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Copper(II) complexes of the beta-blocker pindolol: properties, structure, biological activity

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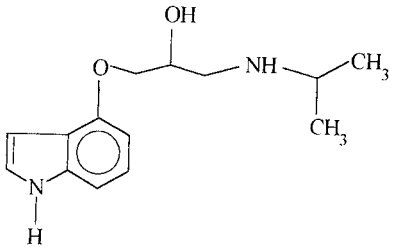
Abstract

The complex formation between copper(II) and the antihypertensive drug pindolol (HPin) was studied both in aqueous and methanolic media. Two complexes are formed at different metal-to-ligand molar ratios. The mononuclear complex $Cu(Pin)_2(HPin)_2$ contains two ligands in an anionic bidentate form and two - in a neutral form bound monodentately. The second complex $Cu_2Pin_2Cl_2$ is dinuclear and its structure was determined by X-ray diffraction. The compound crystallizes in the monoclinic group C2/c with cell components a = 14.4998(13)Å, b = 18.511(2)Å, c = 14.2982(13)Å, $\alpha = 90\,^{\circ}$, $\beta = 109.556(2)^{\circ}$, $\gamma = 90\,^{\circ}$ and Z = 12 at 293K. A pharmacological study on the influence of pindolol and its mononuclear complex on the heart rate of rats was performed. The complex is more active and has a longer effect in comparison with the pure non-coordinated pindolol in equitoxic doses.

Introduction

One of the most important achievements realized in the last century in the field of life-sciences was the finding that some biometals play important role in the regulation and control of essential biological and physiological processes. Here, together with the main groups biometals sodium, potassium, calcium and magnesium, a series of other ions most often of transition metals such as iron, copper, cobalt, nickel, etc. reveal a big variety of biological functions at much lower concentrations. Especially large number of important bioprocesses are copper- and zinc-dependent and this is the reason that any change in the homeostasis of the two metals reflects in disorders of different kind here including widely spread diseases such as diabetes, collagenoses, gastric and duodenal ulcer, arthritis etc. (da Silva & Williams 1991).

Recently it was found that the regulation of the arterial hypertension is also a copper and zinc dependent process. On that ground it was assumed that the effect of some of the most effective antihypertensive agents could be related at least partially to their ability for complexation with these two metals (Gathen & Ritz 1985; Nachev 1988).



Scheme I.

The β-blockers are at present a group of widely used drugs for treatment of the arterial hypertension and disrhythmia. From chemical point of view they are derivatives of aminoalcohols containing adjacent hydroxo- and amino-groups, capable as we have already shown (Bontchev *et al.* 1992, 2000, 2001) of chelate formation with Cu(II) and Zn(II).

The aim of the present work was to investigate the interaction between copper(II) and one of the unselective β-blockers - pindolol (1-(1H-indol-4-iloxy)-3-((1-methylethyl)amino)-2-propanol) (Scheme I), to study and characterize the products obtained in the course of this interaction.

The next step in the investigation was to check the biological efficiency of these complexes and especially in comparison with the pure, non-coordinated drug.

Experimental

Synthesis of the complexes

Mononuclear complex $Cu(Pin)_2(HPin)_2$

The mononuclear complex was obtained in aqueous solution at pH 9.5 (raised with dil. NaOH) in the presence of an excess of the ligand at molar ratio Cu:HPin = 1:10 (0.1240 g pindolol base in 20 ml H_2O were mixed with 0.0085 g $CuCl_2.2H_2O$ in 20 ml H_2O). Under these conditions the complex precipitated as a violet substance, that was filtered, washed with water and dried at 80 °C. The analytical data were as follows: Found: Cu, 5.78; C, 64.70; H, 7.76; N, 10.15. Calc. for $Cu(Pin)_2(HPin)_2$: Cu, 6.01; C, 63.64; H, 7.63; N, 10.60%. The complex was separated as a very fine powder and unfortunately it was not possible to obtain monocrystals suitable for X-ray diffraction study. The same complex in a soluble form was prepared also in methanolic solution at molar metal-to-ligand ratio 1:10.

Dinuclear complex $Cu_2Pin_2Cl_2$

Mixing of methanolic solutions with a molar ratio Cu:HPin = 1:2 (0.0426 g $CuCl_2.2H_2O$ in 10 ml MeOH added to 0.1240 g pindolol base in 20 ml methanol) leads to the appearence of a dark-green coloration and after 14–16 days green monocrystals suitable for X-ray study precipitate from the solution. Found: Cu, 17.52; C, 48.22; H, 5.71; N, 8.80; Cl, 9.95. Calc. for $Cu_2Pin_2Cl_2$: Cu, 18.35; C, 48.56; H, 5.53; N, 8.09; Cl, 10.24%. The complex is soluble in water.

Biological experiments

The literature data have shown that the LD_{50} values for intraperitoneal (*i.p.*) administration of pindolol in mice and rats are very close and practically indistinguishable (Merck Index 2000). For that reason a group of mice was used in the toxicological experiments, whereas the pharmacological runs were performed on rats.

Toxicological studies

The experiments were performed on 60 male white mice with body weight 18–22 g. Acute toxicity of the compounds studied (pindolol and its mononuclear copper(II) complex) was assessed after dissolution in saline (0.9% NaCl with 1–2 drops of Tween 80) and *i.p.* administration to mice in 5 different doses (6 animals in group for each dose). The acute toxicity was determined by the method of Litchfield-Wilcoxon (1949). The lethality of the animals was recorded on the 24^{th} h, and all changes in the mice behaviour were monitored until 14 days after the drug administration.

The animals were kept at standard conditions, having free access to food and water. All experimental procedures were conducted in accordance with the NIH guidelines of the Care and Use of Laboratory animals.

Heart rate studies

The experiments were performed on male Wistar rats (180–220 g body weight). The decrease of the heart rate in a group of animals (5 rats) by pindolol and $Cu(Pin)_2(HPin)_2$ (6 rats) was measured from 0 to 480 min after the *i.p.* administration of the substances. The compounds studied were applied in equitoxic doses (1/10 LD_{50}) after their dissolution in saline with 1–2 drops of Tween 80.

Instrumentation

The electronic spectra in solution were obtained on a spectrometer Specord UV-VIS (Carl-Zeiss, Jena), the electronic reflectance spectra - on a VSU-2P spectrometer (Carl-Zeiss, Jena), the IR-spectra - both on a 75-IR Specord in the range 4,600–700 cm^{-1} and on a Perkin-Elmer spectrometer, model 983, in the far IR range (1,000–200 cm^{-1}) in nujol, KBr or CsI pellets. The EPR-spectra were obtained using a Bruker ER 420 X-band spectrometer, connected with PC for data acquisition. The thermogravimetric measurements were performed using a thermogravimetric system model TGS-2 (Perkin-Elmer) and the magnetochemical experiments - by a Faraday balance. The complexes were analyzed according to the standard

Table 1. Crystal data and structure refinement for $Cu_2Pin_2Cl_2$

Empirical formula	$C_{28}H_{38}Cl_2N_2O_4$	
Formula weight	792.64	
Temperature	293(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	C2/c (#15)	
Unit cell dimensions	a = 14.4998(13) Å	α = 90 deg.
	b = 18.511(2) Å	β = 109.556(2) deg.
	c = 14.2982(13) Å	γ = 90 deg.
Volume, Z	3616.4(6) $Å^3$, 12	
Density (calculated)	1.515 g/cm^3	
Absorption coefficient	1.372 mm^{-1}	
F(000)	1672	
Crystal size	0.15 × 0.15 × 0.20 mm	
θ range for data collection	1.85 to 22.50 deg.	
Index ranges	$-17 \le h \le 19$, $-24 \le k \le 23$, $-15 \le l \le 19$	
Reflections collected	7060	
Independent reflections	2353 [R(int) = 0.0664]	
Refinement method	Full-matrix least-squares on F^2	
Data [I>2σ (I)]/parameters	2286/183	
Goodness-of-fit on F^2	1.056	
Final R indices [I>2σ (I)]	R1 = 0.0692, wR2 = 0.1236	
R indices (all data)	R1 = 0.0835, wR2 = 0.1486	
Extinction coefficient	0.0002(2)	
Largest diff. peak and hole	0.54 and −0.49 e. $Å^{-3}$	

procedures for C and H (as CO_2 and H_2O), for N - by the Dumas method, for Cu(II) - complexometrically with EDTA (pH 8, Murexide) after ignition of a sample and dissolution of the residue in HNO_3.

The heart rate of the rats was measured with a ECG apparatus NEC-9001 (Germany).

Potentiometric titrations and data evaluation

Solutions containing 2.50×10^{-4} mol l^{-1} Cu(II) ($Cu(ClO_4)_2.6H_2O$) and 2.50×10^{-3} mol l^{-1} of the pindolol base with a Cu:HPin ratio 1:10 were titrated with 1.051×10^{-2} mol l^{-1} $HClO_4$ in 50% w/w CH_3OH/H_2O to obtain the stability constants. The ionic strength was adjusted to 0.1 mol l^{-1} with $NaClO_4.H_2O$. Argon was bubbled through the samples to remove oxygen and carbon dioxide and for stirring the solutions. Measurements were performed on Radiometer PHM 84 pH-meter equipped with a GK2402C combined electrode and an ABU80 automatic burette for pH-metric titration. The stability constants were calculated by means of a general computational program PSEQUAD (Zekány & Nagipál 1985).

Crystal structure

A transparent brownish-green crystal of the dinuclear copper(II) complex was selected and mounted on an Enraf-Nonius CAD-4 diffractometer. A hemisphere of 7,060 reflections of which 2,353 independent [R(int) = 0.069] and 2286 with $I > 2\sigma(I)$ was collected at room temperature using graphite monochromated Mo-K_α radiation (λ = 1.71073 Å). The structure was solved by direct methods (Sheldrick 1995) and refined by least squares on F^2 (Sheldrick 1996). First the Cu and Cl atoms were located and their positions refined isotropically. Next the C, N and O atoms of the pindolol molecule were found and all atoms refined anisotropically. Finally some of the H atoms were refined using a riding model. The main crystal and refinement data are presented in Table 1.

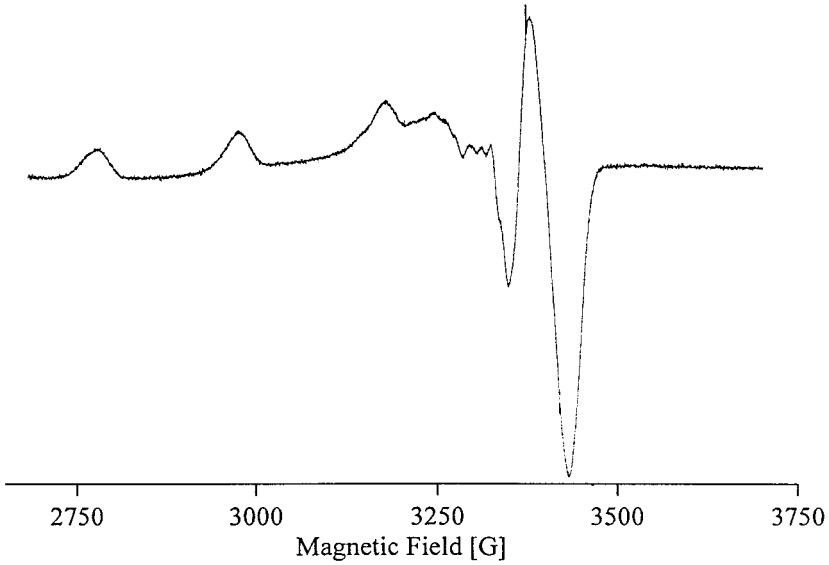


Figure 1. X-band EPR-spectrum of the frozen methanolic violet solution at 150K.

Results and discussion

Mononuclear copper(II) complex Cu(Pin)$_2$(HPin)$_2$

The complex shows an absorption band at 525 nm ($\varepsilon \approx 120$ l mol^{-1} dm^{-1}) with a shoulder at 620 nm both in aqueous and methanolic solutions. The electronic reflectance spectrum of the solid substance exhibits a reverse intensity of the two peaks. No absorbance was found both in solution and in the solid state in the range 350–400 nm, typical for dinuclear Cu(II) complexes with oxygen-bridges from O-containing ligands. The bands observed are due to d-d transitions from copper(II), engaged in Cu-O and Cu-N bond formation. The location of the bands at relatively lower wavelengths suggests a considerable degree of tetragonal distortion of the complex (Melnik 1981; Hathaway 1987). The analogous spectra recorded for both phases studied, have shown that the violet mononuclear complex retains its structure in solution and solid state.

EPR-signals from the complex were observed both in solution and in solid state. An isotropic signal with a resolved hyperfine splitting from ^{63,65}Cu was obtained from the violet methanolic solution at room temperature. The parameters $g_{iso} = 2.12$ and $A_{iso} = 87 \times 10^{-4}$ cm^{-1} are typical for mononuclear Cu(II) complexes (Drago 1981). At 150K anisotropic spectra from both liquid (Figure 1) and solid phases were recorded with the following parameters: $g_\parallel = 2.209$, $A_\parallel = 205 \times 10^{-4}$ cm^{-1}, $g_\perp = 2.071$, $A_\perp = 30.7 \times 10^{-4}$ cm^{-1}. The g- and A-values together with the relations $g_\parallel > g_\perp$ and $A_\parallel > A_\perp$ have shown that the unpaired electron of Cu(II) occupies predominantly the $d_{x^2-y^2}$ orbital, leading to an elongated octahedral coordination around the metal. A superhyperfine splitting (SHFS) from ^{14}N was observed in the perpendicular magnetic field with $A_\parallel(^{14}N) = 13.6$ G, that confirms the coordination of nitrogen to copper(II).

The magnetic susceptibility of the violet complex was measured at 293K and the effective magnetic moment was found to be 1.82 B.M. This value is in accordance with the assumption for a mononuclear structure of the complex studied.

The free ligand pindolol (base) shows an IR spectrum with one broad (3,390–3,410 cm^{-1}) and two resolved bands (3,310 and 3,220 cm^{-1}), due to ν(OH) and ν(NH) respectively, engaged in inter- or intramolecular H–bonds. The last assumption was supported also by the strong absorption in the 2,900–2,300 cm^{-1} range. The bands due to the stretching NH–vibrations remained in the spectrum of the complex, although with a decreased intensity and a new weak band appeared at 3,400 cm^{-1}, assigned to OH-groups, coordinated with Cu(II). The doublet from the free ligand spectrum is observed here as a broad band, shifted to 3,280 cm^{-1}, while the absorbance in the H-bond range decreases. These facts suggest complexation of copper(II) with O^- and NH from the deprotonated aminoalcohol fragment of some ligand molecules and coordination of other neutral ligands

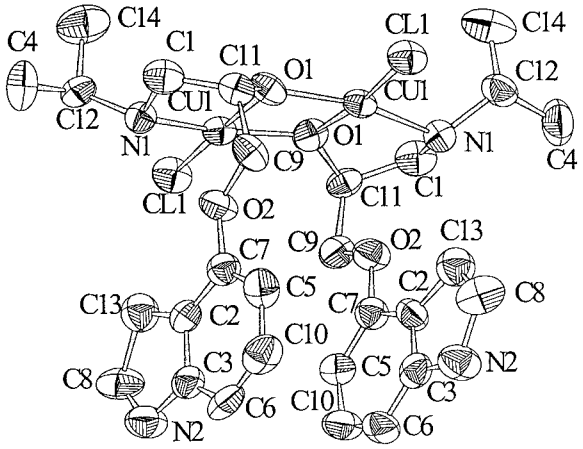
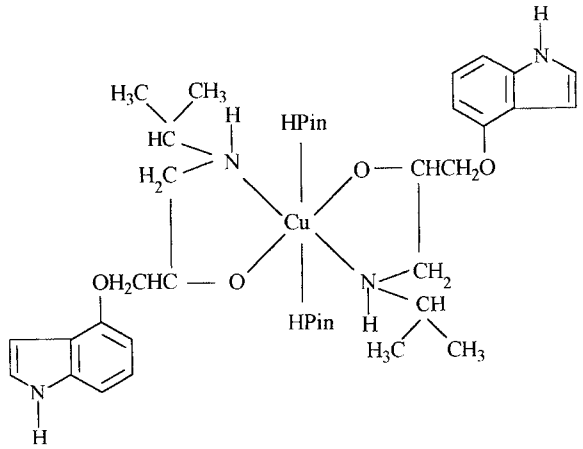


Figure 2. Connectivity in $Cu_2Pin_2Cl_2$, 50% thermal ellipsoids.



Scheme II.

(OH → Cu) as well. The formation of Cu–N and Cu–O bonds was confirmed also by the appearance of new bands at 580 and 490 cm^{-1} in the spectrum of the complex (Nakamoto 1997).

Thermogravimetric studies have shown that no water molecules participate in the complex and therefore the presence of OH-groups observed by vibrational spectra is related to non-dissociated hydroxy-groups from the ligand. At temperatures over 170 °C the ligand undergoes thermal decomposition.

The M:L ratio 1:4 obtained by the elemental analysis together with the other experimental data is consistent with the following structure (Scheme II) with a general formula $Cu(Pin)_2(HPin)_2$ and with the neutral character of the complex as well. The latter follows from the fact that the complex precipitates from aqueous solutions and is soluble in methanol and other solvents of lower polarity.

The autoprotolysis constant of the solvent used in the determination of the stability constants (50% w/w CH_3OH) was previously found to be pKs = 14.57 ± 0.03 (Bontchev *et al.* 1992). The dissociation (acidity) constant of pindolol as a free base was determined to be pKa = 9.04 ± 0.05 in the 50% w/w CH_3OH medium used. In an excess of the ligand (Cu:HPin = 1:4) in the same medium the first two stability constants of the mononuclear complex are: $\lg\beta_1 = 5.64 \pm 0.04$ and $\lg\beta_2 = 10.58 \pm 0.05$. The first two pindolol molecules are coordinated to copper(II), forming consequently the complexes $[CuPin]^+$ and $CuPin_2$, where pindolol acts as a bidentate monoanionic ligand. The addition of the next two ligands HPin leads to the formation of the mononuclear $Cu(Pin)_2(HPin)_2$, where HPin in axial positions is coordinated as a monodentate neutral ligand. The stability constants thus found correlate very well with the known data for copper(II) complexes of other β-blockers (Bontchev *et al.* 1992, 2000, 2001).

This coordination mode realized both in solution and in a solid phase was confirmed by the spectral, EPR and magnetochemical data obtained.

Dinuclear complex $Cu_2Pin_2Cl_2$

X-ray structure determination

The structure consists of centrosymmetric copper(II) dimers, the copper centers being linked by oxygen bridges. Copper(II) has fourfold coordination, built by two O atoms from two ligands, one N from the ligand and one Cl, forming a slightly distorted square (Figure 2). In the structure the Cu atoms occupy one crystallographically independent position. The Cu-O distances are 1.930(7) and 1.899(6)Å, Cu-N and Cu-Cl are 2.016(8) and 2.220(3)Å, respectively (Table 2). Each two structures are linked together by a common edge to form planar dimers of composition $Cu_2Cl_2N_2O_2$ (Figure 2). The L-Cu-L angles within the dimers vary between 76.0(3) and 98.0(2)° (Table 2). The dimers are oriented with their planes perpendicular to the *b*-axis. The coordinated pindolol molecules are located in such a way that the planes of the 5- and 6- membered rings of the pindolol molecules have their planes parallel to each other and perpendicular to the plane of the $Cu_2Cl_2N_2O_2$ dimers (Figure 3). The closest Cu-Cu distances are 3.008(3) within and 8.901(4)Å between the dimers. No evidence of hydrogen bonding between the Cu-dimers has been found and the title compound could be considered as a typi-

Table 2. Selected bond lengths (Å) and angles (°).

Cu(1)-O(1)#1	1.899(6)	O(1)#1-Cu(1)-O(1)	76.0(3)
Cu(1)-O(1)	1.930(7)	O(1)#1-Cu(1)-N(1)	85.2(3)
Cu(1)-N(1)	2.016(8)	O(1)-Cu(1)-N(1)	160.8(3)
Cu(1)-Cl(1)	2.220(3)	O(1)#1-Cu(1)-Cl(1)	172.0(3)
Cu(1)-Cu(1)#1	3.008(1)	Cu(1)#1-O(1)-Cu(1)	103.6(3)

cal molecular crystal with a 3D structure hold together by weak Van der Vaals forces.

Spectral properties of the dinuclear complex

The electronic spectra both of the initial green solution and the solid state have shown an absorbance at 700 ($\varepsilon \approx 100$) and 385 nm ($\varepsilon \approx 1,000$). The lower energy band is assigned to d-d transition in a square-planar copper surrounding, while the second band is related to a charge-transfer transition in the Cu-O bridge formed. The transition is realized between the non-bonding orbitals of bridging oxygen atoms and the vacant copper d-orbitals (Hathaway 1987). The parameters of the other d-d band reveal a strongly distorted octahedral structure, practically very close to a square-planar one. The spectral data are in a good agreement with the X-ray diffraction results for the dinuclear structure.

The most informative spectral feature was the disappearance of the ν(OH) in the IR spectrum of the complex in contrast to the spectrum of the non-coordinated pindolol. This fact together with a decreased absorption in the interval 2,900–2,300 cm^{-1} shows a deprotonation of the hydroxy-group through its coordination to the copper(II) ions. The shift of the stretching (-20 cm^{-1}) and deformation ($+10$ cm^{-1}) modes of the NH-group from the aminoalcohol fragment of the pindolol molecule is a result of its bonding to the metal. In the same time new bands appeared in the far-IR area at 454, 340 and 280 cm^{-1}, due to the stretching Cu-N, Cu-O and Cu-Cl vibrations, respectively (Nakamoto 1997). The thermogravimetric investigation in the range 300–500K has shown that no solvent molecules (CH_3OH) are included into the solid phase complex.

The close Cu-Cu distance in the framework of the dinuclear structure (3.008 Å) leads to the appearance of a strong magnetic exchange coupling (antiferromagnetism) between the two paramagnetic centres (Uhlig & Staiger 1966; Mikuriya *et al.* 1980). This is the reason for the absence of any EPR-signals in the 100–6,000 G range and for the enhancement of the magnetic susceptibility with temperature in the range 298–353K from 1.2×10^{-7} to 5.1×10^{-7}.

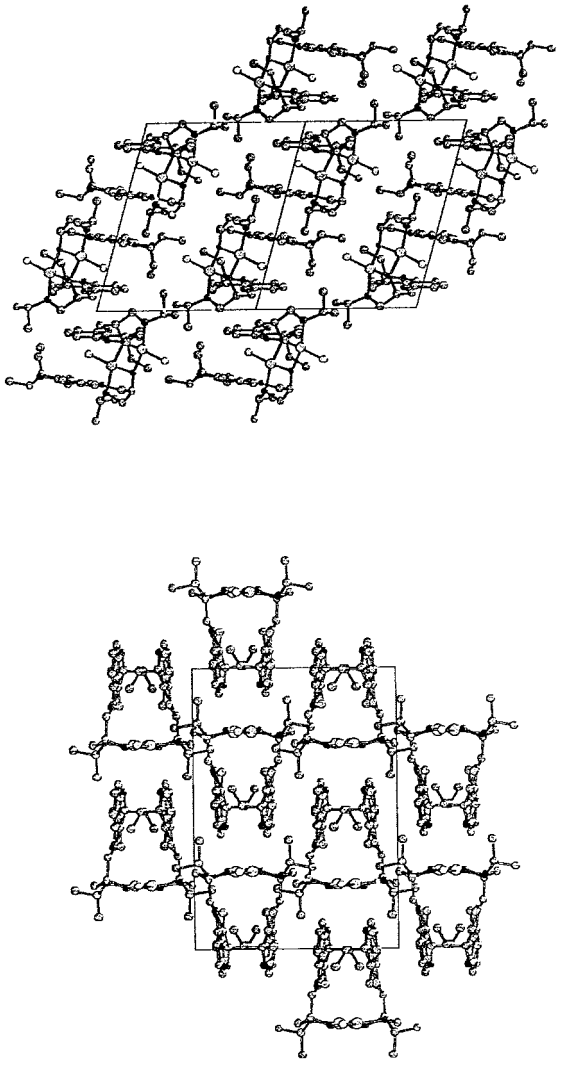


Figure 3. View of the dinuclear structure along [110] - top and [100] - bottom.

Biological investigations

The evaluation of the acute toxicity has shown that the mononuclear complex $Cu(Pin)_2(HPin)_2$ is more toxic than the non-coordinated ligand: $LD_{50} = (5.2 \pm 1.4) \times 10^{-5}$ M (complex) in comparison with $LD_{50} = (5.7 \pm 1.6) \times 10^{-4}$ M (pindolol).

The pindolol pharmacokinetics shows $t_{1/2} = 3$–4 h according to the literature data (Guerett *et al.* 1983).

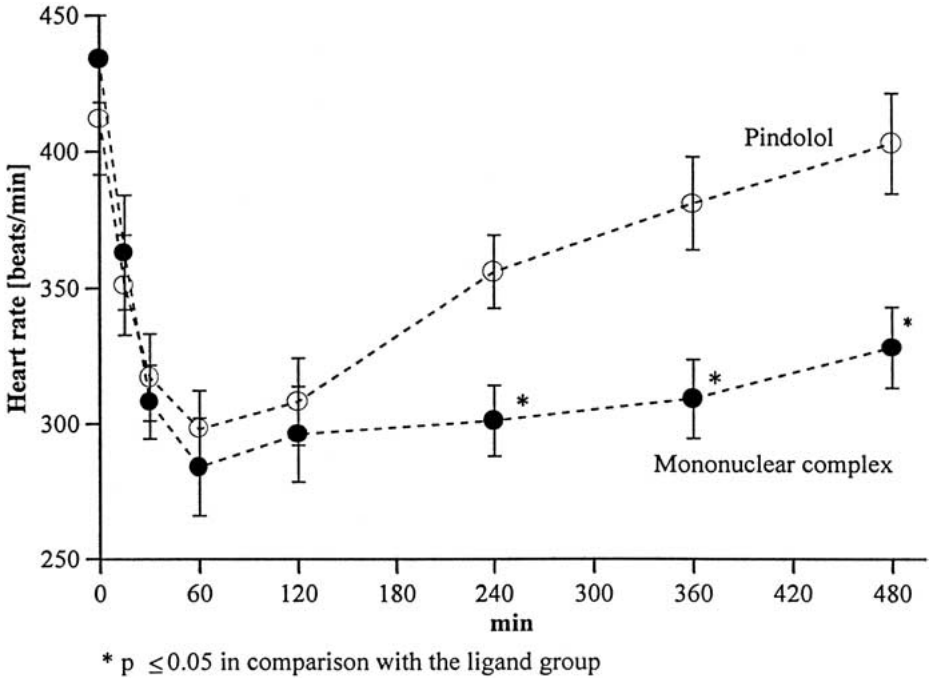


Figure 4. Heart rate of rats after *i.p.* administration of pindolol and its mononuclear complex.

The results for the influence of pindolol and its complex on the heart rate of normotensive animals indicate (Figure 4) a more effective and prolonged action of the complex during the 8-h period of observation. Pindolol is practically uneffective 480 min after its *i.p.* administration, while the complex still retains 24% of its initial activity.

These results reveal that the complexation with copper(II) increases the effect of pindolol as beta-blocking agent. This positive result could be probably explained by the influence of the complex formation on the pharmacodynamic properties or on the pindolol pharmacokinetics.

Acknowledgement

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