COPPER(II) DIHYDROXYBENZOATES; SYNTHESIS AND PROPERTIES

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Received May 20, 1992
Accepted October 27, 1992

Dedicated to Assoc. Professor František Hanic in honour of his 65th birthday.

Compounds of composition $Cu(2,5\text{-}DHB)_2 . 4 H_2O$ (DHB, dihydroxybenzoate anion) and $Cu(2,Y\text{-}DHB)_2 . 8 H_2O$ (Y = 4 or 6) were prepared as potential antiinflammatory agents with a view of pharmacological screening. The room-temperature magnetic moments allow to classify all compounds into the group of magnetically diluted copper(II) complexes. According to EPR and electronic spectra, the degree of tetragonal distortion increases passing from $Cu(2,6\text{-}DHB)_2 . 8 H_2O$ (*III*) through $Cu(2,4\text{-}DHB)_2 . 8 H_2O$ (*I*) to $Cu(2,5\text{-}DHB)_2 . 4 H_2O$ (*II*). The complexes differ also in the cooperative ordering in their structures. All tested compounds exhibit higher antiinflammatory activities (on dextran edema) than free carboxylic acids. However, their effects were accompanied with relatively high toxicity. Remarkable results were also achieved on evaluating the antipyretic activity.

Revived interest in carboxylatocopper(II) complexes, besides from the point of view of pure coordination chemistry, has led to investigations of their pharmacological properties mainly antiinflammatory and antipyretic activities. This trend is based on many proofs on the favourable influence of copper(II) compounds in reducing inflammation on various experimental models. In addition, it was also reported that Cu(II) complexes of antiinflammatory agents, including salicylic and acetylsalicylic acids, gave marked increases in antiphlogistic activity compared with the activities of their parent drugs[1 - 5].

Sodium and cupric salicylates were successfully used in rheumatological practice as injection mixture for several years[6]. Recently developed antiarthritic preparations for human and veterinary uses also contain ethanol adduct of copper(II) salicylate[7,8].

Salicylates, the most widely used non-steroidal antiinflammatory drugs, involve also gentisic (2,5-dihydroxybenzoic) and γ-resorcylic (2,6-dihydroxybenzoic) acids.

Aqua(2,Y-dihydroxybenzoato)copper(II) complexes represent a group in which antiphlogistic effects (including their accompanying antipyretic component) of free acids and their Cu(II) salts could be compared. Copper(II) 2,Y-dihydroxybenzoates were prepared previously[9 - 11] and new conditions of their synthesis, some physical and biological properties of these complexes are now reported.

EXPERIMENTAL

Materials and Methods

2,4-Dihydroxybenzoic acid was prepared according to ref.[12]. From sodium gentisate dihydrate (pharmacopoeia grade) free acid was obtained using concentrated HCl. 2,4-Dihydroxybenzoic or 2,6-dihydroxybenzoic acids (5.0 g) were dissolved in a hot solution of sodium hydrogencarbonate (2.7 g in 50 ml of water). The solutions of sodium salts (freed of CO_2 by boiling) were adjusted to pH 5 with small amounts of corresponding acids. After cooling, a solution of $CuSO_4 . 5 H_2O$ (4.05 g in 25 ml of water) was added. The resulting mixtures, having pH 4.5 – 5.0, yielded homogeneous compounds only when they were cooled down rapidly (to about 5 °C). Microcrystalline substances were collected, washed with cooled water and airdried, giving 80 – 90% yields. These emerald-green products were obtained as octahydrates. Sodium gentisate (6.9 g), dissolved in 150 ml of water, was adjusted by free acid to pH 5. By mixing with a solution of copper(II) sulfate pentahydrate (4.05 g in 50 ml of water), a dark-green solution arised. At room temperature, pale green crystalline precipitate of stoichiometry $Cu(2,5\text{-DHB})_2 . 4 H_2O$ (*II*) was obtained (70% yield, see Table I).

The thermal decomposition was studied by thermogravimetric method at a heating rate of 3 °C/min on an apparatus described in ref.[13]. Electronic spectra were measured on Specord M-40 (Zeiss, Jena) in the range 11 000 – 35 000 cm^{-1}, using Nujol suspension technique. The X-band EPR spectra of polycrystalline samples were scanned on Varian E-4 spectrometer at 77 K. DPPH was used as an internal standard for determination of g values. Magnetic susceptibility measurements were carried out by Gouy method at room temperature (instrument produced by Newport Instruments Ltd.). The values of effective magnetic moments were calculated according to ref.[14] (Table II).

TABLE I
Analytical data of copper(II) complexes *I* – *III*

Complex	Formula (M. w.)	Calculated/Found			
		% C	% H	% Cu	% H_2O[a]
$Cu(2,4\text{-DHB})_2 . 8 H_2O$ (*I*)	$C_{14}H_{26}O_{16}Cu$	32.72	5.10	12.36	28.05
	(513.9)	33.13	5.02	12.43	28.0
$Cu(2,5\text{-DHB})_2 . 4 H_2O$ (*II*)	$C_{14}H_{18}O_{12}Cu$	38.06	4.11	14.38	16.30
	(441.8)	37.93	4.09	14.48	16.0
$Cu(2,6\text{-DHB})_2 . 8 H_2O$ (*III*)	$C_{14}H_{26}O_{16}Cu$	32.72	5.10	12.36	28.05
	(513.9)	33.00	4.86	12.31	28.0

[a] Determined thermogravimetrically.

Biological Assays

Prepared complexes as well as free acids, homogenized through 0.08 mm sieve, were dispersed in 0.9% aqueous sodium chloride and stabilized by adding 0.05 % Tween 80 (Merck). All animals were purchased from Velaz (Praha) and bred at standard conditions.

Acute antiinflammatory activity was measured by reduction in rat paw dextran edema. All substances were applied in a dose of 10 mg/kg intraperitoneally (i.p.). The found values of volume change were related to the starting state and compared with control group of animals (Fig. 1). Antipyretic effect was evaluated in rabbits on standard pyretic reaction induced by endotoxin (acquired from *Proteus vulgaris* T 145). Used doses are given in Table III. Both methods and statistical evaluations are described detailly in ref.[15]. Acute toxicity in i.p. and peroral (p.o.) applications was determined orientationally as a range of the probable lethal dose (LD_{50}). Different doses of the tested compounds were given to a pair of mice and perishing of animals was registered 24 h after (see Table III).

RESULTS AND DISCUSSIONS

Reactions of Cu^{2+} with dihydroxybenzoate ions in aqueous medium under conditions used for synthesis copper(II) salicylate tetrahydrate[16] resulted in product formation of stoichiometry identical to that of salicylate only in the case of gentisate ions. On the other hand, the systems containing further two studied anions yielded samples with

TABLE II
Spectral and magnetic properties of copper(II) complexes *I* – *III*

Complex	$\Delta_{max} \cdot 10^{-3}$ cm^{-1}	μ_{eff} B.M.	g_z $g_{\parallel}$	g_1^c	g_y	g_2^c	g_x	g_3^c $g_{\perp}$	g_{av}	G
I	14.2	1.96		2.309		2.134		2.060	2.168	–
II	15.2	1.90	2.314		2.078		2.039		2.144	5.5
III	13.0	1.97	2.386					2.083	2.184	4.7

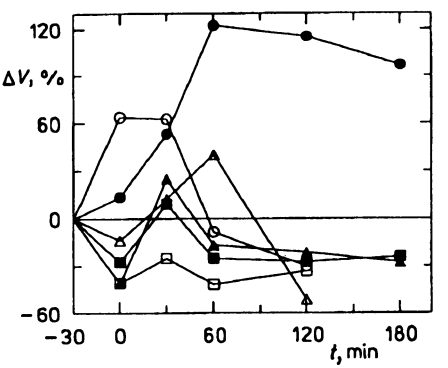

FIG. 1
Time course of changes in edema volume, ΔV (in %) compared to those in the control edema (0% level). The complexes *I* (○), *II* (△), *III* (□); parent free carboxylic acids *I* (●), *II* (▲), *III* (■)

varying degree of hydration at room temperature. Only at about 5 °C the well defined octahydrates of mentioned complexes were found to be formed in sufficiently pure state. Previously, copper(II) dihydroxybenzoates prepared at higher temperature were described to have the same composition[9,10], with the exception of $Cu(2,4\text{-}DHB)_2 . 7 H_2O$ (see ref.[11], cf. Table I).

On storing the complex *I* in the air and the complex *III* over $CaCl_2$ for 3 – 5 days at room temperature, the products of the composition $Cu(2,Y\text{-}DHB)_2 . 2.5 H_2O$ (Y = 4 or 6) were obtained. TG curves showed that the dehydration of both octahydrates proceeded as an endothermic two-step process. In the first step (90 – 100 °C) six water molecules are released. The residual water molecules are liberated in the 100 – 180 °C and 100 – 160 °C range for the complexes *I* and *III*, respectively. In contrast, the dehydration of the complex *II* proceeds in one-step with a maximum (DTA) at 138 °C. The thermal decomposition of this complex was found to be very similar to that of copper(II) salicylate tetrahydrate[16].

Spectral and Magnetic Properties

According to the values of magnetic moments at room temperature, the investigated Cu(II) complexes are magnetically diluted (Table II).

The EPR spectra provide information about the stereochemistry of Cu(II) complexes. Since all *g* factors are greater than 2.04, the ground electronic state[17] is $d_{x^2-y^2}$. Then the following equations are valid for the molecular *g* factors:

TABLE III

Results of pharmacological screening of dihydroxybenzoic acids and their copper(II) salts. Antipyretic activity – body temperature differences $\Delta t . 10$ (in °C); acute toxicity – range of LD_{50} (in mg/kg)

Compound[a]	Antipyretic activity (time interval, min)								Acute toxicity	
	15	30	45	60	75	90	105	120	i.p.	p.o.
CG	1.0	3.2	6.2	12.2	11.3	9.4	6.2	4.2	–	–
I[b]	−0.1	−0.5	−1.7[d]	−2.3[d]	−3.0[d]	−3.4[d]	−4.8[d]	−7.5[d]	>500	≈ 2 000
I	0[c]	3.5	5.7	7.3	4.8	4.0	3.7	3.3	50 – 100	800 – 1 200
II[b]	−3.7	−2.6	−1.5	−0.1[d]	−0.5[d]	−1.4[d]	−2.5	−3.3	>500	>2 000
II	−1.7[c]	0.5	2.8	3.3[d]	4.1[d]	4.3[d]	4.1[d]	4.1	≈ 50	1 200 – 1 500
III[b]	−3.8	−3.5	−1.6	−0.9[d]	−0.3[d]	−0.6[d]	−1.4	−0.6	>500	1 500 – 2 000
III	2.4	−0.5	−1.7	−3.1[d]	−3.2[d]	−3.7[d]	−3.7	−4.1	50 – 100	700 – 1 000

[a] CG control group of animals; numbering of complexes see Table I. [b] Corresponding free carboxylic acids. [c] Applied dose of 10 mg/kg i.p.; the other compounds were applied in twofold dose. [d] Statistically significant results with $P < 0.05$.

$$g_{\parallel} = 2.0023 - 8k_{\parallel}^2 \lambda_0 / \delta E_{xy}$$

$$g_{\perp} = 2.0023 - 2k_{\perp}^2 \lambda_0 / \delta E_{xz,\,yz}\,, \qquad (1)$$

where $\lambda_0 = -830$ cm^{-1} is the spin-orbital splitting parameter for the free Cu^{2+} ion, $k_{\parallel}$, $k_{\perp}$ are the spin-orbital reduction parameters and δE_n are the values of the splitting of the *d* orbital set by the ligand field. According to Eqs (*1*) the average *g* factor $g_{av} = \frac{1}{3}(2g_{\perp} + g_{\parallel})$ is inversely proportional to the values of splitting δE_n.

The high value of $g_{av} = 2.184$ for Cu(2,6-DHB)$_2$. 8 H_2O together with the small wavenumber of *d–d* transition at 13 000 cm^{-1} indicate a low ligand-field energy. In accordance with ref.[9] the powder diffraction pattern of this complex shows that it is isostructural with $[Zn(H_2O)_6](2,6\text{-DHB})_2$. 2 H_2O. The parameters of the EPR spectrum (Table II) are very similar to the values for copper(II) hydrogenmaleate hexahydrate[18], where $[Cu(H_2O)_6]^{2+}$ cations with strongly elongated tetragonal structure were found by X-ray structural analysis. So our results are in agreement with ref.[9], where for Cu(2,6-DHB)$_2$. 8 H_2O a structure consisting of $[Cu(H_2O)_6]^{2+}$ cations and outer-sphere bound 2,6-DHB anions was postulated. The steric hindrance owing to phenolic groups in positions 2 and 6 contributes probably to the fact that the anionic ligands are not bound in the inner coordination sphere of the Cu(II) central atom.

In contrast, the parameters of the EPR and ligand-field spectra of Cu(2,4-DHB)$_2$. 8 H_2O (*I*) and Cu(2,5-DHB)$_2$. 4 H_2O (*II*) are comparable with those characteristic for the most common structural type of magnetically diluted carboxylatocopper(II) aquacomplexes in which 4 oxygen atoms – 2 from the carboxylate groups and 2 from water molecules – are bound in the equatorial plane[9,16]. Here the axial positions are occupied by water molecules and/or the phenolic oxygen atoms from the adjacent molecules. The first type of axial interaction was found by X-ray structural analysis in copper(II) salicylate tetrahydrate[16] and the second one in copper(II) 2,6-dihydroxybenzoate dihydrate[9].

According to Eqs (*1*) the value of g_{av} is inversely proportional to the strength of the axial ligand field provided that the coordination in the equatorial plane remains unaltered[19]. The value of $g_{av} = 2.144$ obtained for Cu(2,5-DHB)$_2$. 4 H_2O is slightly lower than $g_{av} = 2.154$ reported for copper(II) salicylate tetrahydrate[9], where the Cu–O axial distance is 2.8 Å (ref.[16]). On the other hand, this value is higher than that reported for copper(II) acetylacetonate, a typical square planar Cu(II) complex[20]. It can be assumed that the $Cu–O_{ax}$ distance in the investigated complex is slightly higher than 2.8 Å, but some degree of axial interaction is still operating. The EPR spectrum characteristic of orthorhombic symmetry indicates some distortion from tetragonal arrangement in the equatorial plane.

The value of $g_{av} = 2.168$ for Cu(2,4-DHB)$_2$. 8 H_2O (*I*) is nearly equal to the g_{av} value found for copper(II) 2,6-dihydroxybenzoate dihydrate, where the distance from Cu

atom to the axial phenolic oxygen is 2.559 Å (ref.[9]). In the present case similar axial interaction can be assumed.

For $Cu(2,5\text{-}DHB)_2 . 4\ H_2O$ and $Cu(2,6\text{-}DHB)_2 . 8\ H_2O$ the parameter

$$G = (g_{\parallel} - g_0)/(g_{\perp} - g_0) \qquad (2)$$

is higher than 4.0. This indicates that the observed g factors are molecular, e.g. they characterize the isolated Cu(II) coordination polyhedron[17]. A parallel (ferrodistortive) ordering of the complex molecules in the structure is possible for these complexes. This arrangement in which the distance between the clost Cu(II) atoms with non-parallel g axes is so large that the exchange interaction J_{ex} is smaller than $4(g_{\parallel} - g_{\perp})\beta H$ can also account for such spectra[21]. Only EPR spectroscopy of single crystals can distinguish between these two alternatives.

In contrast, the value of $G < 4.0$ for $Cu(2,4\text{-}DHB)_2 . 8\ H_2O$ (*I*) shows that in this compound the exchange interaction between Cu(II) ions with locally misaligned symmetry axes causes the crystal EPR spectrum to appear. In the simplest case of two molecules of tetragonal symmetry with an angle 2γ between the tetragonal axes the experimentally observed crystal g values are given by the following equations[21]:

$$g_1^c = \cos^2\gamma\, g_{\parallel} + \sin^2\gamma\, g_{\perp}$$

$$g_2^c = \sin^2\gamma\, g_{\parallel} + \cos^2\gamma\, g_{\perp}$$

$$g_3^c = g_{\perp} . \qquad (3)$$

For $Cu(2,4\text{-}DHB)_2 . 8\ H_2O$, the values $g_{||} = 2.383$ and $g_{\perp} = 2.060$ are calculated from Eqs (*3*). The angle $2\gamma = 57°$, calculated from the equation[21]:

$$\cos 2\gamma = (g_1^c - g_2^c)/(g_1^c + g_2^c - 2\, g_3^c) \qquad (4)$$

corresponds to disturbed-antiferrodistortive ordering of the molecules in the structure.

Biological Properties

The present copper(II) complexes were synthesized with the aim to investigate their antiinflammatory and antipyretic activities in comparison with parent 2,Y-dihydroxybenzoic acids.

To evaluate the antiinflammatory paw dextran edema was induced in rats, because this edema type was found the most sensitive to salicylates[22]. Except for the complex *III*, all tested compounds in a single dose of 10 mg/kg caused elevation of dextran irritant effect in the initial phase of established inflammation. In the following course of edema reaction, the copper(II) complexes exhibited higher antiphlogistic activities

compared to the free acids. Cupric gentisate tetrahydrate achieved the highest degree in edema reducing (with 51.5% volume contraction). However, results for all Cu(II) complexes were statistically insignificant (in the 5.0% level). In addition, observed effects of cupric salts in doses used were accompanied with toxicity leading to animals' exitus after second hour of experiments (Fig. 1).

More important results were obtained on evaluating the antipyretic effect (Table III). The standard pyretic reaction in rabbits was affected by tested compounds in single dose of 20 mg/kg i.p. except for the complexes *I* and *II* which were applied in the half doses because of their toxicity. Pretreatment of rabbits by these compounds blocked the appearance of endotoxin fever. All compounds tested caused evident lowering in the body temperature of fevered animals and so they could be considered to have antipyretic activities. Figure 2 illustrates these effects in culminating phase of pyretic reaction. The complex *III* was found to be most effective as in 30th min of assay it caused the decrease of animals' temperature below the normothermic state. The antipyretic action of this complex is significantly higher than that of γ-resorcyclic acid.

From all complexes 2,6-dihydroxy derivative was the most active with respect to the pair acid–Cu(II) complex in both studied activities. In relation to its structure, hexaaquacopper(II) ordering surrounded by 2,6-DHB anions in outer-sphere of the complex $[Cu(H_2O)_6]$ $(2,6\text{-DHB})_2 \cdot 2\ H_2O$ seems to be advantageous for possible controlling of liberation of Cu^{2+} and carboxylate ions. As organic carboxylate component represents only 60% of this complex, higher activity of the complex *III* can be understood in terms of synergistic effect operating in favour of absorption of both carboxylate and cupric ions.

Examination of acute toxicity showed that the copper(II) complexes were more toxic than the corresponding free acids (Table III). The observed differences at i.p. application scattered in the range of one order, while they were approximately twofold at p.o. administration. Such degrees of acute toxicity correspond to those found in sali-

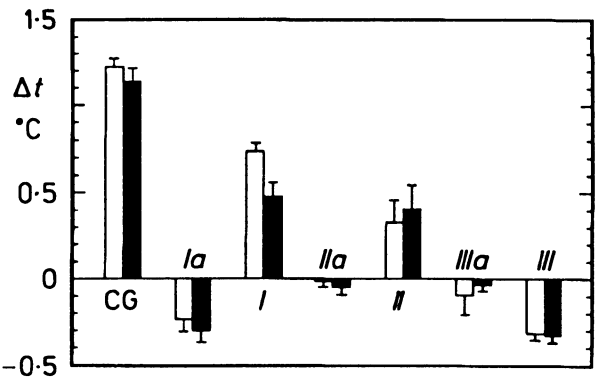

FIG. 2
Column graph of the effects of tested compounds on endotoxin-induced pyretic reaction, Δt (°C) in the 60th (white column) and 75th min (dark column). Mean standard deviations are represented as bars on columns. The compounds are detailed in Table I; *Ia*, *IIa*, *IIIa* free carboxylic acids

cylate-like compounds[5,15]. Undesirable toxic effects of studied complexes could be eliminated by alkyl or acyl substitution at phenolic groups.

This work was supported by the Ministry of Education, Youth and Sport of The Slovak Republic in Grant No. 05/63.

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Translated by the author (B. L.).