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Complexes of Azithromycin with Some Divalent Metal Ions

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The formation of 2 : 1 complexes of azithromycin with copper(II), zinc(II), cobalt(II), nickel(II), magnesium(II) and calcium(II) ions was examined. These compounds (**2–7**) showed the same *in vitro* biological potency as azithromycin (**1**). The complexes (**3–7**) are isostructural and different when compared with azithromycin-Cu(II)- complex. Copper(II)-complex (**2**) was most stable and selected for structure determination by X-ray crystallography. Some physical characteristics and analyses of the complexes are also presented.

INTRODUCTION

Azithromycin (**1**),[1–3] a semisynthetic 15-membered azalide antibiotic derived by chemical modification of erythromycin A, shows a broader antimicrobial spectrum, longer elimination half-life and higher tissue concentrations than erythromycin.[4–8] It differs structurally from erythromycin A in the insertion of a 9a-methyl substituted nitrogen in the aglycone moiety, while the sugar moieties (desosamine and cladinose) stay unchanged.

The presence of metals and the formation of metal complexes might substantially influence the stability, distribution, biotransformation, elimination and other characteristics of the antibiotic. According to the early literature, the formation of a weak complex with erythromycin A was established in dilute solution only with Co(II) while with the other metal ions, like Cu(II), Ca(II), Mg(II), Ni(II) and Zn(II), such complexes were not observed.[9] However, later on, the erythromycin complex with Zn(II) was prepared and structurally characterized in the solid state, but in this structure the zinc atom is coordinated only by one erythromycin molecule while the other ligands are one monodentately and one bidentately bonded acetate ions and one water molecule.[10] In

this paper, we describe the formation of 2:1 complexes of azithromycin with some divalent ions (**2–7**). One of these complexes, the azithromycin-Cu(II) complex (**2**), was most stable and therefore selected for structural elucidation by X-ray crystallography.

EXPERIMENTAL

Azithromycin was produced by Pliva, Zagreb. All inorganic salts and other chemicals used were of analytical grade (Aldrich). Potentiometric (pH) measurements were carried out on a 672 Titroprocessor, Metrohm, and the UV spectra were recorded on a Pye Unicam SP8-100 instrument. The melting points were determined by a Fisher-Johns apparatus and are uncorrected. Atomic absorption spectrometry of metals was done using a Perkin-Elmer Model 5000 and polarographic measurements using an Amel Multipurpose Unit Model 563/564. X-ray diffraction data were collected at room temperature on a Philips PW 1100 automatic diffractometer by using monochromatized Mo Kα radiation (λ = 0.71069 Å). Powder diffraction patterns were taken on a Philips diffractometer (monochromatized Cu Kα radiation).

Preparation of Complexes

Azithromycin-Cu(II) complex (**2**). To a stirred suspension of **1** (0.750 g, 1 mmol) in 48 ml of water, 1.7 ml of 0.1 M HCl was added to acidify the reaction mixture to pH 6.0. Then, 0.086 g (0.5 mmol) $CuCl_2 \times 2H_2O$ was added and the stirring was continued under a stepwise addition of 0.1 M NaOH until a pH value of 8.5 was achieved. Subsequently, the reaction mixture was stirred for 2 hours at constant pH value (pH stat), then the violet-coloured precipitate was filtered off, washed with water and dried, to obtain 0.64 g (81.8%) of **2**. After crystallization from methanol, pale-violet prisms were obtained, m.p. 188–192 °C; UV(EtOH) λ_{max} 292,485 nm.

Anal. Calcd. for $C_{76}H_{142}O_{24}N_4Cu$: C 58.54, H 9.17, N 3.59, Cu 4.08; found: C 58.22, H 9.28, N 3.49, Cu 4.10.

Activity (*in vitro* biological potency): 834 U/mg against *Micrococcus luteus* ATCC 9341.

Azithromycin-Zn(II) complex (**3**). was prepared from **1** (0.750 g, 1 mmol) and $ZnCl_2$ (0.068 g, 0.5 mmol) at the pH value of 8.6, in a similar manner as described for **2**, to give a colourless powder of **3**, 0.61 g (77.9%); m.p. 120–123 °C.

Anal. Calcd. for $C_{76}H_{142}O_{24}N_4Zn$: C 58.47, H 9.16, N 3.59, Cu 4.19; found: C 58.17, H 9.17, N 3.55, Cu 4.50.

Acitivity: 852 U/mg.

Azithromycin-Co(II) complex (**4**). was prepared from **1** (0.750 g, 1 mmol) and $CoCl_2 \times 6H_2O$ (0.118 g, 0.5 mmol) at the pH value of 8.6, as described in the preparation of **2**, to give a light-green coloured product of **4**, 0.63 g (80.7%); m.p. 128–131 °C; UV(EtOH) λ_{max} 290,655 nm.

Anal. Calcd. for $C_{76}H_{142}O_{24}N_4Co$: C 58.71, H 9.21, N 3.60, Co 3.79; found: C 58.51, H 9.29, N 3.56, Co 4.10.

Activity: 849 U/mg.

Azithromycin-Ni(II) complex (**5**). was prepared from **1** (0.750 g, 1 mmol) and $NiCl_2$ x $6H_2O$ (0.119 g, 0.5 mmol) at the pH value of 8.6, as described in the preparation of **2**, to give a light-green product of **5**, 0.62 g (79.6%); m.p. 118–123 °C.

Anal. Calcd. $C_{76}H_{142}O_{24}N_4Ni$: C 58.72, H 9.21, N 3.60, Ni 3.77; found: C 58.42, H 9.29, N 3.56, Ni 4.30.

Activity: 853 U/mg.

Azithromycin-Mg(II) complex (**6**). was prepared from **1** (0.750 g, 1 mmol) and $MgCl_2$ x $6H_2O$ (0.102 g, 0.5 mmol) at the pH value of 8.6, as described in the preparation of **2**, to give a colourless product of **6**, 0.55 g (75.0%); m.p. 121–124 °C

Anal. Calcd. $C_{76}H_{142}O_{24}N_4Mg$: C 60.05, H 9.43, N 3.69, Mg 1.60; found: C 59.85, H 9.42, N 3.60, Mg 1.54.

Activity: 850 U/mg.

Azithromycin-Ca(II) complex (**7**). was prepared from **1** (0.750 g, 1 mmol) and $CaCl_2$ x $2H_2O$ (0.074 g, 0.5 mmol) at the pH value of 8.6, as described in the preparation of **2**, to give a colourless product of **7**, 0.60 g (77.9%); m.p. 120–123 °C.

Anal. Calcd. for $C_{76}H_{142}O_{24}N_4Ca$: C 59.43, H 9.32, N 3.65, Ca 2.61; found: C 59.15, H 9.40, N 3.52, Ca 2.30.

Activity: 856 U/mg.

Structure Determination of Azithromycin-Cu(II) Complex by X-ray analysis

The pale-violet crystals obtained from methanolic solution of the title compound were of irregular shape and poor quality and, above all, very sensitive. Left in the air, they started immediately to lose solvent, became opaque and white and clove into very thin layers. For protection they were sealed in Lindemann glass capillaries together with a droplet of methanol. However, in spite of that, they decomposed significantly during the data collection.

Accurate cell dimensions were determined from the least-squares refinement based on 18 reflections within the Θ range from 4.4 to 7.2°.

Crystal data: $Cu(C_{38}H_{71}N_2O_{12})_2$, monoclinic, space group $P2_1$, a = 19.143(5), b = 23.491(5), c = 12.486(4), Å, β = 95.71(3)°, V = 5586.9 Å^3, Z = 2, D_m = 1.182 Mg m^{-3} (pycnometrically).

An irregular crystal of approximate dimensions 0.2 x 0.3 x 0.4 mm was used for intensity data collection by the ω-2Θ scan technique (scan width 1.2°, scan speed 0.04° s^{-1}) in the range of $2 \leq \Theta \leq 25°$. Three check reflections monitored every two hours indicated crystal decay of *ca* 20% and the intensities were corrected accordingly. A total of 2396 unique reflections were collected, of which 2336 were greater than $1\sigma(I)$ and used in the structure solution and refinement. The data were corrected for the Lorentz and polarization factors but not for absorption.

The copper atom position was obtained from a three-dimensional Patterson synthesis, all other non-hydrogen atoms by using direct methods and subsequent Fourier map calculations Due to the limited number of diffraction data, the least-squares refinement was performed assuming anisotropic temperature factors for the copper atom and isotropic temperature factors for all other atoms. Hydrogen atoms attached to the azithromycin ligands were placed on geometrical grounds and their contributions included in the structure factor calculations. At R = 0.154, ten water-oxygen atoms were located in the cavities formed between the complex molecules. The refinement converged at R = 0.112. The final atomic parameters, with temperature factors and lists of the interatomic distances and angles, and of the observed and calculated structure factors are obtainable from the authors on request. All calculations were performed using the SIR,[11] SHELX76[12] and SHELX93[13] programmes, the distances and angles were calculated by means of the CSU[14] and the diagrams drawn by the EUCLID programme.[15]

RESULTS AND DISCUSSION

Chemical Preparation

The process of complexing azithromycin with particular metal ions was carried out by adjusting the pH of the solution to values convenient for precipitation of the product (see Experimental). The following metal complexes were prepared and analyzed: Cu(II), Zn(II), Co(II), Ni(II), Mg(II) and Ca(II). Some physical characteristics and analyses of complexes are also presented in the Experimental section. It is obvious that these compounds showed similar *in vitro* biological potency as azithromycin (867 U/mg against *Micrococcus luteus* ATCC 8341). It appears that an introduction of metal atoms and the formation of the corresponding complexes with the azithromycin molecule do not change its antibacterial activity.

Complexes of EPR and polarographic active metals were chosen for recording the EPR spectra and for polarographic measurements. The EPR spectra of the azithromycin-copper(II) (**2**) and the azithromycin-cobalt(II) complexes (**4**) (Figure 1)

confirmed the presence of new species (azithromycin itself showed no EPR ability). Formation of the azithromycin-Zn(II) complex (**3**) was detected by shifting the polarographic wave of Zn(II) by about 0.2 V (Figure 2).

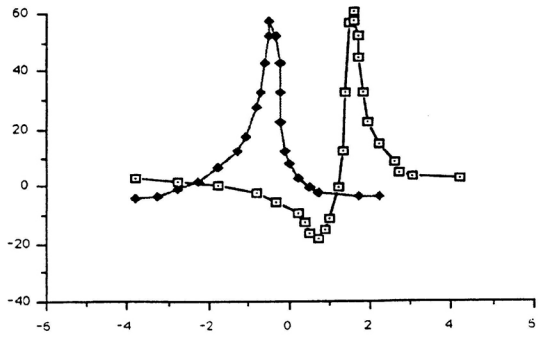

Figure 1. EPR Spectra of the azithromycin-copper(II) complex (⊡) and azithromycin-cobalt(II) complex (◆)

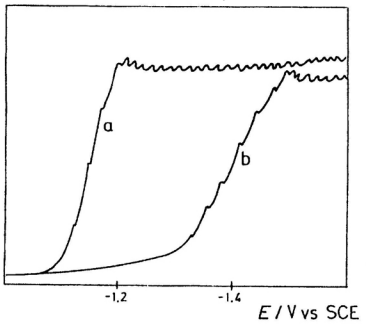

Figure 2. Polarographic curves of Zn(II) (curve a) and the azithromycin- zinc(II) complex (curve b); 10^{-3} M solutions in methanol –0.1 N KCl (1:1).

X-ray Structure Analysis of Azithromycin-Cu(II) Complex

The structure of azithromycin-Cu(II) complex (**2**) is shwon in Figure 3. The copper atom links two azithromycin molecules (A) and (B) , being bonded to two tertiary nitrogen [N(3'A) and N(3'B)] and two hydroxyl oxygen [O(2'A) and 0(2'B)] atoms from two desosamine moieties. Analogous bonding to the desosamine moiety was found in the above mentioned zinc acetate complex with erythromycin.[10] The coordination is very irregular, with the angles at the copper atom ranging from 88(1) to 97(1)°. The bond lengths Cu-O of 1.88(2) and 1.85(2) Å and Cu-N of 2.00(3) and 2.02(3) Å are as expected.[16,17] The bond lengths and angles in the 15-membered rings and

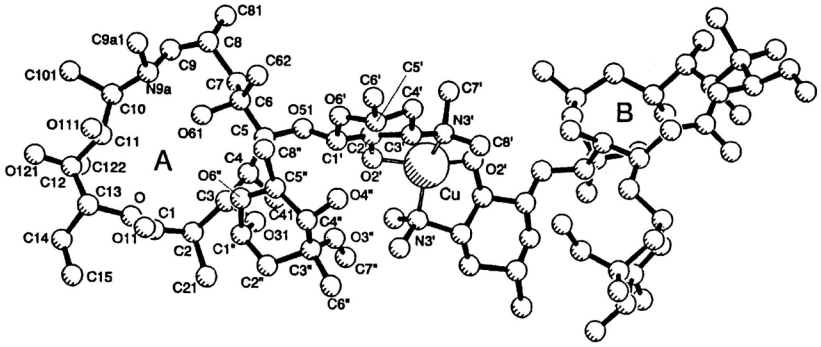

Figure 3. A perspective view of the azithromycin-Cu(II) complex molecule. The copper atom is coordinated by two azithromycin ligands, (A) and (B), and both have the analogous atom-numbering scheme. Hydrogen atoms are omitted for clarity. Bond lengths (Å) and angles (°) within the copper coordination sphere: Cu-O(2'A), 1.88(2); Cu-O(2'B), 1.85(2); Cu-N(3'A), 2.00(3); Cu-N(3'B), 2.02(3); O(2'A)-Cu-N(3'A), 88(1); O(2'B)-Cu-N(3'B), 88(1); O(2'B)-Cu-N(3'A), 96(1); N(3'B)-Cu-O(2'A), 97(1); O(2'A)-Cu-O(2'B),162(1); N(3'A)-Cu-N(3'B), 152(1).

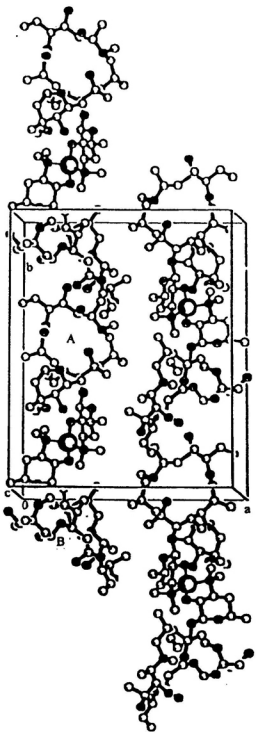

Figure 4. Packing of molecules in the unit-cell seen along the crystallographic *c* axis. A cavity formed between the molecules is extended along the *b* axis.

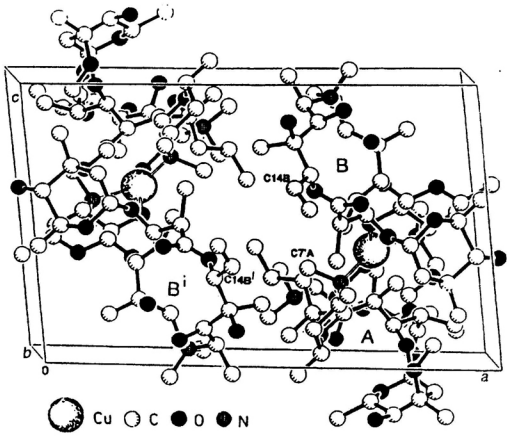

Figure 5. Packing of molecules in the unit-cell showing the cavity formed around the two-fold screw axis. Transformation of the asymmetric unit (*x,y,z*): (*i*) = 1 − *x*, 1/2 + *y*, 1 − *z*.

sugar components are similar to those observed in azithromycin itself,[3] as well as in the analogous but differently substituted 15-membered aglycone rings.[2,18,19] In the C(2)-C(5) region, both aglycone rings exhibit the »folded in« in contrast to the »folded out« conformation observed in the free azithromycin. Azithromycin adopts the same »folded in« conformation in solution, as proved by the NMR spectroscopy.[20]

An outstanding feature of the structure is a significant cavity formed around the two-fold screw axis, *i.e.* between the columns built up from the complex molecules and extended along the crystallographic *b* axis (Figure 4). Remarkable distances between the complex molecules, such as C(7'A)...C(14B^i) (transformation of the asymmetric unit (*x,y,z*): (*i*) = 1 − *x*, 1/2 + *y*, 1 − *z*) and C(7'A)...C(14B) amounting to 3.81(6) and 11.69(6) Å, respectively, indicate that such a cavity can accommodate a considerable number of solvent (water and/or methanol) molecules (Figure 5). This is exactly what has been observed: if freshly prepared crystals were left in dessicator over calcium chloride up to the constant weight, they lost about 22% of their original weight. This loss corresponds approximately to 24 water or 14 methanol molecules per one complex molecule. This is also in good agreement with the difference observed between the measured and calculated density, 1.18 *versus* 0.925 Mg m^{-3}. However, only ten water molecules were located in the crystal structure. Therefore, the loss of solvent may be the reason for the observed crystal decomposition as well as for the low accuracy of the diffraction data. Unfortunately, all attempts to prepare better crystals from a number of different solvents were unsuccessful.

According to the powder diffraction data, the complexes (**3–7**) are isostructural and differ from those of the azithromycin-Cu(II) complex.

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SAŽETAK

Kompleksi azitromicina s nekim dvovalentnim metalnim ionima

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Proučavano je nastajanje 2 : 1 kompleksa azitromicina s ionima bakra(II), cinka(II), kobalta(II), nikla(II), magnezija(II) i kalcija(II). Ti spojevi (**2–7**) pokazuju jednaku biološku aktivnost *in vitro* kao azitromicin (**1**). Kompleksi (**3–7**) su izostrukturni, ali različiti u usporedbi s kompleksom Cu(II). Kompleks (**2**) bakra(II) bio je najstabilniji i odabran je za određivanje strukture metodom rentgenske kristalografije. Također su navedena neka fizikalna svojstva i analize kompleksa.