Synthesis, complexation properties, antimicrobial and anticoagulant activity of 2-{3-[4-(dimethylamino)phenyl]-1-hydroxyprop-2-en-1-ylidene}-4-nitro-1*H*-indene-1,3(2*H*)-dione

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I Nikolova[1], I Kostova[1], M Staneva[2], M Marinov[3]

[1] "Angel Kanchev" University of Ruse, Razgrad Branch, Department of Chemical, Food and Biotechnologies, 7200 Razgrad, 47 "Aprilsko Vastanie" Blvd., Bulgaria
[2] Institute of Polymers, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
[3] Agricultural University – Plovdiv, Faculty of Plant Protection and Agroecology, Department of Chemistry and Phytopharmacy, 4000 Plovdiv, 12 "Mendeleev" Blvd., Bulgaria

e-mail: ikostova@uni-ruse.bg

Abstract. This article presents the synthesis of a new 4-nitro-cinnamoyl derivative of 2-acyl-1,3-indandione. The resulting compound was subjected to interaction with $ErCl_3x6H_2O$, which was obtained from Er_2O_3 and HCl. The synthesized compounds were proven by IR and NMR spectroscopy. The spectral data clearly proves the formation of a chelate complex. A probable structural formula of the coordination compound is also proposed. The obtained compounds were used for analysis of their antimicrobial and anticoagulant activity.
Keywords: 2-acyl-1,3-indandione, chelate complex, ligand, antimicrobial activity, anticoagulant activity.

1. Introduction

Erbium as a representative of lanthanides is characterized by exceptional reactivity, easily enters into reactions, even under normal conditions, until the formation of relatively stable and resistant compounds. Of the coordination compounds that form the rare-earth elements (REE), the chelate ones with the participation of polydentate ligands prove to be the most stable.

Lanthanide complexes with carboxylic acids have interesting photochemical and structural features [1-6]. They have a wide range of applications - luminescence [6-10], catalysis [11], chemical materials [12-17].

The luminescent properties in a solution of complexes of lanthanide(III) ions Nd^{3+}, Eu^{3+}, Tb^{3+}, Er^{3+} and Yb^{3+}, in which an organic chromophore is attached to the metal centre as a sensitizer, were studied. The metallic ions Eu^{3+} and Tb^{3+} emitting in the visible range require sensitizers that absorb light in the UV or near-UV region, whereas VIS absorbing sensitizers can be used for the NIR (near infrared) emitting ions Nd^{3+}, Er^{3+} and Yb^{3+}. These complexes are of great interest due to their potential applications as luminescent markers in biological systems [10].

Quaternary erbium tellurite glass, whose thermal characteristics show higher thermal stability of the glass in a system, which suggests its applicability to scratching by fibers, has been synthesized and characterized from Eu_2O_3 combined with TeO_2-20ZnO-5Na_2O [18].

2-Cinnamoyl-1,3-indanedione derivatives are of great interest due to their photophysical properties and

are the subject of constant research [19-23]. Of interest are the resulting complexes. In the literature there are data for those with Cu(II), Cd(II), Zn(II), Co(II), Ni(II), *etc.* [24-27].

Since the data on the nitro derivatives of 2-cinnamoyl-1,3-indandione are scarce [28], even though successful attempts have been made for complexation with Cu(II), Ni(II) and Co(II) [29-31], we decided to try to synthesize new derivatives and this time to obtain a complex with Er(III).

2. Experimental

2.1. General

All used chemicals were purchased from Merck and Sigma-Aldrich. The melting point was determined by a SMP-10 digital melting point apparatus. The IR spectra were taken on Perkin-Elmer FTIR-1600 spectrometer in nujol. The NMR spectra were obtained on Bruker Avance III HD (500.13 MHz for ^{1}H and 125 MHz for ^{13}C NMR) spectrometer. The chemical shifts are given in parts per million (δ) relative to tetramethylsilane as internal standard for spectra in $CDCl_3$ solutions.

2.2. Synthesis of 2-{3-[4-(dimethylamino)phenyl]-1-hydroxyprop-2-en-1-ylidene}-4-nitro-1h-indene-1,3(2h)-dione

The method is described in ref. [28].

2.3. Preparation of $ErCl_3x6H_2O$

To 0.1 mol of Er_2O_3 (0.3665 g) we add 0.6 mol (0.2188 g) of HCl by heating. The resulting mixture is evaporated to dryness.

2.4. Preparation of the complex compound

To 0,36 g of ligand dissolved in dioxane, we add 0,13 g of complexing agent dissolved in methanol by refluxing. A precipitate falls out of the complex compound, which, after complete precipitation and cooling, is filtered off.

2.5. Antimicrobial assay

To determine the antimicrobial activity of the substances, the agar diffusion method and the following test microorganisms were used: Gram-positive bacteria *Staphylococcus aureus* ATCC 6538*, Bacillus subtilis* ATCC 6633*,* and *Kocuria rhizophila* ATCC 9341, Gram-negative bacteria *Escherichina coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NTCC 6017, yeast *Candida albicans* ATCC 10231, mold *Aspergillus brasiliensis* ATCC 16404. A 1% solution in solvent dimethyl sulfoxide (DMSO) was prepared from each test substance. The experiments were performed on Tryptic soy agar (Merck) growth medium - for bacteria, and Sabouraud-Dextrose agar (Merck) for yeast and mold fungi. The media are melted and cooled to 50°C. They were inoculated with 1% of pre-prepared suspensions of test microorganisms at a cell concentration of 10^8 CFU/mL (0.5 McFarland turbidity standard) and mixed well. 20 ml of the inoculated media was poured into sterile petri dishes (∅ = 90 mm). The agar was allowed to solidify. A cork borer was used to punch holes (∅ = 8 mm) in the agar. 50 µl of the pre-prepared solutions were added dropwise to each hole and, after 30 min of pre-infusion at room temperature, the petri dishes were placed in a thermostat at 37°C for 24 h for the bacteria; at 28°C for 48 h for yeast and for 96-120 hfor mold fungi. After cultivation with a digital caliper, the diameters of the zones of growth inhibition were measured in mm, as: up to 15 mm the microbial culture was weakly sensitive; from 15 to 25 mm - sensitive and over 25 mm - highly sensitive. The experiments were performed in parallel with the control sample of the solvent, taking into account its action as well. The data on antimicrobial activity were arithmetic average of three measurements.

2.6. Determination of the anticoagulant activity

From the obtained compounds, solutions with a concentration of 0.9% were obtained, and DMSO was used as a solvent. Venous blood was used to determine the anticoagulant activity. The study was conducted in a laboratory for taking biological material.

3. Results and Discussion

2-Acetyl-1,3-indandione and its derivatives are a group of compounds with high biological activity and wide application nowadays. An increase in their activity has been demonstrated when they are included in coordination compounds.

A 4-nitrocinnamoyl derivative with the following structure was synthesized (figure 1):

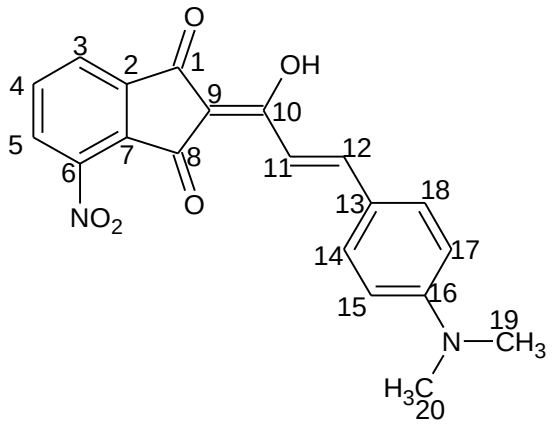

Figure 1. Structure of 2-{3-[4-(dimethylamino)phenyl]-1-hydroxyprop-2-en-1-ylidene}-4-nitro-1*H*-indene-1,3(2*H*)-dione.

The numbering of the atoms given there is only for spectral assignment. The determination of the *Z* and *E* isomeric forms is not the subject of this article.

The compound obtained has a melting point of 258-259°C and yield of 74%.

The data from the NMR analysis show the following values: ^{1}H NMR ($CDCl_3$, *δ,* ppm): 3.23 (s, 3H, CH_3), 7.41-7.74 (m, 4H, CH), 7.72-7.83 (m, 3H, CH), 7.85-8.12 (m, 2H, CH), 11.82 (s, 1H, OH); ^{13}C NMR ($CDCl_3$, *δ,* ppm): C(19, 20) – 43.1, C(10) – 102.6, C(15, 17) – 112.6, C(13) – 124.1, C(11) – 125.3, C(14, 18) – 126.9, C(12) – 127.9, C(5) – 128.7, C(7) – 133.0, C(4) – 135.2, C(3) – 135.4, C(2) – 138.8, C(16) – 142.8, C(6) – 149.8, C(10) – 177.5, C=O(1) – 188.4, C=O(8) – 190.1; ^{13}C DEPT 135 ($CDCl_3$, *δ,* ppm): C(19, 20) – 43.1, C(15, 17) – 112.6, C(11) – 125.3, C(14, 18) – 126.9, C(12) – 127.9, C(5) – 128.7, C(4) – 135.2, C(3) – 135.4.

An IR analysis was performed, which proved the structure of the new compound (table 1).

Table 1. Values from IR (nujol) ligand spectroscopy.

υ_{OH}	$\upsilon_{C=O}$	$\upsilon_{C=O}$; $\upsilon_{C=C}$	$\upsilon_{C=O}$; $\upsilon_{C=C}$	$\upsilon_{C=O}$; $\upsilon_{C=C}(Ph)$	υ_{NO_2}	υ_{NO_2}
3448	1695	1640	1630	1590	1532	1351

2-{3-[4-(Dimethylamino)phenyl]-1-hydroxyprop-2-en-1-ylidene}-4-nitro-1*H*-indene-1,3(2*H*)-dione was subjected to complexation with $ErCl_3x6H_2O$. The resulting precipitate was filtered off and dried at room temperature, and the clear filtrate was further precipitated with H_2O. IR spectroscopy was performed on the obtained compounds, which data are presented in table 2.

Table 2. Values from IR (nujol) spectroscopy of the complex compound.

υ_{OH}	$\upsilon_{C=O}$	$\upsilon_{C=O};\ \upsilon_{C=C}$	$\upsilon_{C=O};\ \upsilon_{C=C}$	$\upsilon_{C=O};\ \upsilon_{C=C}(Ph)$	υ_{NO_2}	υ_{NO_2}
-	1691	1632	1544	1518	1382	1355

The data show that the oscillations in the range of 3250-3500 cm^{-1} are missing, which proves the formation of a new complex. This, together with the change in the values for one carbonyl group from 1630 to 1544 cm^{-1}, proves that the newly obtained compound has a chelate structure.

When obtaining the coordination compounds, a colour difference is also found:

- Colour of ligand in solution - tile red
- Color of the complex

= before drying - reddish-brown

= after drying - dark brown.

This is another proof that complex formation has taken place.

The presumed structure of the coordination compound is (figure 2):

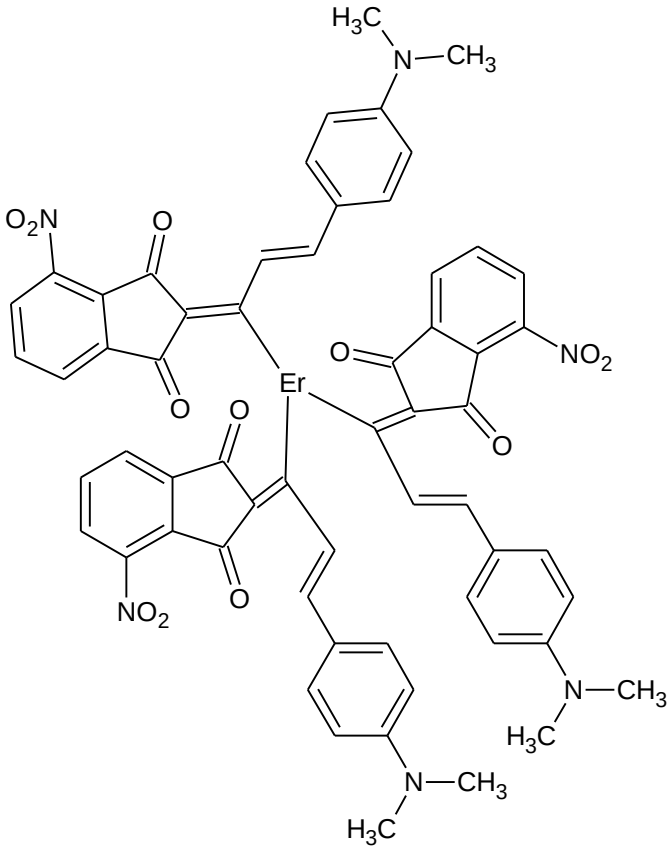

Figure 2. Structure of the coordination compound.

The percentage yield of the coordination compound is 92%. The ratio between the ligands and the complexing agent is 3:1.

The data from the antimicrobial activity study are presented in table 3.

Table 3. Antimicrobial activity of the compound and the complex.

Microorganism	Inhibition zone (mm)			
	L	L + Er^{3+}	L + Er^{3+} + H_2O	DMSO
Staphylococcus aureus ATCC 6538	10.5	10.0	0	0
Bacillus subtilis ATCC 6633	14.4	13.5	0	0
Kocuria rhizophila ATCC 9341	0	0	0	0
Escherichia coli ATCC 8739	0	0	0	0
Pseudomonas aeruginosa ATCC 9027	0	0	0	0
Salmonella abony NCTC 6017	13.2	0	0	0
Candida albicans ATCC 10231	0	0	0	0
Aspergillus brasiliensis ATCC 16404	0	0	0	0

The study of 2-{3-[4-(dimethylamino)phenyl]-1-hydroxyprop-2-en-1-ylidene}-4-nitro-1*H*-indene-1,3(2*H*)-dione revealed weak antibacterial activity against Gram-positive bacteria *S aureus* and *B. subtilis* and none against *K. rhizophila*. Of the Gram-negative bacteria, only *S. abony* is weakly sensitive to this compound. The other Gram-negative bacteria *E. coli* and *P.s aeruginosa* are insensitive. The compound does not show fungicidal activity against the studied yeasts and fungi.

The complex compound showed weak antibacterial activity against Gram-positive bacteria *S.taureus, B. subtilis*, and no activity against *K. rhizophila*. All Gram-negative bacteria, yeasts and molds are resistant to this compound. No antimicrobial activity was detected against the test microorganisms used in the complex with water included.

A study of the anticoagulant activity of the ligand and the complex compound was performed. The data from the study are as follows:

	PT, %	INR	PT, s
I	31.4	2.74	32
II	43.4	2.08	24.4
III	101.8	0.81	10.8

PT – Prothrombin Time

INR – International Normal Ratio

I - 2-{3-[4-(dimethylamino)phenyl]-1-hydroxyprop-2-en-1-ylidene}-4-nitro-1*H*-indene-1,3(2*H*)-dione

II - 2-{3-[4-(dimethylamino)phenyl]-1-hydroxyprop-2-en-1-ylidene}-4-nitro-1*H*-indene-1,3(2*H*)-dione + Er^{3+} + H_2O

III - 2-{3-[4-(dimethylamino)phenyl]-1-hydroxyprop-2-en-1-ylidene}-4-nitro-1*H*-indene-1,3(2*H*)-dione + Er^{3+}

From these values it can be seen that in terms of percentage the highest is the prothrombin time in the complex compound and only in it the result is higher than the reference values, which are > 70%. The ligand has the highest protombin time in terms of time, followed by the coordination compound further precipitated with water, both values being above the reference (10-14 s). The value of

prothrombin time in seconds for the coordination compound (10.8 s) is at the reference limit. The reference values for INR are in the range of 0.8-1.2. From the obtained results it can be seen that of the tested compounds only the coordination compound falls within the limits, and the ligand, as well as the complex further precipitated with water, have significantly higher values.

4. Conclusions

A new 4-nitrocinnamoyl derivative of 2-acyl-1,3-indandione was synthesized. The resulting compound was characterized by IR and NMR spectroscopy. The melting point was determined. The newly synthesized compound was subject to complexation with Er^{3+} and it was proved by IR spectrum that the experiment was successful.

The studied ligand and complex have weak antimicrobial activity against Gram-positive bacteria *Staphylococcus aureus, Bacillus subtilis,* and have no activity against *Kocuria rhizophila*. The compound 2-{3-[4-(dimethylamino)phenyl]-1-hydroxyprop-2-en-1-ylidene}-4-nitro-1*H*-indene-1,3(2*H*)-dione has weak activity against the Gram-negative microorganism *Salmonella abony*. The complex compound with included water did not show antimicrobial activity against the Gram-positive and Gram-negative test microorganisms used in the experiment. All compounds tested did not have antimicrobial activity against yeasts and molds.

The resulting coordination compound was characterized by high values of prothrombin time in terms of percentage. The ligand and the complex further precipitated with water were characterized by high INR values and prothrombin time expressed in seconds. In the coordination compound, INR values and prothrombin time expressed in seconds were established, which fell within the reference values.

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