



**SYNTHESIS, STRUCTURAL CHARACTERIZATION AND
BIOLOGICAL APPLICATION OF MIXED LIGANDS
COMPLEXES OF COUMARIC ACID AND COUMARINE WITH
SOME TRANSITION METAL CATIONS**

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Four new mixed ligand transition metal (Co(II), Ni(II), Cu(II) and Zn(II)) complexes of *p*-coumaric acid and coumarine have been synthesized and the structurally characterized. These complexes contain hydrate water except the Cu(II) complex. Coumaric acid is a monodentate ligand except Cu(II)-complex in which a bidentate coordination mode could be observed. The thermal decomposition starts with dehydration of Co(II), Ni(II) and Zn(II) complexes while in Cu(II) complex decomposition starts with fragmentation of organic ligands at 145 °C. Studies on their biological activity have also been performed. Molecular formulas of the obtained complex are as follows: **(I)** [Co(C₉H₇O₃)₂(C₉H₆O₂)₂(H₂O)₂]⁺4H₂O; **(II)** [Ni(C₉H₇O₃)₂(C₉H₆O₂)₂(H₂O)₂]⁺6H₂O; **(III)** [Cu(C₉H₇O₃)₂(C₉H₆O₂)₂]⁺ and **(IV)** [Zn(C₉H₇O₃)₂(C₉H₆O₂)₂(H₂O)₂]⁺3H₂O. The ratio of metal: *p*-coumaric acid: coumarine in complexes are found to be 1:2:2. The complex **IV** is diamagnetic, and the other complexes are paramagnetic. According to BM data and result of electronic spectra, the geometry for all of the complexes has thought to be octahedral coordination around the metal ions.

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Introduction

Coumarin (Coum), chemically 2H-chromene-2-one, belongs to the class of benzo[*a*]pyrones isolated from Tonka beans (Semen Tonca), removed from the tree called *Dipteryx odorata* (coumarone odorata), by Vogel in 1822. This species belongs to Fabaceae family and grows in South America.¹ The coumarin derivatives are present in various plants² such as Tonka bean, lavender, sweet clover grass and licorice and in food such as strawberries, cherries, apricots and cinnamon. Colored coumarin derivatives with fluorescing properties are widely used in solar energy and laser systems.³

Several coumarin derivatives were isolated from various plants and microorganisms, and these generally were called after the name of the plant or place where the plant grown, which has resulted various trivial names for the same compound. For example, 7-hydroxycoumarin is also known as umbelliferone, skimmetin and hydrangea.¹ The coumarin has widespread application as an anticoagulant, spasmolytic and bacteriostatic agents and derivatives are also used as antibiotics, fungicides, anti-inflammatory, anticoagulant and anti-tumor agents.²⁻⁴ The coumarin has sedative and anti-inflammatory effect and widely used in the perfumery and tobacco industry and as odorizer in some insecticides.⁵ Coumarin and its metal complexes inhibit the proliferation of bacteria such as *Bacillus cereus*, *Pseudomonas aeruginosa* and *Escherichia coli*.⁶ Coumarin plays an important role in the growth of plants because it inhibits enzymes such as amylase, invertase and stimulates oxidase and peroxidase enzymes.

The *p*-coumaric acid (p-CoumH) is derived from cinnamic acid and plays important role in color, odor and taste of several plant materials.⁷ It is found in orange,⁸ cherry, coffee, chocolate and wine.⁹ It has been reported that *p*-coumaric acid provides protection against stress. Besides the ability to kill tumor cells, it has been reported that it may cause oxidative damage in DNA. In the case of taken in large amounts, it exerts toxic effect.¹⁰ It is known that the *p*-coumaric acid is very effective against gastric cancer.

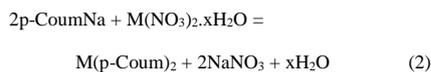
Experimental

In the synthesis of complexes, Coum and p-CoumH, obtained from Sigma-Aldrich, were used.

Monosodium salt of *p*-coumaric acid (p-CoumNa) was prepared by the reaction of 0.02 mol p-CoumH and 0.02 mol of sodium bicarbonate solutions prepared in 50:50 (v/v) EtOH:H₂O. The solutions of starting materials are mixed and stirred until cessation of CO₂ evolution.

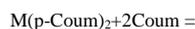


The aqueous solution of metal salts (0.01 mol) and the solution of p-CoumNa (0.02 mol) were mixed and stirred at 55 °C for a day.



x=6 (Ni, Co); x=7 (Zn) and x=3(Cu);

The aq. EtOH=1:1 solution of *p*-coumaric acid transition metal salts (0.01 mol) was added to the 50:50 (v/v) EtOH:H₂O solution of coumarin (0.02 mol) and the mixture was stirred and refluxed approximately for a day.



$m=n=0$ (Cu), $m=2, n=3$ (Zn), $m=2, n=6$ (Ni); $m=2, n=4$ (Co)

The solutions were allowed to stand at room temperature until crystallization. The resulting crystalline material was filtered off and dried at room temperature. The composition and structure of the complexes were investigated by the following methods.

Results and discussion

The transition metals salts of *p*-coumaric acid were synthesized in the reaction of monosodium salt of *p*-coumaric acid and the appropriate hydrated metal(II) nitrates in aqueous ethanol (1:1, v/v). These complexes were reacted with coumarine in aqueous ethanol (1:1, v/v), the yield and elemental analysis data of the new complexes synthesized (Co = (1), Ni = (2), Cu = (3), and Zn = (4)) are given in Table 1. The experimental and theoretical compositions of the synthesized complexes agreed well. The magnetic susceptibility of the products are in harmony with the literature data.¹¹⁻¹³

Infrared Spectroscopy

The IR spectra of coumarine, *p*-coumaric acid and the synthesized mixed ligand complexes are given in Supplementary material (Figure S1) and the peak values and their assignment are given in Table 2.

The peak of stretching vibration belong to the C=O group of coumarine observed at 1753 cm⁻¹ was shifted to 1704, 1706, 1697 and 1705 cm⁻¹ in the spectra of Co^{II}, Ni^{II}, Cu^{II} and Zn^{II} complexes, respectively, as a result of coordination bond form between the carbonyl group and the metal cations.¹⁴ Likewise, the peak observed for stretching of carboxylate C=O in *p*-coumaric acid was also shifted from 1688 cm⁻¹ to 1621, 1620, 1628 and 1619 cm⁻¹ in the spectra of Co^{II}, Ni^{II}, Cu^{II} and Zn^{II} complexes, respectively. The higher shifting values for *p*-coumaric acid comparing to coumarine ligand is the consequence of more ionic character of the bond between the metal and its ligand.^{15,16} The most important indicator representing the denticity of carboxylate group bonding is the difference between symmetrical and asymmetrical stretching vibrations of carboxylate groups. These differences were found to be 252, 248 and 255 cm⁻¹ or the complexes of Co^{II}, Ni^{II} and Zn^{II} complexes, respectively, which show monodentate coordination mode of *p*-coumarate ligand, while the difference of 115 cm⁻¹ for the Cu^{II} complexes unambiguously shows the bidentate coordination mode.^{16,17}

Since the phenolic group of *p*-CoupH has not been deprotonated with NaHCO₃, two *p*-coumarate ligands are

required to compensate the positive charge of the metal cation.

The other vibration bands of pyrone ring of coumarine appeared at 2910-2825 cm⁻¹ (aromatic C-H) and 1522-1460 cm⁻¹ (C-C vibrations). Stretching vibrations corresponding to the aromatic C-H bands were observed between 2860 and 2960 cm⁻¹ in the mixed ligand complexes. The strong and broad band observed at 3600-3000 cm⁻¹ belongs to the -OH group vibrations. The asymmetric and symmetric COO⁻ bands of carboxylate group are located between 1600-1610 cm⁻¹ and 1380-1390 cm⁻¹, respectively. The absorption bands of the C-O groups can be observed between 890 and 900 cm⁻¹. The vibrations of metal-oxygen bonds belong to the pyrone and carboxylate oxygens could not be distinguished, the stretching vibrations were appeared at 610 and 526 cm⁻¹ for Co^{II}; 611 and 526 cm⁻¹ for Ni^{II}; 645 and 515 cm⁻¹ for Cu^{II}, and 609 and 625 cm⁻¹ for Zn^{II} complexes.^{2,17,18}

Thermal Analysis

Thermal analysis curves of the complexes (TG, DTG, DTA) are given in Figure 1-4 and the results of thermal analysis are summarized in Table 3.

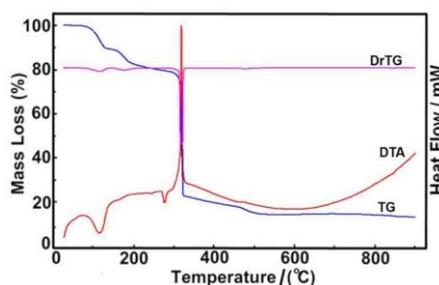


Figure 1. Thermal analysis curves of the complex $[\text{Co}(\text{Coup})_2(\text{p-Coup})_2(\text{H}_2\text{O})_2] \cdot 4\text{H}_2\text{O}$

The decomposition of the pink compound I consists of five steps as it can be seen from TGA curve. The decomposition temperatures are 84, 158, 236, 315 and 518 °C, respectively. The mass decrease of the first decomposition step confirm leaving of the 4 mol of crystallization water, the coordinated water (2 mol) leaves in the temperature interval of 123-182 °C

The organic ligands are lost between 185 and 345 °C, the final black product remained is CoO.

As it can be seen from Figure 2, the green Ni(II) complex decomposes in six steps at 75-131, 151, 210, 400 and 760 °C. The mass loss of the first of decomposition step corresponds the losing of all six crystallization water. The coordinated water (2 mol) is lost between 142 and 165 °C. The ligand loss is occurred between 167 and 807 °C, the black residue was found to be NiO.

Table 1. The elemental analysis data of complexes

Complex	MW g mol ⁻¹	Yield, %	Contents % Found (calcd.)		Color	dec. °C	μ_{eff} BM
			C	H			
[Co(C ₉ H ₇ O ₃) ₂ (C ₉ H ₆ O ₂) ₂ (H ₂ O) ₂]·4H ₂ O (I) C ₃₆ H ₃₈ CoO ₁₆	785.61	89	55.72 (55.04)	5.23 (4.88)	Dark pink	84	3.52
[Ni(C ₉ H ₇ O ₃) ₂ (C ₉ H ₆ O ₂) ₂ (H ₂ O) ₂]·6H ₂ O (II) C ₃₆ H ₄₂ NiO ₁₈	821.40	91	53.51 (52.64)	5.15 (4.88)	green	72	2.47
[Cu(C ₉ H ₇ O ₃) ₂ (C ₉ H ₆ O ₂) ₂] (III) C ₃₆ H ₂₆ CuO ₁₀	682.13	95	62.92 (63.39)	3.77 (3.84)	blue	60	1.32
[Zn(C ₉ H ₇ O ₃) ₂ (C ₉ H ₆ O ₂) ₂ (H ₂ O) ₂]·3H ₂ O (IV) C ₃₆ H ₃₆ ZnO ₁₅	774.04	86	57.59 (58.59)	5.11 (4.69)	white	71	Diam.

Table 2. IR spectra of the ligands and mixed ligand metal complexes

Groups	Coumarin	<i>p</i> -Coumaric acid	I (Co)	II (Ni)	III (Cu)	IV (Zn)
V(OH) _{H2O}	-	-	3600-3000	3600-3000	3600-2900*	3600-3000
V(OH) _{carboxyl}	-	3000-2900	-	-	-	-
V(CH ₂)	2910,2825	2879,2685	2961,2857	2958, 2887	2956,2870	2931,2866
V(C=O) _{carboxyl}	-	1688	1621	1620	1628	1619
V(C=O) _{pyrone}	1753	-	1704	1706	1697	1705
V(COO) _{asym.}	-	-	1606	1605	1567	1604
V(COO) _{sym.}	-	-	1384	1386	1452	1382
Δ v _{asym-sym}	-	-	252	248	115	255
δ (OH) _{H2O}	-	-	1473	1467	1463	1486
V(C-C) _{ring}	1522,1460	1506,1432	-	-	-	-
V(C-O) _{pyrone}	917	-	893	896	886	891
δ (OH) _{carboxyl}	-	973	-	-	-	-
V(M-O) _{pyrone}	-	-	610	611	645	609
V(M-O) _{carboxyl}	-	-	526	526	515	625

*Due to the -OH group of complex.

Table 3. The thermal analysis data for mixed ligand complexes

Complexes	Step	Temp. Interval, °C	DTA _{max} , °C	Leaving group	Weight loss %		Residue %		Decomp. product
					Exp.	Theo.	Exp.	Theo.	
I Co(II)	1	55-121	84	4H ₂ O	9.27	9.17			
	2	123-182	158	2H ₂ O	4.59	4.68			
	3	185-267	236	2C ₉ H ₈ O;2C ₆ H ₆ O	55.57	56.82			
	4	268-345	315	C ₂ H ₂	6.12	6.63			
	5	352-650	518	CO ₂	10.91	11.72	11.42	9.56	CoO
II Ni(II)	1	61-141	75; 133	6H ₂ O	13.32	13.15			
	2	142-165	151	2H ₂ O	4.48	4.39			
	3	167-248	210	2C ₉ H ₈ O	32.47	31.67			
	4	256-653	400	2C ₆ H ₆ O	21.33	22.65			
	5	657-807	760	2C ₃ H ₃ O ₂	17.91	17.30	10.77	9.10	NiO
III Cu(II)	1	145-266	208; 250	2C ₉ H ₈ O	37.87	38.31			
	2	267-431	310; 408	2C ₆ H ₆ O	25.91	27.27			
	3	433-488	471	2C ₂ H ₂	8.53	7.63			
	4	490-851	722	2CO ₂	12.21	12.90	12.83	11.67	CuO
IV Zn(II)	1	60-101	92	3H ₂ O	6.09	6.97			
	2	156-194	185	2H ₂ O	4.16	4.65			
	3	205-360	288; 295	2C ₉ H ₈ O	34.26	33.59			
	4	363-418	393; 408	2C ₆ H ₆ O	22.13	24.03			
	5	420-772	707	2C ₂ H ₂	7.12	6.72			
	6	773-826	812	CO ₂	10.41	11.37	11.68	10.52	ZnO

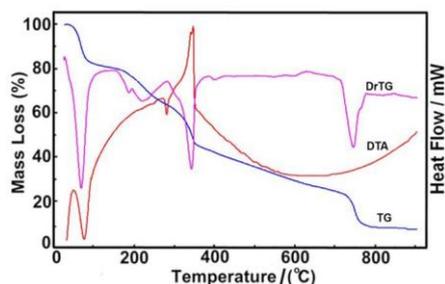


Figure 2. Thermal analysis curves of the complex $[\text{Ni}(\text{Coum})_2(\text{pCoum})_2(\text{H}_2\text{O})_2] \cdot 6\text{H}_2\text{O}$

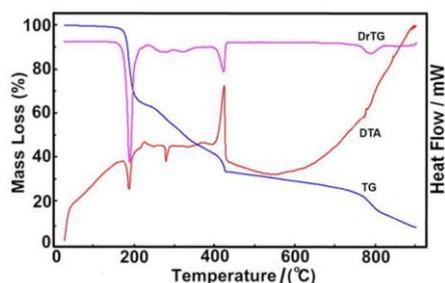


Figure 3. The thermal analysis curves of the complex $[\text{Cu}(\text{Coum})_2(\text{pCoum})_2]$

The compound **III** (Cu-complex) decomposes in four steps with at 208-250, 310-408, 471 and 722 °C. The first decomposition step is the main decomposition step with completely loss of ligands. Based on the mass decreasing, the supposed intermediate is copper(II) carbonate. Copper(II) carbonate decomposes in the temperature interval of 490-851 with formation of black CuO as final decomposition product.

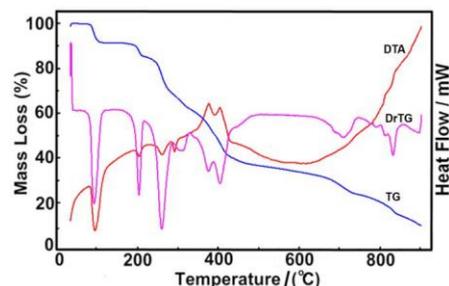


Figure 4. Thermal analysis curves of the complex $[\text{Zn}(\text{C}_9\text{H}_7\text{O}_3)_2(\text{C}_9\text{H}_6\text{O}_2)_2(\text{H}_2\text{O})_2] \cdot 3\text{H}_2\text{O}$

Complex **IV** decomposes in a six stage reaction at 92, 185, 288; 295-393; 408, 707 and 812 °C. The first decomposition step is the loss of 3 mol of crystalline water. The coordinated water (2 mol) is lost around 185 °C. The next steps belong to ligand loss when the final decomposition product is ZnO. The grey color of ZnO shows the presence of contamination from the organic ligand decomposition products.

Solid state UV-Vis spectroscopy

UV-visible spectra of the synthesized complexes were recorded between 900 and 200 nm (Figure 6). In case of complex I, the Co^{II} *d-d* transitions could be assigned to peak at 537.23 nm (${}^4\text{T}_{1g} \rightarrow {}^4\text{T}_{2g}$) (F) and 469.45 nm (${}^4\text{T}_{1g} \rightarrow {}^3\text{T}_{1g}$) (P).

Three spin-allowed *d-d* transitions of Ni(II) in the complex II were found at 821.52 nm (${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}$) (P), 684.87 nm (${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}$) (F) and 402.17 nm (${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{2g}$) (F). Splitting of *d* orbitals confirms the octahedral geometry around the Ni(II) (Figure 4.20). The two of these transitions were found in the visible region, whereas the 402.17 nm (${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{2g}$) (F) transition was shielded by the charge transfer bands.

The multiple absorption bands of the complex III were composed of overlapped peaks, and thus have a broad view in a wide range corresponding to the interval of 877.39-519.63 nm. According to these spectral data, it can be thought that the metal cation Cu(II) has a "pseudo-octahedral" structure with Jahn-Teller distortion. The maximum absorption band of a broad spectrum of the complex III corresponds to the wavelength value of approximately 709.71 nm (${}^2\text{E}_g \rightarrow {}^2\text{T}_{2g}$) comes longitudinal waves.¹⁸

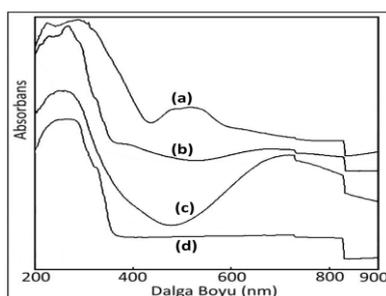


Figure 5. Solid State UV-vis spectra of the complexes I-IV. (a) Co-complex, (b) Ni-complex, (c) Cu-complex, (d) Zn-complex

The zinc complex (IV) is diamagnetic and due to closed *d*-shells there are no *d-d* electronic transitions of the octahedral structure.²³

Table 4. The *in vitro* antimicrobial activity of the complexes synthesized.

Complex	Strains (inhibition zones \pm SD), mm				
	<i>S. aureus</i> ATCC 25923	<i>E. faecalis</i> ATCC 29212	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853	<i>C. albicans</i> ATCC 0231
I (Co)	10,0 \pm 2,0	9,0 \pm 1,0	-*	-	12,0 \pm 1,0
II (Ni)	-	10,0 \pm 0,1	-	-	11,0 \pm 1,0
III (Cu)	-	-	9,0 \pm 1,0	8,5 \pm 0,5	12,5 \pm 0,5
IV (Zn)	11,0 \pm 1,0	10,5 \pm 0,5	12,0 \pm 1,0	12,0 \pm 1,0	12,5 \pm 1,0

(*: no inhibition, the amount of complex used: 25 μ l)

The high intensity absorption bands observed at lower wavelength belong to the metal \rightarrow ligand charge transfer transition.²⁴ While the absorption bands of Co(II), Ni(II) and Cu(II) at the wavelength of 219.35 and 302.85 nm, 211.12 and 284.17 nm, and 257.61 nm belong to metal \rightarrow ligand transitions, the highly intensive peak observed in case of Zn complex (IV) at 281.15 nm could be assigned to ligand \rightarrow metal charge transfer.^{11-14,16-20}

Mass spectral analysis

The thermal decomposition pathway of the [Ni(Coum)₂(pCoum)₂(H₂O)₂.6H₂O] complex was recorded using direct insertion probe pyrolysis mass spectrometry method (Supplementary material, Figure S2). The mass spectra show the fragmentation pattern of the ligands, the molecular ion peak could not be detected.

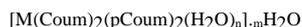
Biological Application

The results obtained in studies of antimicrobial and total antioxidant activity (TAS) studies of the synthesized complexes are given in Table 4 and Table 5, and in the supplementary material (Figure S3).

Table 5. The results of total antioxidant activity for the complexes synthesized.

Metal complexes	Total antioxidant values (mmol Trolox Eq. L ⁻¹)
Co ^{II} (I) complex	1.47
Ni ^{II} (II) complex	1.33
Cu ^{II} (III) complex	1.73
Zn ^{II} (IV) complex	1.66

The coordination mode of ligands, the UV and magnetic measurements suggest octahedral geometry around the metal cations and water occupy the free coordination places in the complexes except the bidentate pCoum anion containing Cu(II) compound. The formula of the complexes can be written as follows as follows.



M=Co, n=2, m=4 (I); M=Ni, n=2, m=6 (II); M=Cu, m=n=0 (III); M=Zn, n=2, m=3 (IV).

Among the studied mixed ligand complexes the lowest activity was observed for Ni^{II} (II) while the highest activity for Cu^{II} (III) complex. The strongest antimicrobial activity was found in case of Zn^{II} complex (IV).

Conclusion

Mixed ligand complexes of coumarine and *p*-coumaric acid with Co(II), Ni(II), Cu(II) and Zn(II) ions were synthesized and their structure were elucidated with using elemental analysis, infrared and ultraviolet-visible spectroscopy and magnetic measurements. The metal:ligand ratio was found to be 1 : 2 : 2 for all the complexes.

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