

Synthesis, Molecular Spectroscopy, Computational, Thermal Analysis and Biological Activity of Some Orotic Acid Complexes

Mamdouh S. Masoud¹, Alaa E. Ali², Ashimaa S. Abd Elfatah², Gomaa E. Amer^{2*}

¹Chemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt ²Chemistry Department, Faculty of Science, Damanhour University, Damanhour, Egypt Email: *jomaaamer@dmu.edu.eg

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Abstract

Binary orotic acid metal complexes of Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II), and two mixed metals complexes of (Co(II), Ni(II)) and (Ni(II), Cu(II)) were synthesized and characterized by elemental analysis, IR, electronic spectra, magnetic susceptibility, and ESR spectra. The Analysis proved that the ligand has different coordination modes and the complexes were of octahedral, tetrahedral, and trigonal bipyramidal geometries. Molecular modeling techniques and quantum chemical methods have been performed for orotic acid to calculate charges, bond lengths, bond angles, dihedral angles, electronegativity (χ), chemical potential (μ), global hardness (η), softness (σ) and the electrophilicity index (ω). The thermal decomposition of the complexes was monitored by TGA, DTA, and DSC techniques under the N₂ atmosphere. The thermal decomposition mechanisms of the complexes were suggested. The biological activity of orotic acid and some of the complexes are tested against antibacterial and antifungal organisms.

Keywords

Orotic Acid, Synthesis, Complexes, Characterization, Thermal Analysis, Biological Activity

1. Introduction

Vitamin B₁₃ is an orotic acid monohydrate, **Figure 1** (2,6-Dioxo-1,2,3,6-tetrahydro-4-pyrimidine carboxylic acid or uracil-6-carboxylic acid) [1]-[6]. It is extracted from cow's milk [7] [8]. It is found in root vegetables,



Figure 1. Chemical structure of Orotic acid (Vitamin B₁₃).

whey and beef [1]. It is synthesized from aspartic acid and takes part in the biosynthesis of pyrimidine nucleotides [8] [9]. It acts as a mono- and bidentate complex ligands in aqueous solutions at pH equal 10 [10] [11]. Different sites in coordination depend on the solvent, pH, and metal ion [12]. It is able to coordinate a metal atom through the two N atoms of the pyrimidine ring, two carbonyl oxygen atoms, and a carboxylate group [13] [14]. It is manufactured by the body by intestinal flora [15] and it is important for the metabolization of vitamin B₆ and vitamin B₁₂ [16] [17]. It helps the absorption of essential nutrients especially calcium and magnesium and helps the production of genetic material [17]. Both vitamin B₁₃ and magnesium orotate have some effects on the prophylaxis and treatment of heart diseases. Complexes of orotic acid and diaminocyclohexane ligands (DACH) with platinum (II) and palladium (II) ions investigated as potential anticancer agents [18] [19] [20].

2. Experimental

Seven metal-orotic acid complexes were prepared. The inorganic salts [Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) as chloride or sulfates] dissolved in 10 mL distilled water and the ligand dissolved as ammonium salt. The molar amount of the metal chloride or sulfate salts were mixed with the calculated amount of the ligand using different mole ratios (M:L) 1:1 and 1:2. In each case, the reaction mixture refluxed for about 15 min, and then left overnight, where the precipitated complexes separated by filtration, then washed several times with a mixture of EtOH-H₂O and dried in a vacuum desiccator over anhydrous CaCl₂. Two mixed metals complexes of orotic acid were prepared from the combination of [Co, Ni and Cu] by dissolving 1 mmol of the first metal chloride and 1 mmol of the second metal chloride in 10 ml of distilled water. The resulting mixed solution was added to the ligand (1 mmol in 10 ml distilled water). The reaction mixture was refluxed for about 15 min where complexes were precipitated and were filtered, then washed several times with a mixture of EtOH-H₂O and dried in a number of etoH-H₂O and dried in a number of distilled water. The resulting mixed solution was added to the ligand (1 mmol in 10 ml distilled water). The reaction mixture was refluxed for about 15 min where complexes were precipitated and were filtered, then washed several times with a mixture of EtOH-H₂O and dried in a vacuum desiccator over anhydrous CaCl₂. The analytical results of

the isolated mixed metals complexes depicted the formation of complexes with different stoichiometry 2:1:2 (M1, M2, L) respectively, the analytical results in **Table 1**.

3. Measurements

The metal contents were determined by two methods: 1) atomic absorption technique using model 2380 Perkin Elmer absorption spectrophotometer and 2) complexometric titration procedures by standard EDTA solution using the appropriate indicator as reported [21]. C, H, N contents of the synthesized complexes were analyzed by an elemental analyzer. The chloride contents were determined by applying the familiar Volhardmethod [22]. The sulphate content was determined gravimetrically as $BaSO_4$ [23], The nujol mull electronic spectra of the solid complexes were measured, used Perkin-Elmer spectrophotometer model lambda 4B covering the wavelength range 190 - 900 nm [24]. There were

Table 1. Elemental analysis, formula, stoichiometry and color of orotic acid (H₃L) complexes.

	0.1.	D			Calcula	ted/(Found	.)%	
Complexes	Color	Formula	С	н	N	SO4	м	Cl
[Fe ₂ (H ₂ L) ₂ Cl ₂ (OH) ₂ (H ₂ O) ₂]·2H ₂ O	Pale brown	$Fe_2C_{10}H_{16}N_4O_{14}Cl_2$	20.02 (20.01)	2.67 (2.65)	9.34 (9.33)	-	18.69 (18.66)	11.84 (11.82)
[Co (H ₂ L) ₂ (H ₃ L)]·3H ₂ O	Pale pink	$CoC_{15}H_{16}N_6O_{15}$	31.07 (31.05)	2.76 (2.75)	14.49 (14.48)	_	10.18 (10.26)	_
$[Ni_2(H_2L)_2(SO_4)]$ ·5H ₂ O	Pale green	$Ni_2C_{10}H_{16}N_4 O_{13}(SO_4)$	19.53 (19.55)	2.60 (2.59)	9.11 (9.10)	15.62 (15.60)	19.21 (19.19)	_
$[Cu_2 (H_2L)_2(SO_4)(H_2O)_2]$	Blue	$Cu_2C_{10}H_{10}N_4O_{10}(SO_4)$	21.04 (21.03)	1.75 (1.72)	9.82 (9.80)	16.83 (16.82)	22.44 (21.42)	_
[Zn (H ₂ L) ₂ (H ₂ O) ₂]·4H ₂ O	White	$ZnC_{10}H_{18}N_4O_{14}$	24.83 (24.81)	3.72 (3.73)	11.58 (11.56)	_	13.45 (13.43)	
[Cd (H ₂ L) ₂ (H ₂ O) ₂]·H ₂ O	White	$CdC_{10}H_{12}N_4O_{11}$	25.19 (25.17)	2.51 (2.50)	11.75 (11.72)	_	23.51 (23.49)	_
$[Hg (H_3L)_2Cl_2(H_2O)_2]$	White	$HgC_{10}H_{12}N_4O_{10}Cl_2$	19.35 (19.32)	2.93 (2.90)	9.03 (9.01)	_	32.41 (32.40)	11.45 (11.43)
[Co ₂ Ni (HL) ₂ (OH) ₂ (H ₂ O) ₄]·H ₂ O	Pale pink	$Co_2NiC_{10}N_4O_{15}H_{16}$	19.70 (19.69)	2.63 (2.67)	9.19 (9.21)	_	Co 19.37 (19.35) Ni 9.68 (9.66)	_
[Ni ₂ Cu (HL) ₂ (OH) ₂ (H ₂ O) ₄]·H ₂ O	Pale blue	$\rm Ni_2 CuC_{10} N_4 O_{15} H_{16}$	19.54 (19.56)	2.60 (2.57)	9.12 (9.11)	_	Ni 19.21 (19.23) Cu 10.42 (10.41)	_

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taken in potassium bromide disc using Perkin Elmer spectrophotometer, Model 1430 covering a frequency range of 200 - 4000 cm⁻¹. Calibration of frequency reading made with polystyrene film (1602 \pm 1 cm⁻¹). DTA, TGA and DSC of orotic acid and its complexes were carried out using a Shimadzu DTA/TGA-50. The rate of heating was 10°C/min. The cell used was platinum and the atmospheric nitrogen rate flow was 20 ml/min. There was recorded with a reflection spectrometer operating at (9.1 - 9.8) GHZ in a cylindrical resonance cavity with 100 KHZ modulation. The magnetic field was controlled with a (LMR Gauss meter). The g values were determined by comparison with 2,2-diphenyl pyridylhydrazone (DPPH) [25]. Molar magnetic susceptibilities, corrected for diamagnetism using Pascal's constants determined at room temperature (298°K) applying Faraday's method. The instrument [26] was calibrated with Hg [Co (SCN)₄]. Molecular modeling calculations of the ligand and Hg-complex as an example performed with ChemBio Office 3D Ultra 11.0. The optimized conformations (lowest energy) of the individual molecules were determined using dynamic simulations followed by energy minimization [27] to give extra spotlights on the bonding properties of these compounds. Apply hyperchem computer program using PM3 semi-empirical and molecular mechanics force field (MM+) methods to calculate theoretically the quantum chemical parameters [28]. The antimicrobial activities of the free ligand and its complexes were examined by using Agar well diffusion method. The bacterial indicators were: Staphylococcus aureas (ATCC 6538P), Bacillus subtilis (ATCC 19659); (Gram positive), Escherichia Coli (ATCC 8739) strain [29] and Pesudomonas aeruginosa (ATCC 9027); (Gram negative) and one fungal species Candida albicans (ATCC 2091).

4. Results and Discussion

4.1. IR Spectroscopy

The IR of orotic acid and its metal complexes, Table 2, assigned the ligand gave characteristic bands at 3517 cm $^{-1}$ due to the $\nu_{\rm O-H}$ of water molecule, 2835 cm $^{-1}$ of $v_{\text{O-H}}$ of acid, 3015, 2990 cm⁻¹ due to v_{NH} of the pyrimidine ring and 1705, 1665 $\text{cm}^{\text{-1}}$ due to $\nu_{\text{C=O}}$ of the keto and the carboxyl groups, respectively. The broad bands at 3408 - 3530 cm⁻¹ may be assigned to v_{O-H} in all prepared simple and mixed complexes of orotic acid of the coordinated and lattice water molecules. In the case of Cu and Hg complexes, water molecules were found in the coordination sphere while in the case of Co and Ni complexes, the water molecules were lattice water. In other complexes, the water molecules were in both the coordination sphere and lattice water. The $v_{C=0}$ band of the keto and the carboxyl groups appeared at 1705 and 1665 cm⁻¹, respectively, of orotic acid. In simple complexes these bands are shifted to 1688 - 1716 cm⁻¹ for keto group and 1618 -1672 cm⁻¹ for carbonyl group. The appearance of two bands of $v_{\rm NH}$ of the pyrimidine ring at 2822 - 3125 cm⁻¹ confirmed that orotic acid acts as a bidentate in keto form, except, Cu and Ni complexes showed one band of v_{NH} of the pyrimidine ring confirmed that orotic acid acts as a bidentate in the enol form. The v_{C-H}

Compound	ν _{0-Η}	ν _{C-H}	ν _{nH}	v _{O-H} of acid	ν (C=O)	ν (COO) asym	ν _{M-0}	ν_{M-N}	ν _{s-O}
Orotic acid	3517	3115	3015 2990	2835	1705 1665	-	-	-	-
$[Fe_2 (H_2L)_2Cl_2(OH)_2(H_2O)_2] \cdot 2H_2O$	3530	3100	3022 2837	-	1700 1660	-	444	549	-
[Co (H ₂ L) ₂ (H ₃ L)]·3H ₂ O	3448	3205	3125 3000	2809	1715 1644	1486	484	550	-
[Ni ₂ (H ₂ L) ₂ (SO ₄)]·5H ₂ O	3435	3292	2880	-	1700 1643	1476	519	556	627
$[Cu_2 (H_2L)_2(SO_4)(H_2O)_2]$	3408	3300	3100	-	1700 1628	-	550	560	612
$[Zn (H_2L)_2 (H_2O)_2] \cdot 4H_2O$	3477 3412	3221	3000 2822	-	1701 1672	1479	550	563	-
[Cd (H ₂ L) ₂ (H ₂ O) ₂]-H ₂ O	3500	3156	3092 2825	-	1688 1618	1491	560	566	-
$[Hg (H_3L)_2Cl_2(H_2O)_2]$	3524	3168	3000 2990	2850	1702 1669	-	567	-	-
[Co ₂ Ni (HL) ₂ (OH) ₂ (H ₂ O) ₄]·H ₂ O	3414	3028	2835	-	1716 1651	1473	443 505	551	-
[Ni ₂ Cu (HL) ₂ (OH) ₂ (H ₂ O) ₄]·H ₂ O	3452	3120	2827	-	1712 1651	1477	455 555	559	-

Table 2. Fundamental infrared bands (cm⁻¹) of orotic acid (H₃L) and its metal complexes.

band in all complexes appears at 3028 - 3300 cm⁻¹. The band appeared at 2809 cm⁻¹ in the cobalt complex and at 2850 cm⁻¹ in mercury complex at assigned the v_{O-H} of the carboxylic group. The band that appears at 1473 - 1491 cm⁻¹ due to the carboxylate asymmetrical in all complexes except Fe, Cu and Hg complexes, where this band disappeared. The presence of v_{M-N} in the range of 549 - 566 cm⁻¹ for the all metal complexes except Hg-complex provided evidence that orotic acid is bonded to the metal ion through nitrogen. The bonding of oxygen is assigned by the presence of bands at 444 - 567 cm⁻¹ due to v_{M-O} . The Ni and Cu complexes possess sulphate attached to the metal ions supported by the presence of v_{S-O} at 627 and 612 cm⁻¹, respectively.

4.2. Electronic Absorption Spectra and Magnetic Susceptibility Studies

The pale brown iron-complex gave bands at 270, 308, 500 nm, where the first two's are due to CT $(\pi \rightarrow \pi^*)$ [30] and the latter is due to ${}^{6}A_{1g} \rightarrow {}^{4}T_{1g}(S)$ or ${}^{6}A_{1g}(S) \rightarrow {}^{4}E_{g}(G) + {}^{4}A_{1g}(G)$ multiplicity forbidden transitions [31] [32]. Its room temperature magnetic moment value of 6.20 BM typified the existence of octahedral configuration. The data assigned a type of Fe-Fe interaction.

The cobalt (II) complex exhibits bands at 450 and 535 nm which may be assigned to ${}^{4}T_{1g} \rightarrow {}^{4}A_{2g}(F)$ (ν_{1} -transition) and ${}^{4}T_{1g}(F) \rightarrow {}^{4}T_{1g}(P)$ (ν_{2} -transition) indicating octahedral structure with magnetic moment 3.4 BM [33] [34]. The nickel complex gives a band at 650 nm which may be assigned to ${}^{3}T_{1}(F) \rightarrow {}^{3}T_{1}(P)$ indicating tetrahedral geometry with a total magnetic moment 5.6 BM [35].

The copper complex, exhibits two bands at 280 and 760 nm. The first δ -band is overlapped with that of orotic acid [30]. The band at 760 nm suggests trigonal bipyramidal (TBP) [36] μ_{eff} = 2.58 BM. The data assigned a type of weak Cu-Cu interaction. The proposed structure depends on bidentate nature of two molecules of orotic acid through carboxylic acid and imino group with the presence of one sulphate ion and two water molecules in the outer sphere. Zn, Cd and Hg complexes are diamagnetic with octahedral environment. The proposed structures of Zn and Cd complexes depend on the bidentate nature of two molecules of orotic acid through carboxylic acid and imino group with the two water molecules in the inner sphere and four, one water molecules in outer sphere for Zn and Cd complexes of orotic acid, respectively. Hg-complex depends on the monodentate nature of two molecules of orotic acid through carboxylic acid through carboxylic acid through carboxylic acid through carboxylic acid through the two water molecules in outer sphere for Zn and Cd complexes of orotic acid, respectively. Hg-complex depends on the monodentate nature of two molecules of orotic acid through carboxylic acid through carboxylic acid through carboxylic acid with the presence of two chloride ions and two water molecules in the inner sphere.

The pale pink Co-Ni complex, showed bands at $\lambda_{max} = 650$ and 320 nm with total room temperature magnetic moment value 9.80 BM, to assume 7.0 and 2.8 BM for Co and Ni, respectively, which may be assigned to the transition ${}^{4}A_{2} \rightarrow {}^{4}T_{1}$ (P) indicating tetrahedral structure for cobalt [33] [37] and ${}^{3}A_{2g} \rightarrow {}^{3}T_{1g}$ (P) indicating octahedral structure for nickel [33]. The pale blue Ni-Cu complex, showed bands at $\lambda_{max} = 630$ and 720 nm with room temperature magnetic moment value 7.33 BM for the complex, where the two nickel are with 5.6 BM and one copper with 1.73 BM. The spectral properties are assigned to the transition ${}^{3}T_{1}(F) \rightarrow {}^{3}T_{1}(P)$ and ${}^{2}E_{g} \rightarrow {}^{2}T_{2g}(D)$ respectively, indicating tetrahedral structure for nickel and octahedral structure for copper [32]. All the data are given in Table 3, while the structures of the complexes are collected in Figure 2.

Complex	λ_{\max} (nm)	μ_{eff}	Geometry
$[Fe_2(H_2L)_2Cl_2(OH)_2(H_2O)_2]\cdot 2H_2O$	270,308,500	6.20	O _h
$[\text{Co}(\text{H}_2\text{L})_2(\text{H}_3\text{L})]\cdot 3\text{H}_2\text{O}$	450,535	3.4	$O_{\rm h}$
$[Ni_{2}(H_{2}L)_{2}(SO_{4})]\cdot 5H_{2}O$	650	5.6	T_{h}
$[Cu_2(H_2L)_2(SO_4)(H_2O)_2]$	280,760	2.58	TBP
$[\text{Co}_2\text{Ni}(\text{HL})_2(\text{OH})_2(\text{H}_2\text{O})_4]\cdot\text{H}_2\text{O}$	650,320	9.80	$\begin{array}{c} \text{Co } T_h \\ \text{Ni } O_h \end{array}$
[Ni ₂ Cu(HL) ₂ (OH) ₂ (H ₂ O) ₄]·H ₂ O	630,720	7.33	Ni T _h Cu O _h

Table 3. Nujol mull electronic absorption spectra λ_{max} (nm), room temperature effective magnetic moment values (μ_{eff} 298°K) and geometries of orotic acid metal complexes.



Figure 2. The structures of the prepared complexes.

4.3. Electron Spin Resonance of Copper Complexes

The ESR spectra of the simple copper complex (1:1) and mixed Ni-Cu complex (2:1) were recorded. The spectra indicate $g_{//}$ and g_{\perp} components, axial compressed and axial elongated for both simple and mixed complexes, respectively. The obtained g values were due to the influence of the exchange interaction, which makes the hyperfine lines smaller [38]. These g parameters were calculated, $g_{//} = (2.0017, 2.3026)$ and $g_{\perp} = (2.2387, 2.0617)$, respectively. The $\langle g \rangle$ values are calculated from the relation [39] $\langle g \rangle = (g_{//} + 2 g_{\perp})/3$ and equal 2.1597 and 2.1420, respectively, **Table 4**. For $[Cu_2(H_2L)_2(SO_4)(H_2O)_2]$ complex, the G = 0.007 reflecting Cu-Cu interaction in solid state ,while in the case of $[Ni_2Cu(HL)_2(OH)_2(H_2O)_4] \cdot H_2O$ complex the value is >4 reflecting that there no Cu-Cu interaction [24] [39]. This agrees with the assumed structure where nickel surrounded the copper preventing Cu-Cu interaction.

4.4. Molecular Modeling

The molecular modeling calculations of orotic acid and Hg-complex were calculated, Figure 3 and Figure 4, concerning the charge, bond lengths, bond angles and dihedral angles. These calculations are based on neglecting the possibility of hydrogen bonding. Orotic acid is coordinated to different metal ions through an oxygen atom of carboxylate group O(11) hydrogen atom of imino group H(13)and lone pair of nitrogen side N(3). Oxygen atom of this group is caring more electronegative charge confirming active sites for coordination. The bond lengths of two N-H are nearly the same and lie within values 1.007 and 1.008 (Å) for N(6)-H(12) and N(3)-H(13), respectively. The bond lengths of C(9)-H(15) is 1.101 (Å) and O(11)-H(14) is 0.971 (Å). All C-C bond lengths lie in the range 1.353 - 1.490 (Å) for C(2)-C(9), C(1)-C(2) and C(7)-C(9). These values reduce the C-N bond lengths in between the range 1.345 - 1.370 (Å) for C(2)-N(3), N(3)-C(4), C(4)-N(6) and N(6)-C(7). However, for all C-O bond lengths lie within the range 1.205 - 1.348 (Å) for C(4)-O(5), C(7)-O(8), C(1)-O(10), and C(1)-O(11) (*i.e.* all C-C > C-N > C-O). This is due to electronegativity, where as it increased the bond length decreased. The angles between atoms in orotic acid are around 120° due to sp2 hybridization of the atoms. The bond angle around 109.5° is due to sp3 hybridization of the atoms for N (3)-C(4)-N(6). The deviation with of angles is due to distorted electronic effects for C(1)-C(2)-N(3), C(2)-N(3)-C(4), C(2)-N(3)-H(13), N(3)-C(4)-N(6),

C(4)-N(6)-C(7), C(4)-N(6)-H(12), C(7)-N(6)-H(12), N(6)-C(7)-C(9),

O(8)-C(7)-C(9). It seems that, some of dihedral angles lie in -179.5° , referred to the distortion in linearity of sp3 hybridization. The dihedral angles proved the

Table 4. Room temperature ESR spectral parameters for copper complexes.

Complexes	G	g,,	g⊥	<g></g>	$A_{\prime\prime} imes 10^{-4}$	$A_{\perp} imes 10^{-4}$	a²	F ²
$[Cu_2(H_2L)_2(SO_4)(H_2O)_2]$	0.0071	2.0017	2.2387	2.1597	178	231	0.6351	1.2239
$[\mathrm{Ni}_{2}\mathrm{Cu}(\mathrm{HL})_{2}(\mathrm{OH})_{2}(\mathrm{H}_{2}\mathrm{O})_{2}]\cdot\mathrm{H}_{2}\mathrm{O}$	4.9044	2.3026	2.0617	2.1420	227	448	0.9963	0.9931

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Figure 3. Molecular modeling of orotic acid.



Figure 4. Molecular modeling of mercury complex.

near planarity, where the angles are of nearly 180°C and 0°C. The difference is due to the syn and anti-positions of the investigated atoms, the anti gave 180°C and the syn gave 0°C. It's observed that, the negative charge is located at O(11), while positive charges at N(1), so, the deprotonation occurred from O(11) from OH of carboxylate group, where most of bond angles are around 120° of the configurations with sp2-hybridization, and the dihedral angles are with $179^{\circ} \pm$ 1°, where the distribution of the atoms are in the same plane. For mercury complex [Hg (H₃L)₂Cl₂(H₂O)₂], all N-H bond lengths between 0.998 - 1.002 (Å) for N(6)-H(36), N(12)-H(32), N(14)-H(33) and N(3)-H(35). The bond lengths of two C-H within values 1.107 and 1.100 (Å) for C(9)-H(39) and C(15)-H(38), respectively while the bond lengths for all O-H lie within the range 0.961 - 0.980 (Å) for O(25)-H(31), O(25)-H(29), O(24)-H(30), O(24)-H(28), O(21)-H(34) and O(11)-H(37) (*i.e.* all C-H > N-H > O-H) also all C-C > C-N > C-O. These are due to increased electronegativity, decrease leading to bond length. The charge density of Hg in its complex proved that there is a type of charge transfer to metal. The angles around 120° and 109.5° are due to sp2 and sp3 hybridization of the atoms. The deviations appeared in the region where the rings are fused together. Some dihedral angles lie in the range of $(158.910^{\circ}) - (-179.684^{\circ})$, referred to the distortion in linearity of sp3 hybridization. However, the dihedral angles in the range of $(121.446^{\circ}) - (-145.416^{\circ})$ are due to deviation from sp2 hybridization, while the dihedral angles from $(43.157^{\circ}) - (-71.697^{\circ})$ pointed to the strong deviation from perpendicular angle attributed to the distortion effect. More ever, the dihedral angles proved the near planarity, where the angles are of nearly 180°C and 0°C. The difference was due to the syn and anti-positions of the investigated atoms, the anti gave 180°C and the syn gave 0°C.

The absolute hardness (η) and softness (σ) are important properties to measure the molecular stability and reactivity. In a complex formation system, the ligand acts as a Lewis base while metal ion acts as a Lewis acid. Metal ion is soft acid and thus soft base ligand is most effective for complex formation [33]. A hard molecule has a large energy gap and a soft molecule has a small energy gap. Soft molecules are more reactive than hard ones because they could easily offer electrons to an acceptor. Orotic acid has the highest (σ) value, (9.615 eV obtained by PM3 semi-empirical method to be more soft molecule compared with Hg-complex, *i.e.* more reactive than hard one because it could easily offer electrons to an acceptor metal and possess high ability for complexation. Critical changes for the quantum chemical parameters for orotic acid on complexation with Hg(II), **Table 5**.

4.5. Thermal Analysis

The thermal data of orotic acid, **Table 6**, gave three peaks. Two of them are endothermic at 429.40 and 636.10 $^{\circ}$ K with activation energies of 11.31 and 168.53 kJ/mole and their orders are 1.27 and 0.80. The rest exothermic peak at 774.10 $^{\circ}$ K with the activation energy of 72.50 kJ/mole and the reaction order is 0.97.

The DTA data of $[Fe_2(H_2L)_2Cl_2(OH)_2(H_2O)_2]\cdot 2H_2O$ complex give two peaks, **Table 6**, at 359.30 and 641.90 °K with activation energies 25.86 and 128.80 kJ/mole, respectively and the orders of reactions are 2.67 indicating 3rd order, and 1.45 of 1st order. The first peak is endothermic and the second one is exothermic in nature. The TGA data confirmed these results where it gave two peaks, the first one is due to dehydration of five lattice and coordinated water molecules and loss of CO while the second one is due to elimination of 4HCN, 2HCl and 5CO and formation of Fe₂O₃.

The DTA of Co-orotic acid complex, $[Co (H_2L)_2(H_3L)]\cdot 3H_2O$, give three peaks, **Table 6** at 369.50, 555.40 and 745.80 °K. All peaks are endothermic except

Table 5. Quantum chemical parameters (eV) of the orotic acid and mercury complex calculated by PM3 method.

Compound	E _H	EL	ΔE	x	μ	η	ω	σ
Orotic acid	3.682	3.889	0.207	-3.786	3.786	0.104	68.912	9.615
$[\mathrm{Hg}(\mathrm{H_3L})_2\mathrm{Cl}_2(\mathrm{H_2O})_2]$	-10.072	-1.675	8.397	5.874	-5.874	4.199	4.109	0.238

Table 6. DTA analysis of orotic acid (H3L) and its simple and mixed metal complexes.

						ΔS *		_		Wt.	Loss %	
Complex	Туре	1m (°K)	E₄ kJ∙mol ⁻¹	n	am	kJ·K ^{−1} · mol ^{−1}	∆H* kJ·mol ⁻¹	Z S ⁻¹	Temp. (°C) TGA	Calc.	Found	Assignment
	Endo	429.40	11.31	1.27	1.47	-0.13	-55.88	7.74	67°C - 232.3°C	10.34	10.07	Dehydration of water molecule
Orotic acid	Endo	636.10	168.53	0.80	2.38	-0.11	-70.78	50.46	232.3°C - 360°C	25.27	26.38	Elimination of CO ₂
	Exo	774.10	72.50	0.97	2.10	-0.12	-90.18	22.13	360°C - 580.2°C	57.43	56.49	Elimination of the rest of ligand and formation of carbon residue
	Endo	359.30	25.86	2.67	1.47	-0.12	-43.36	28.84	56.4°C - 264.1°C	19.69	19.92	Elimination of 5H ₂ O and CO
$[Fe_{2}(H_{2}L)_{2}Cl_{2}(OH)_{2}$ $(H_{2}O)_{2}]2H_{2}O$	Exo	641.90	128.80	1.45	1.88	-0.11	-69.98	65.55	264.1°C - 468.7°C	53.57	53.32	Elimination of 4HCN, 2HCl, 5CO and formation of Fe ₂ O ₃
	Endo	369.5	26.04	1.85	1.63	-0.12	-44.91	25.30	60.1°C - 240.9°C	4.66	4.80	Dehydration of 1.5H ₂ O
[Co(H ₂ L) ₂ (H ₃ L)]	Endo	555.4	117.86	1.32	1.95	-0.11	-61.16	66.43	240.9°C - 335.9°C	12.26	12.30	Elimination of 1.5H ₂ O and CO ₂
3H ₂ O	Exo	745.8	275.01	0.88	2.31	-0.11	-79.07	80.97	335.9°C - 524.8°C	55.60	55.40	Elimination of 2NH ₃ , 2CO ₂ , 2NO, CO, $C_4N_2H_4O_2$ and formation of CoO+7C
	Endo	379.4	31.30	0.83	2.20	-0.12	-47.41	16.30	55.3°C - 139.2°C	17.42	17.32	Loss of $5H_2O$ and NH_3
	Endo	457.2	93.95	1.50	1.86	-0.11	-50.91	69.63	139.2°C - 258.3°C	27.19	26.92	Elimination of 2NO, HCN and SO ₃
[Ni ₂ (H ₂ L) ₂ (SO ₄)] 5H ₂ O	Exo	653	27.73	1.17	1.75	-0.12	-80.40	11.82	258.3°C - 470.2°C	10.75	10.86	Elimination of 2O ₂ and H ₂
	Exo	787.9	402.96	1.41	1.94	-0.10	-78.25	171.60	470.2°C - 593.5°C	2.60	2.60	Elimination of $0.5O_2$ and formation of $2NiO+9C$

Continued

[Cu ₂	Endo	366.40	12.36	2.43	1.35	-0.13	-46.58	13.03	34.1°C - 200°C	11.22	11.42	Dehydration of two molecules coordination water and CO
$(H_2L)_2(SO_4)(H_2O)_2]$	Exo	655.50	412.17	2.35	1.63	-0.10	-63.69	267.20	200°C - 428°C	66.29	65.94	Elimination of the rest of complex and formation of 2Cu
	Endo	403.50	28.84	1.30	1.84	-0.12	-49.28	21.53	77.2°C - 196.6°C	11.58	11.64	Loss of 3H ₂ O and H ₂
	Endo	535.30	162.52	1.21	2.03	-0.11	-57.67	91.76	196.6°C - 360.8°C	12.00	11.94	Dehydration of 3H ₂ O and 2H ₂
$[Zn(H_2L)_2$ $(H_2O)_2]4H_2O$	Exo	615.70	28.51	1.35	1.70	-0.12	-75.19	14.13	360.8°C - 500.8°C	38.07	38.00	Elimination of $C_2N_2O_2$ and $C_2N_2O_3$
	Exo	774.80	153.50	1.53	1.84	-0.11	-83.19	66.23	500.8°C - 599.1°C	9.10	8.85	Elimination of CO_2 and formation of ZnO and 5C
	Endo	381.10	16.90	1.41	1.66	-0.13	-48.10	13.98	44.8°C - 108.8°C	11.34	11.27	Dehydration of water molecules from outer and coordination sphere
[Cd(H ₂ L) ₂ (H ₂ O) ₂]	Endo	533.10	74.01	1.41	1.87	-0.11	-60.66	44.52	108.8°C - 280.3°C	16.80	16.86	Elimination of CO and C ₂ N ₂
H ₂ O	Endo	612	337.30	1.00	2.21	-0.10	-62.85	147.15	200.2*0 400.7*0	22.54	22.05	Elimination of
	Endo	646.10	373.06	1.56	1.87	-0.10	-64.15	210.18	280.3 C - 400.7 C	32.70	52.95	$\rm CH_4$ and 5CO
	Exo	804.70	164.07	1.01	2.15	-0.11	-87.90	51.00	400.7°C - 540.9°C	12.18	12.02	Elimination of NO, CH ₂ N and formation of CdO
	Endo	363.80	15.61	1.55	1.61	-0.13	-46.00	14.20	59.8°C - 128.1°C	8.71	8.33	Loss of 3H ₂ O
$[Hg (H_3L)_2Cl_2(H_2O)_2]$	Exo	629.40	18.21	1.37	1.50	-0.13	-79.17	8.90	128.1°C - 462.6°C	89.32	89.63	Elimination of the rest of complex and formation of carbon residue
	Exo	588.46	511.06	1.09	2.14	-0.10	-57.65	270.33	60.4°C - 335.9°C	19.20	18.72	Loss of 5H ₂ O + HCN
$[Co_2Ni(HL)_2$	Exo	617	298.89	1.20	2.06	-0.10	-63.26	148.99				Elimination of the
(011) ₂ (11 ₂ 0) ₄]·Π ₂ 0	Endo	628	363.11	1.17	2.29	-0.10	-63.38	177.55	335.9°C - 488.3°C	43.83	44.54	rest of complex and formation of 2CoO + NiO

	Exo	469.52	124.24	1.09	2.11	-0.11	-51.95	73.80	80.1°C - 359.7°C 14.65	15.12	Dehydration of water molecules from outer and coordination
[Ni ₂ Cu(HL) ₂	Exo	480.18	162.07	1.78	1.77	-0.11	-50.87	127.12			sphere
$(OH)_2(H_2O)_4] \cdot H_2O$	Exo	494.24	400.81	1.54	1.88	-0.10	-48.43	321.26			
	Exo	680.75	352.57	1.45	1.91	-0.10	-68.19	179.20	359.7°C - 515.9°C 47.87	47.63	Elimination of 4HCN, $2CO_2$, 3CO, CH_2 and formation of 2NiO + CuO

the third one of exothermic nature. The TGA data gave three steps; the first one was due to the evolved outer sphere water molecules, while the last two steps were due to the decomposition steps and formation of CoO + 9C as a final product.

The DTA data of $[Ni_2(H_2L)_2(SO_4)]$ ·5H₂O complex, **Table 6**, showed four peaks, at 379.40, 457.20, 653 and 787.90 °K with activation energies 31.30, 93.95, 27.73 and 402.96 kJ/mole, and the orders of reactions were 0.83, 1.50, 1.17 and 1.41, respectively. All peaks are of the first order type. The first and second peaks are of endothermic type while the third and the fourth peaks are of exothermic agitation types [40]. This can be proved by TGA data which gave well defined four peaks, the first is due to the evolving of lattice water molecules and loss of NH₃. The last three peaks are due to the decomposition steps and formation of 2NiO + 7C.

The $[Cu_2(H_2L)_2(SO_4)(H_2O)_2]$ complex, **Table 6**, showed two well defined peaks at 366.40 and 655.50 K with activation energies of 12.36 and 412.17 kJ/mole, their orders of reactions are 2.43 and 2.35 indicating second order, respectively. The first peak is endothermic and the second is exothermic. However, the TGA data gave two peaks, the first one is due to dehydration process of coordinated water molecules and elimination of CO while the last peak is due to the decomposition step ended with the formation of 2Cu.

However, the Zinc complex $[Zn(H_2L)_2(H_2O)_2]\cdot 4H_2O$, **Table 6**, showed four peaks at 403.50, 535.30, 615.70 and 774.80 °K. The first and second are endothermic in nature while the last two peaks are of exothermic behavior. The calculated energies of activation are 28.84, 162.52, 28.51 and 153.50 kJ/mole accompanied with order of reactions 1.30, 1.21, 1.35 and 1.53, respectively. All orders are of the first type except the fourth is the second order. Also, the TGA data gave four peaks, the first and second peaks were due to a dehydration reaction of lattice and coordinated water molecules and loss of $3H_2$ while the last two strong exothermic peaks were due to the decomposition reactions ended with

the formation of ZnO + 5C as a final product.

The DTA data of $[Cd (H_2L)_2(H_2O)_2]\cdot H_2O$ complex, gave five peaks. Four of them are endothermic at 381.10, 533.10, 612 and 646.10 °K with activation energies of 16.90, 74.01, 337.30 and 373.06 kJ/mole. The last exothermic peak at 804.70 °K with activation energies is 164.07 kJ/mole. Also, the TGA data gave four peaks, the first endothermic is due to dehydration process of lattice and coordinated water molecules and the last three strong endothermic and exothermic are due to decomposition steps with the formation of CdO as a final product with 26.89% (calc. 26.87%). From DTA, two endothermic peaks in the temperature range 280.3°C - 400.7°C overlapped with one peak in TGA which corresponds to elimination of CH₄ + 5CO, **Table 6**.

The [Hg (H₃L)₂Cl₂(H₂O)₂] complex, **Table 6**, showed two well defined peaks at 363.80 and 629.40 °K from the DTA data with activation energies of 15.61 and 18.21 kJ/mole. Their orders of reactions are 1.55 indicating 2nd order and 1.37 indicating 1st order. The two lines are intercepting with each other at 505.05 °K (phase transition). The first peak is endothermic and the second is exothermic. Also, the TGA data gave two peaks, the first one is due dehydration of coordinated water molecules while the rest peak is due to thermal decomposition of ligand and sublimation of Hg in temperature range 180° C - 560° C with the formation of carbon residue as a final, **Figure 5**.

The $[CO_2Ni(HL)_2(OH)_2(H_2O)_4]$ ·H₂O complex, **Table 6**, showed three peaks at 588.46, 617 and 628 °K with activation energies 511.06, 298.89 and 363.11 kJ/mole, their orders of reactions are 1.09, 1.20 and 1.17, respectively. All peaks are of the first order. The first and second peaks are exothermic and the third is endothermic. However, the TGA data gave two peaks, the first one is due to







Dichloro(2,6-Dioxo-1,2,3,6-tetrahydro-pyrimidine-4-carboxylic acid) (2,6-Dioxo-1,2,3,6-tetrahydro-pyrimidinato-4-car baldehyde)mercury(II)

Figure 5. Thermolysis of [Hg(H₃L)₂Cl₂(H₂O)₂] complex.

elimination of water molecules and HCN and the second step is due to the decomposition step ended with the formation of 2CoO + NiO as a final product. From DTA, the last two peaks in the temperature range 335.9°C - 488.3°C overlapped to give one peak in TGA which corresponds to the loss of $5H_2O + HCN$.

The thermolysis of mixed $[Ni_2Cu(HL)_2(OH)_2(H_2O)_4]$ ·H₂O complex, Table 6, showed four peaks at 469.52, 480.18, 494.24 and 680.75 °K with activation energies 124.24, 162.07, 400.81 and 352.57 kJ/mole. Their orders of reactions were 1.09, 1.78, 1.54 and 1.45, respectively. All peaks are exothermic. The first and the fourth peaks are of the first order, the second and the third peaks are of the second order type. However, the TGA data gave two peaks, the first one is due to dehydration process of outer and coordinated water molecules and the second step is due to the decomposition step ended with the formation of 2NiO + CuO as a final product with 37.25% (calc. 37.45%). From DTA, three exothermic peaks in the temperature range 80.1°C - 359.7°C overlapped to give one peak in TGA which corresponds to the loss of water molecules, Figure 6.

4.6. Differential Scanning Calorimetry

DSC curves are obtained for $[Fe_2(H_2L)_2Cl_2(OH)_2(H_2O)_2]\cdot 2H_2O$, $[Co(H_2L)_2(H_3L)]\cdot 3H_2O$, $[Cu_2(H_2L)_2(SO_4)(H_2O)_2]$, $[Zn(H_2L)_2(H_2O)_2]\cdot 4(H_2O)$ and



1,2,3,6-tetrahydro-pyrimidinato-4-carboxylato) copper(II) nickel(II) monohydrate

µ-dihydroxo bis(2,6-Dioxo-1,2,3,6-tetrahydropyrimidinato-4-carboxylato) copper(II) nickel(II)



Figure 6. Thermolysis of $[Ni_2Cu (HL)_2(OH)_2(H_2O)_4] \cdot H_2O$ complex.

 $[Cd (H_2L)_2(H_2O)_2]$ ·H₂O complexes, recorded under a flow of N₂. The glass transition temperature (Tg) exhibits dehydration process followed by thermal agitation [41]-[46]. The crystallization temperature (Tc), will have gained enough energy to move into very ordered arrangements after that it gave off heat through an exothermic transition. This is compatible with the explanation of TGA for these complexes. For all systems (Tg) is at 137.2°C - 220°C, [Fe₂ (H₂L)₂Cl₂(OH)₂(H₂O)₂]·2H₂O complex have the highest value Tg, where this complex of octahedral geometry with two water molecules in the inner sphere,

Figure 7 The crystallization temperature (Tc) is at 227.3 °C - 277.9 °C. DSC



Figure 7. DSC curves for $[Fe_2L_2Cl_2(OH)_2(H_2O)_2]\cdot 2H_2O$. (a) Dependence of heat flow on temperature. (b) Dependence of specific heat on temperature © Variation of C_p/T versus *T*.

plot is used to carefully determine the melting temperature through an endothermic transition. There is no melting temperature (Tm) in all these complexes except $[Fe_2(H_2L)_2Cl_2(OH)_2(H_2O)_2]\cdot 2H_2O$ has Tm, 305.8°C, Table 7. However, the Debye model [47] [48] is applied to describe capacity change over a large temperature range. The C_p can be represented as the following empirical form: $C_p = aT + b$, Plotting C_p versus *T*, a straight line is obtained, a and b parameters can be determined from the slope and intercept of the line, respectively. Debye model on selected complexes is given from the following equations [47] [48].

$$C_p = \alpha T^3 + \gamma T$$
 $\frac{C_p}{T} = \alpha T^2 + \gamma$

where, γ and α are the coefficients of electronic and lattice capacities, respectively. C_p is the heat capacity. Plots of C_p/T versus T^2 should yield straight lines with α slope and intercept γ , **Table 8**.

4.7. Biological Activity

Five microorganisms representing different microbial categories, {two Gram-positive (Staphylococcus Aureas ATCC6538P and Bacillus subtilis ATCC19659), two Gram negatives (Escherichia coli ATCC8739 strain and Pseudomonas aeruginosa ATCC9027) [49] [50] bacteria and one fungal species Candida albicans (ATCC 2091) were used. The study included orotic acid and some of its metal complexes. Two different broad antibiotics (Ciprofloxacin and Clotrimazole) are used as references. Ligand showed antimicrobial activity

Table 7. Glass, c	crystallization an	d melting	temperatures	of selected	complexes
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Commission	Therm	al transitions (°C	C)
Complexes	Tg	Тс	Tm
$[Fe_2(H_2L)_2Cl_2(OH)_2(H_2O)_2]\cdot 2H_2O$	220	null	305.8
$[\text{Co}(\text{H}_2\text{L})_2(\text{H}_3\text{L})]\cdot 3\text{H}_2\text{O}$	180.3	277.9	null
$[Cu_2(H_2L)_2(SO_4)(H_2O)_2]$	166.0	260.9	null
$[Zn(H_2L)_2(H_2O)_2]\cdot 4H_2O$	137.2	227.3	null
$[Cd(H_2L)_2(H_2O)_2]\cdot H_2O$	137.4	238.7	null

Table 8. Debye equation parameters for selected complexes.

Complexee	$C_p =$	aT+b	$C_{_{p}}/T = aT + \gamma$		
Complexes	а	Ь	$\alpha \times 10^{-6}$	γ	
$[Fe_2(H_2L)_2Cl_2(OH)_2(H_2O)_2]\cdot 2H_2O$	2.5873	-692.38	1	0.8202	
$[\text{Co}(\text{H}_2\text{L})_2(\text{H}_3\text{L})]\cdot 3\text{H}_2\text{O}$	0.8248	-253.29	1	0.0205	
$[Cu_2(H_2L)_2(SO_4)(H_2O)_2]$	0.0041	2.1754	5	0.3498	
$[Zn(H_2L)_2(H_2O)_2]\cdot 4H_2O$	0.6228	-77.483	4	-0.2693	
$[Cd(H_2L)_2(H_2O)_2]\cdot H_2O$	1.6451	-569.19	4	-0.3427	

Complexes	DMSO	<i>Staphylococcus aureus</i> (ATCC 6538P)	<i>Bacillus subtilits</i> (ATCC 19659)	Pseudomonas aeruginosa (ATCC 9027)	<i>Escherichia coli</i> (ATCC 8739)	<i>Candida albicans</i> (ATCC 2091)
Orotic acid	8	10	10	10	10	8
$[\mathrm{Ni}_2(\mathrm{H}_2\mathrm{L})_2(\mathrm{SO}_4)]{\cdot}5\mathrm{H}_2\mathrm{O}$	8	10	10	10	10	10
$[Cu_2(H_2L)_2(SO_4)(H_2O)_2]$	8	8	8	8	8	8
$[Fe_2(H_2L)_2Cl_2(OH)_2(H_2O)_2]\cdot 2H_2O$	8	8	8	8	8	8
$[\mathrm{Hg}(\mathrm{H_{3}L})_{2}\mathrm{Cl}_{2}(\mathrm{H_{2}O})_{2}]$	8	29	26	23	26	27
$[\mathrm{Ni}_{2}\mathrm{Cu}(\mathrm{HL})_{2}(\mathrm{OH})_{2}(\mathrm{H}_{2}\mathrm{O})_{4}]{\cdot}\mathrm{H}_{2}\mathrm{O}$	8	8	8	8	8	8
$[\text{Co}_2\text{Ni}(\text{HL})_2(\text{OH})_2(\text{H}_2\text{O})_4]\cdot\text{H}_2\text{O}$	8	8	8	8	8	8
Ciprofloxacin	9	30	30	30	30	-
Clotrimazole	10	-	-	-	-	17

Table 9. Antibacterial and antifungal activity against some reference strains expressed in absolute activity (AU).

against Gram-positive bacteria and Gram negative and has no activity against Candida albicans. $[Ni_2(H_2L)_2(SO_4)]\cdot 5H_2O$ and $[Hg(H_3L)_2Cl_2(H_2O)_2]$ complexes showed antimicrobial activity against all the test organisms, **Table 9**. Hg- complex is the most effective.

5. Conclusion

The complexes of orotic acid were synthesized and characterized by different spectroscopic methods. The stoichiometry of complexes was determined by the analytical data. The complexes have different geometries. The Nujol mull electronic spectra confirmed the expected results. An ESR spectrum of copper was studied for binary and mixed complexes. The spectral data confirmed that orotic acid acts as a bidentate ligand. Some complexes showed antibacterial and antimicrobial activity against some strains. The kinetic and thermodynamic parameters were calculated from the differential thermal analysis curves. All complexes were thermally decomposed under nitrogen atmosphere.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this research article.

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