaOA (at concentration 100 μ M), in non-enzyme-induced lipid peroxidation, on isolated rat microsomes.

** P < 0.01 vs control (non-treated microsomes)

+ P < 0.05 vs toxic agent (Fe²⁺/AA).

centrifugation, contents fragments from the endoplasmatic reticulum and preserve the enzyme activity, mostly cytochrome P450 enzymes. Microsomes are used as a model of lipid membrane in experiments, related to the process of lipid peroxidation [58]. Here, we show that only the complex GaOA revealed statistically significant antioxidant effect in non-enzyme-induced lipid peroxidation in isolated microsomes. The effects of GaOA might be due to the presence of metal ions.

Conclusion

The complex of gallium(III) with orotic acid has been synthesized and characterized by elemental and vibrational (IR, Raman) analyses. The vibrational analysis performed for the studied species, orotic acid and its Ga(III) complex, helped to explain the vibrational behavior of the ligand vibrational modes, sensitive to interaction with Ga(III). The most probable metal-ligand binding mode in the Ga(III) complex of orotic acid was elucidated. It is suggested that orotic acid binds through the oxygen atoms of the carboxylic groups from the ligands.

The results from the preliminary pharmacological investigations of orotic acid, sodium salt of orotic acid and Ga(III) complex demonstrate the antioxidant potential of the Ga(III) complex which is in line with our preceding papers concerning the activity of lanthanide coordination compounds with diverse biologically active ligands. The complex formation proved to be beneficial for the exerted efficacy of the Ga(III) complex vs. the corresponding ligand and its sodium salt. Thus, the newly synthesised Ga(III) complex necessitates further more detailed pharmacological evaluation.

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References

- Dodin G, Dubois JE (1980) Tautomerism of orotic acid dianion. Effect of calcium and magnesium cations on the tautomeric constant and on tautomerization dynamics. *J Am Chem Soc* 102: 3049–3056. [Link: https://doi.org/10.1021/ja00541a011](https://doi.org/10.1021/ja00541a011)
- Arrizabalaga P, Castan P, Dahan F (1983) Coordination sites of 5-nitro-6-carboxyuracil: UV study and x-ray structure determination

of diammine(5-nitroorotato)copper(II) hydrate and hexaamminebis(5-nitroorotato)tricopper(II) pentahydrate. *Inorg Chem* 22: 2245–2252. [Link: https://doi.org/10.1021/in00133a011](https://doi.org/10.1021/in00133a011)

- Arrizabalaga P, Castan P, Laurent JP (1984) Intramolecular influence of a carboxylic function on platinum blue synthesis. A systematic study of complexes originating from acid amides. *J Am Chem Soc* 106: 4814–4818. [Link: https://doi.org/10.1021/ja00133a011](https://doi.org/10.1021/ja00133a011)
- Lea MA, Luke A, Assad A, Patel M, Reddy PA (1992) Inhibitory action of orotate, 2-thioorotate and iso-orotate on nucleotide metabolism and nucleic acid synthesis in hepatoma cells. *Int J Biochem* 24: 1453–1459. [Link: https://doi.org/10.1080/09637489208839563](https://doi.org/10.1080/09637489208839563)
- Casas JS, Sordo J, Hiller W, Strahle J (1991) Reaction of dimethylhydroxythallium(III) with 2-thioorotic acid. Crystal structure of dimethyl(2-thioorotato)thallium(III) monohydrate. *Inorg Chim Acta* 181: 43–49. [Link: https://doi.org/10.1016/0162-0138\(91\)00133-3](https://doi.org/10.1016/0162-0138(91)00133-3)
- Hueso-Ureña F, Moreno-Carretero MN, Salas-Peregrin JM, Alvarez de Cienfuegos-Lopez G (1995) Silver(I), palladium(II), platinum(II) and platinum(IV) complexes with iso-orotate and 2-thioiso-orotate ligands: synthesis, i.r. and n.m.r. spectra, thermal behaviour and antimicrobial activity. *Transition Metal Chemistry* 20: 262–269. [Link: https://doi.org/10.1007/BF00132777](https://doi.org/10.1007/BF00132777)
- Hueso-Ureña F, Moreno-Carretero MN, Romero-Molina MA, Salas-Peregrin JM, Sanchez-Sanchez MP, et al. (1993) *J Inorg Biochem* 51: 613–632. [Link: https://doi.org/10.1016/0162-0138\(93\)00133-3](https://doi.org/10.1016/0162-0138(93)00133-3)
- Kumberger O, Riede J, Schmidbaur H (1993) Preparation and crystal structure of zinc bis[orotate(1-)] octahydrate. *Zeits Naturforsch B* 48: 961-964. [Link: https://doi.org/10.1515/znb/1993/48/961](https://doi.org/10.1515/znb/1993/48/961)
- Bach I, Kumberger O, Schmidbaur H (1990) Orotate complexes. Synthesis and crystal structure of lithium orotate(I) monohydrate and magnesium bis[orotate(I)] octahydrate. *Chem Berich*, 123: 2267-2271. [Link: https://doi.org/10.1002/chem.199012311](https://doi.org/10.1002/chem.199012311)
- Kostova I, Trendafilova N, Momekov G (2005) Theoretical and spectroscopic evidence for coordination ability of 3,3'-benzylidenedi-4-hydroxycoumarin. New neodymium(III) complexes and its cytotoxic effect. *J Inorg Biochem* 99: 477-487. [Link: https://doi.org/10.1016/j.jinorgbiochem.2005.05.011](https://doi.org/10.1016/j.jinorgbiochem.2005.05.011)
- Kostova I, Traykova M (2006) Cerium(III) and neodymium(III) complexes as scavengers of X/XO-derived superoxide radical. *Med Chem* 2: 463-470. [Link: https://doi.org/10.1002/med.10011](https://doi.org/10.1002/med.10011)
- Kostova I, Peica N, Kiefer W (2006) Theoretical and spectroscopic studies of lanthanum(III) complex of 5-aminoorotic acid. *Chem Phys* 327: 494-505. [Link: https://doi.org/10.1016/j.cpl.2006.03.011](https://doi.org/10.1016/j.cpl.2006.03.011)
- Kostova I, Momekov G (2008) Synthesis, spectral and pharmacological studies on lanthanide(III) complexes of 3, 5-pyrazoledicarboxylic acid. *J Coord Chem* 61: 3776-3792. [Link: https://doi.org/10.1080/00938410802288888](https://doi.org/10.1080/00938410802288888)
- Kostova I, Stefanova Ts (2009) Synthesis, characterization and cytotoxic/cytostatic activity of Sm(III) and Gd(III) complexes. *J Coord Chem* 62: 3187-3197. [Link: https://doi.org/10.1080/00938410903288888](https://doi.org/10.1080/00938410903288888)
- Kostova I, Valcheva-Traykova M (2015) New samarium(III) complex of 5-aminoorotic acid with antioxidant activity. *Appl Organomet Chem*, 29: 815–824. [Link: https://doi.org/10.1080/00938410903288888](https://doi.org/10.1080/00938410903288888)
- Kostova I, Peica N, Kiefer W (2007) Theoretical and spectroscopic studies of 5-aminoorotic acid and its new lanthanide(III) complexes. *J Raman Spectrosc* 38: 205-216. [Link: https://doi.org/10.1002/jrs.1211](https://doi.org/10.1002/jrs.1211)
- Collery P, Keppler B, Madoulet C, Desoize B (2002) Gallium in Cancer Treatment. *Crit Rev Oncol Hematol* 42: 283–296. [Link: https://doi.org/10.1080/07321830208839563](https://doi.org/10.1080/07321830208839563)
- Mohammadi K, Thompson KH, Patrick BO, Storr T, Martins C, et al. (2005) Synthesis and characterization of dual function vanadyl, gallium and indium curcumin complexes for medicinal applications. *J Inorg Biochem* 99: 2217–2225. [Link: https://doi.org/10.1016/j.jinorgbiochem.2005.05.011](https://doi.org/10.1016/j.jinorgbiochem.2005.05.011)

19. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, et al. (2003) Gaussian 03, Revision B. 04, Gaussian Inc., Pittsburgh PA, 12. [Link: https://goo.gl/E8qFBt](https://goo.gl/E8qFBt)
20. Becke AD (1993) Densityfunctional thermochemistry. III. The role of exact exchange. *J Chem Phys* 98: 5648-5652. [Link: https://goo.gl/8A5bHP](https://goo.gl/8A5bHP)
21. Perdew JP (1991) Electronic structure of solids; Eds. P. Ziesche and H. Eschrig; Akademie Verlag, Berlin, 11.
22. Perdew JP, Wang Y (1992) Pair-distribution function and its coupling-constant average for the spin-polarized electron gas. *Phys Rev B* 46: 12947. [Link: https://goo.gl/rZfMCT](https://goo.gl/rZfMCT)
23. Becke AD (1992) Densityfunctional thermochemistry. II. The effect of the Perdew-Wang generalized gradient correlation correction. *J Chem Phys* 97: 9173-9177. [Link: https://goo.gl/90GMO5](https://goo.gl/90GMO5)
24. Gelfand LS, Iaconianni FJ, Pytlewski LL, Specca AN, Mikulski CM, et al. (1980) Nicotinic and isonicotinic acid n-oxide interactions with 3d metal perchlorates. *J Inorg Nucl Chem*, 42: 377-385. [Link: https://goo.gl/rKbC4h](https://goo.gl/rKbC4h)
25. Lee C, Yang W, Parr RG (1988) Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Phys Rev B* 37: 785. [Link: https://goo.gl/Z4RfrJ](https://goo.gl/Z4RfrJ)
26. Dunning TH, Hay PJ (1976) *Modern Theoretical Chemistry*; Eds. HF Schaefer; Plenum, New York, 3: 805.
27. Deby C, Goutier R (1990) New perspectives on the biochemistry of superoxide anion and the efficiency of superoxide dismutases. *Biochem Pharmacol* 39: 399-405. [Link: https://goo.gl/iXJps3](https://goo.gl/iXJps3)
28. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ (1951) Protein measurement with the Folin Phenol reagent. *J Biol Chem* 193: 265-275. [Link: https://goo.gl/s2brsJ](https://goo.gl/s2brsJ)
29. Kim HJ, Chun YJ, Park JD, Kim SI, Roh JK, et al. (1997) Protection of rat liver microsomes against carbon tetrachloride-induced lipid peroxidation by red ginseng saponin through cytochrome P450 inhibition. *Planta Med* 63: 415-418. [Link: https://goo.gl/6xg5vr](https://goo.gl/6xg5vr)
30. Oh KY, Roberts VH, Schabel MC, Grove KL, Woods M, et al. (2015) Gadolinium chelate contrast material in pregnancy: fetal biodistribution in the nonhuman primate. *Radiology* 276: 110-118. [Link: https://goo.gl/KocHLQ](https://goo.gl/KocHLQ)
31. Hernanz A, Billes F, Bratu I, Navarro R (2000) Vibrational analysis and spectra of orotic acid. *Biopolym* 57: 187-198. [Link: https://goo.gl/RBAQA6](https://goo.gl/RBAQA6)
32. Icbudak H, Olmez H, Yesilel OZ, Arslan F, Naumov P, et al. (2003) Syntheses, characterization and crystal structures of novel amine adducts of metal saccharinates, orotates and salicylates. *J Mol Struct* 657: 255-270. [Link: https://goo.gl/4dFO8m](https://goo.gl/4dFO8m)
33. Papaefstathiou GS, Manessi S, Raptopoulou CP, Behrman, E J, Zafiropoulos TF (2004) The first metal complex of 5-hydroxyorotic acid: dimethylammonium bis (N, N-dimethylformamide) bis (5-hydroxyorotato(-2)) gallate (III). *Inorg Chem Comm* 7: 69-72. [Link: https://goo.gl/auYTpQ](https://goo.gl/auYTpQ)
34. Kostova I, Peica N, Kiefer W (2007) Theoretical and spectroscopic studies of new lanthanum (III) complex of orotic acid. *Vibr Spectrosc* 44: 209-219. [Link: https://goo.gl/xi9ou9](https://goo.gl/xi9ou9)
35. Lencioni S, Pellerito A, Fiore T, Giuliani AM, Pellerito L, et al. (1999) Organometallic complexes with biological molecules. X: Dialkyltin (IV) and trialkyltin (IV) orotates: Spectroscopic and in vivo investigations. *Appl Organomet Chem* 13: 145-157. [Link: https://goo.gl/rEOwOZ](https://goo.gl/rEOwOZ)
36. Exner K, Fischer G, Bahr N, Beckmann E, Luga M, et al. (2000) Proximate, synperiplanar bisdiazene skeletons: Syntheses, structures, homoconjugate reactivity and photochemistry. *Eur J Org Chem* 2000: 763-785. [Link: https://goo.gl/BiYbEc](https://goo.gl/BiYbEc)
37. Castaneda JP, Denisov GS, Kucherov SY, Schreiber VM, Shurukhina AV (2003) Infrared and ab initio studies of hydrogen bonding and proton transfer in the complexes formed by pyrazoles. *J Mol Struct* 660: 25-40. [Link: https://goo.gl/bcbM4L](https://goo.gl/bcbM4L)
38. González-Sánchez F (1958) Infra-red spectra of the benzene carboxylic acids. *Spectrochim Acta* 12: 17-33. [Link: https://goo.gl/lwuZe2](https://goo.gl/lwuZe2)
39. Hadzi D, Sheppard N (1953) The infra-red absorption bands associated with the COOH and COOD groups in dimeric carboxylic acids. I. The region from 1500 to 500 cm⁻¹. *Proc Roy Soc Ser A* 216: 247-266. [Link: https://goo.gl/8piHQb](https://goo.gl/8piHQb)
40. Lin-Vien D, Colthup NB, Fateley WG, Grasselli JG (1991) *The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules*. Edited by Jovanovich HB, Academic Press Inc., San Diego 140. [Link: https://goo.gl/P1NSII](https://goo.gl/P1NSII)
41. Dolish FR, Fateley WG, Bentley FF (1974) *Characteristic Raman Frequencies of Organic Compounds*. John Wiley&Sons Inc. 128. [Link: https://goo.gl/dds9Yq](https://goo.gl/dds9Yq)
42. Orza JM., García MV, Alkorta I, Elguero J (2000) Vibrational spectra of 3, 5-dimethylpyrazole and deuterated derivatives. *Spectrochim Acta A* 56: 1469-1498. [Link: https://goo.gl/aO4RGD](https://goo.gl/aO4RGD)
43. Szabó A, Češljević VI, Kovács A (2001) Tautomerism, hydrogen bonding and vibrational properties of 4-acetyl-3(5)-amino-5(3)-methylpyrazole. *Chem Phys* 270: 67-78. [Link: https://goo.gl/ZxH2sy](https://goo.gl/ZxH2sy)
44. Sourisseau C, Fouassier M, Mauricot R, Boucher F, Evain M (1997) Structure and bonding in cerium oxysulfide compounds. II Comparative lattice dynamics calculations on Ce₂O₂S and Ce₂O₂.5S. *J Raman Spectrosc* 28: 973-978. [Link: https://goo.gl/i0l62h](https://goo.gl/i0l62h)
45. Lu Y, Deng G, Miao F, Li Z (2003) Metal ion interactions with sugars. The crystal structure and FT-IR study of the NdCl₃-ribose complex. *Carbohydr Res* 338: 2913-2919. [Link: https://goo.gl/TgKW1s](https://goo.gl/TgKW1s)
46. Sohn JR, Chun EW, Pae YI (2003) Spectroscopic studies on ZrO₂ modified with MoO₃ and activity for acid catalysis. *Bull Korean Chem Soc* 24: 1785-1792. [Link: https://goo.gl/ylzPgr](https://goo.gl/ylzPgr)
47. Jayaraman A, Sharma SK, Wang SY, Shieh SR, Ming LC, et al. (1996) Pressureinduced phase transitions in KDy(MoO₄)₂ and KY(MoO₄)₂: a high pressure Raman study. *J Raman Spectrosc* 27: 485-490. [Link: https://goo.gl/0KU3wZ](https://goo.gl/0KU3wZ)
48. Fielicke A, Meijer G, Von Helden G (2003) Infrared multiple photon dissociation spectroscopy of transition metal oxide cluster cations. *Eur Phys J* 24: 69-72. [Link: https://goo.gl/9dNLRm](https://goo.gl/9dNLRm)
49. Lewandowski W, Dasiewicz B, Koczóń P, Skierski J, Dobrosz-Teperek K, et al. (2002) Vibrational study of alkaline metal nicotinate, benzoate and salicylates. *J Mol Struct* 604: 189-193. [Link: https://goo.gl/8zkcQn](https://goo.gl/8zkcQn)
50. Koczón P, Lewandowski W, Mazurek AP (1999) Vibrational (FT-IR and FT-Raman) and NMR studies on selected metal (Ca, Mn, Zn) complexes with ortho-, meta-, and para-iodobenzoic acids. *Vibr Spectrosc* 20: 143-149. [Link: https://goo.gl/uZI9JH](https://goo.gl/uZI9JH)
51. Kakiuchi M, Abe T, Nakayama H (2001) D/H fractionation factor between water vapor and crystal water of copper chloride dihydrate: Statistical mechanical approach based on Raman spectra. *Geochem J* 35: 285-293. [Link: https://goo.gl/9rjz02](https://goo.gl/9rjz02)
52. Wang K, Li YS (1997) Silver doping of polycarbonate films for surface-

- enhanced Raman scattering. *Vibr Spectrosc* 14: 183-188. [Link: https://goo.gl/iYqTTh](https://goo.gl/iYqTTh)
53. Boerio FJ, Hong PP, Clark PJ, Okamoto Y (1990) Surface-enhanced Raman scattering from model acrylic adhesive systems. *Langmuir* 6: 721-727. [Link: https://goo.gl/xNMVQE](https://goo.gl/xNMVQE)
54. Kwon YJ, Dong HS, Sang JA, Myung SK, Kwan K (1994) Vibrational spectroscopic investigation of benzoic acid adsorbed on silver. *J Phys Chem* 98: 8481-8487. [Link: https://goo.gl/lHajKz](https://goo.gl/lHajKz)
55. Galdecka E, Galdecki Z, Huskowska E, Amirkhanov V, Legendziewicz J (1997) Crystal structure and optical properties of Ln(III) octahedral complexes with hexamethylphosphotriamide; $[\text{Ln}(\text{HMPA})_6](\text{ClO}_4)_3$. *J Alloys Comp* 257: 182-190. [Link: https://goo.gl/3N1uUd](https://goo.gl/3N1uUd)
56. De Andres A, Taboada S, Martínez JL, Salinas A, Hernández J, et al. (1993) Optical phonons in R_2BaMO_5 oxides with M= Co, Ni, Cu, and R= a rare earth. *Phys Rev B* 47: 14898-14904. [Link: https://goo.gl/sASTsv](https://goo.gl/sASTsv)
57. Cho BO, Lao SX, Chang JP (2003) Origin and effect of impurity incorporation in plasma-enhanced ZrO_2 deposition. *J Appl Phys* 93: 9345-9351. [Link: https://goo.gl/AuoKHP](https://goo.gl/AuoKHP)
58. Zaidi SI, Agarwal R, Eichler G, Rihter BD, Kenney ME, et al. (1993) Pharmacodynamic effects of new silicon phthalocyanines: In vitro studies utilizing rat hepatic microsomes and human erythrocyte ghosts as model membrane sources. *Photochem Photobiol* 58: 204-210. [Link: https://goo.gl/kzaanS](https://goo.gl/kzaanS)