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Synthesis, structural studies and biological activity of new Cu(II) complexes with acetyl derivatives of 7-hydroxy-4-methylcoumarin

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ABSTRACT

The new Cu(II) complexes with 6-acetyl-7-hydroxy-4-methylcoumarin (HL1) and 8-acetyl-7-hydroxy-4-methylcoumarin (HL2) have been obtained by the electrochemical method. The density functional theory calculations and X-ray absorption spectroscopy techniques have been used to geometrically describe a series of new compounds. The studies have been focused on the coordination mode of the hydroxy ligands to the metallic centre. The complexes, $Cu(HL1)_2$ and $Cu(HL2)_2 \cdot 0.5H_2O$, have flat square geometry with oxygen atoms in the first coordination sphere. Two bidentate anionic coumarins are bonded to the metal cation via the acetyl and deprotonated hydroxyl O atoms. Biological activity, including microbiological and cytotoxic, has been evaluated and found to be enhanced in comparison with the parent ligands. Moreover, the Cu(II) complex with 8-acetyl-7-hydroxy-4-methylcoumarin shows similar antifungal activity as commercially used fluconazole.

1. Introduction

The natural as well as synthetic coumarins, therein hydroxycoumarins, have a large spectrum of biological activity. Such derivatives proved usefulness as anti-coagulants [1], antibacterial agents [2], antifungal agents [3], biological inhibitors [4], chemotherapeutics [5] and as bio-analytical reagents [6]. It has been found out that coordination of metal ions to therapeutic agents (such as simple coumarins) can improve their efficacy and accelerate bioactivity. In many cases such metal complexes are more potent and less toxic compared to the parent drug. Therefore, among others, also biologically active metal complexes of coumarin based ligands are being widely investigated.

Among them, triorganotin(IV) derivatives of umbelliferone (7-hydroxycoumarin) have shown good antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans* and *Microsporum gypseum*, and this activity was slightly enhanced upon adduct formation with 1,10-phenanthroline. Creaven et al. have investigated the antimicrobial activity of a number of coumarin complexes with silver(I), copper(II) and manganese(II) ions. For example, the Cu(II) complexes exhibit antifungal activity against a clinical strain of *C. albicans* comparable to that of the commercially available antifungal drugs, i.e. ketoconazole and Amphotericin B [7].

This paper is focused on the synthesis and characterization of copper complexes of two hydroxyligands: HL1 and HL2 (Fig. 1). These ligands have acetyl group attached at two different positions, C6 and C8, to the rigid coumarin ring. The electrochemical method was applied for the synthesis of the complexes.

In order to determine the geometry of metal-ligand interactions X-ray crystallography is usually used. However, this technique requires compound in the crystal form, which is not always possible to obtain. Since that was the case for the investigated complexes, the X-ray absorption spectroscopy (XAS) was applied. The great advantage of XAS is that it can be used for crystal as well as amorphous materials at different states: solid, liquid or gaseous. XAS provides information about the local atomic order, coordination number, kind of atoms, oxidation state, relative disorder and even angles between central atom and near neighbours [8–10]. Data obtained from XAS combined with the density functional theory (DFT) calculations allowed building models of the complexes. Additionally to

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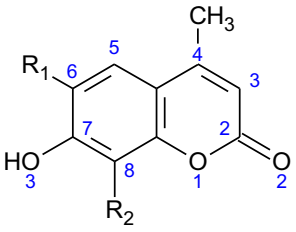

	R_1	R_2
HL1	$C(=O)CH_3$	H
HL2	H	$C(=O)CH_3$

Fig. 1. Molecular structure of ligands.

geometrical characterization of complexes also the biological activity tests, including microbiological and cytotoxic studies, were performed.

2. Experimental

2.1. Instrumentation

The elemental analysis was performed using the Perkin-Elmer 2400 CHN elemental analyzers. The thermal stability and decomposition process of complexes were studied in air using the Setsys 16/18 (Setaram) TG, DTG and DSC instruments. The experiments were carried under air flow in the temperature range of 293–823 K at a heating rate of 5 K/min. The initial masses of samples of complexes used for measurements changed from 4.59 mg to 4.56 mg for **1** and 2.01 mg to 1.96 mg for **2**. Samples of these compounds were heated in Al_2O_3 crucibles.

The IR spectra were gathered between 400 and 4000 cm^{-1} in the ATR mode on a Thermo Scientific Nicolet iS5 spectrometer.

2.2. Synthesis of ligands

The initial reagents were bought from Sigma-Aldrich. The synthesis of 6-acetyl-7-hydroxy-4-methylcoumarin (HL1) and 8-acetyl-7-hydroxy-4-methylcoumarin (HL2) was performed as described in literature [11,12].

2.3. Synthesis of complexes

The copper complexes, **1**–**2**, were obtained by an electrochemical synthesis [13]. For the complexation reaction the 7-hydroxycoumarins, HL1 and HL2, were used as ligands. The copper plate was used as a metal anode and a platinum wire was the cathode. A 96% ethanol solution of HL1 and HL2 containing about 80 mg of tetrabutylammonium perchlorate (electrolyte) was electrolyzed for 2 h (see Table 1). After filtration of the resulting solution, the powder product was purified by washing thoroughly with water.

2.4. X-ray absorption spectroscopy

The K-edges of Cu for the complexes **1** and **2** were measured at the beamline I811 at MAX-lab in Lund, Sweden. Both XANES (X-ray absorption near edge structure) and EXAFS (extended X-ray absorption fine structure) spectra were recorded in the transmission mode. To confirm the oxidation state of metal in the complexes, the XANES spectra of the reference compounds (two copper oxides, Cu_2O and CuO) were also measured.

The quantitative analysis of EXAFS spectra of compounds **1**–**2** was performed as follows: k^2 weighted $\chi(k)$ data were Fourier transformed in the k range 2.4–11 for **1** and 2.5–10 for **2**. The Fourier's back transformation ranges were from 1 to 2 Å for both complexes. For the XAS analysis, the Athena and Artemis programs included in the IFEFFIT package [14] were applied.

2.5. DFT calculations

The DFT calculations were performed using Turbomole 6.5 code [15]. Relying on reasonable assumption, that weak interactions keeping molecular solids together should not affect strongly the properties of molecules, structural models consisting of single molecule were used. Such models were successfully applied in elucidation of structural and spectroscopic properties of metal complexes with coumarin derivatives [16]. Geometry optimization was carried out using gradient Perdew–Burke–Ernzerhof (PBE) [17] and hybrid PBE0 (i.e. PBE with 25% of Hartree–Fock exchange) [18] exchange-correlation functionals with def2-TZVPP basis set [19,20]. The convergence criteria for total energy and gradient norm were 10^{-6} Eh and 10^{-4} Eh/bohr, respectively. Harmonic vibrational analysis was performed at PBE level to verify, if true minimum of energy was achieved and support quantitative analysis of FT-IR spectra.

2.6. Biological activity

In order to check the biological activity of complexes **1**–**2** microbiological and cytotoxic tests were carried out and afterwards compared with the parent ligands (HL1–HL2) described before [21].

2.6.1. Microbiological assays

The following microbial strains were chosen from American Type Culture Collection (ATCC): bacteria Gram-positive strains: *Micrococcus luteus* (9341, 10240), *B. subtilis* 6633, *Bacillus cereus* 11778, *S. aureus* (6538, 6538P), bacteria Gram-negative strains: *Escherichia coli* (8739, 10536), *Pseudomonas aeruginosa* (15442, 9027), fungi (yeast strains): *C. albicans* (10231, 2091), *Candida parapsilosis* 22019, *Zygosaccharomyces rouxii* 28253, *Saccharomyces cerevisiae* 9763 and a mould strain *Aspergillus brasiliensis* 16404. The MRSA hospital strain 573/12 (methicillin resistant *S. aureus*) isolated from peripheral blood was obtained from MUW Museum Collection. According to disc-diffusion Kirby-Bauer method, the 573/12 strain is resistant to: penicillin, erythromycin, amikacin, clindamycin, and ciprofloxacin.

2.6.2. Antimicrobial activity — preliminary test

In the preliminary tests antimicrobial activity was determined by a modified cylinder-plate method [22]. The inhibition of bacterial growth was observed as a halo around the cylinder containing the tested compound. The size of inhibition zone reflected an antimicrobial activity of the examined compounds.

2.6.3. Minimum inhibitory concentration (MIC)

For the compound which showed some activity against any of the tested strains an MIC was determined based on M7-A6 method [23]. The lowest concentration of tested compound, which totally inhibited

Table 1
Experimental conditions for the synthesis of complexes **1**–**2**.

Complex	Amount of solved copper [g]	Ligand	Amount of HL [g]	Voltage* [V]	Colour of complex	Elemental analysis (C, H calc**/found %)
1	0.0415	HL1	0.0814	29	green	57.89, 3.64/57.22, 3.72
2	0.0515	HL2	0.0814	18	green	56.86, 3.85/56.78, 3.75

* Voltage to produce a current of 10 mA.
** Anal. calc. for $Cu(HL1)_2$ and $Cu(HL2)_2 \cdot 0.5\ H_2O$.