Antimicrobial Activities of Iron Perchlorate Complexes of Triphenylphosphine and Triphenylarsine Oxides

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Antimicrobial activities of Fe(II) and Fe(III) complexes of the composition $Fe(OEPh_3)_4(ClO_4)_n$ (E = P or As; $n = 2$ or 3) are described. The perchlorate Fe(II) complexes show higher antimicrobial activity than the Fe(III) complexes and the triphenylphoshine oxide complexes are generally more antimicrobially effective than the triphenylarsine oxide ones. Properties of the studied perchlorate complexes are compared with those of other iron—$OEPh_3$ complexes.

Generally iron—$OEPh_3$ complexes (E = P or As) are possible to prepare by the reaction of $OEPh_3$ with appropriate iron compounds [1, 2]. Another way is based on the autocatalytic oxidation of EPh_3 by O_2 in the presence of corresponding iron compounds in acetonitrile (ACN) [1, 3]. However, in the case of iron perchlorates the oxidation of PPh_3 by O_2 takes place only in the presence of $Fe(ClO_4)_3$. During the oxidation of PPh_3 Fe(III) is reduced to Fe(II) and stable complex β-$[Fe(OPPh_3)_4](ClO_4)_2$ is formed [1]. Formation of iron—triphenylarsine oxide complexes from $AsPh_3$ and Fe(II) or Fe(III) perchlorate has not been observed because oxidation of $AsPh_3$ by O_2 did not occur. This is probably caused by a lower reactivity of $AsPh_3$ and a weaker energy of the As—O bond in comparison with PPh_3 [4].

The complexes α-$[Fe(OPPh_3)_4](ClO_4)_2$ (*I*) and β-$[Fe(OPPh_3)_4](ClO_4)_2$ (*Ia*) have the same composition, but they have different spectral and magnetic properties. We have supposed that these complexes have tetrahedral arrangement of ligands $OPPh_3$ with a different degree of distortion [1].

EXPERIMENTAL

$Fe(ClO_4)_2 \cdot 6H_2O$ was prepared by dissolution of powder of iron in perchloric acid as green needles [5].

$Fe(ClO_4)_3 \cdot 12H_2O$ was prepared by the reaction of $Fe_2O_3 \cdot xH_2O$ with perchloric acid. The substance crystallized as yellowish crystals. Elemental analysis: w_{Fe}(calc.): 9.79 %; w_{Fe}(found): 9.81 %.

The iron perchlorate complexes (*I*—*IV*) were prepared by reaction of $OEPh_3$ (*V*, *VI*) with Fe(II) and Fe(III) perchlorates, respectively. The violet α-$[Fe(OPPh_3)_4](ClO_4)_2$ (*I*), β-$[Fe(OPPh_3)_4](ClO_4)_2$ (*Ia*), and yellow $Fe(OPPh_3)_4(ClO_4)_3$ (*III*) were prepared previously [1].

The light violet $Fe(OAsPh_3)_4(ClO_4)_2$ (*II*) was prepared by the reaction of $Fe(ClO_4)_2 \cdot 6H_2O$ in acetonitrile solution with acetonitrile solution of *VI* in the mole ratio 1 : 4 at ambient temperature. Microcrystals which precipitated were filtered off and dried at room temperature. Yield of *II* was 65 %. For $C_{72}H_{60}O_{12}Cl_2As_4Fe$ (M_r = 1543.63) w_i(calc.): 3.62 % Fe, 56.02 % C, 3.92 % H; w_i(found): 3.54 % Fe, 55.88 % C, 3.84 % H.

The light yellow $Fe(OAsPh_3)_4(ClO_4)_3$ (*IV*) was prepared when acetonitrile solutions of $Fe(ClO_4)_3 \cdot 12H_2O$ and $OAsPh_3$ were mixed in the mole ratio 1 : 4 at ambient temperature. Immediately after a small amount of white powder formed was filtered off and mother liquid was left to crystallize. After 3 h a light yellow powder which precipitated was filtered off and dried at room temperature. Yield of *IV* was 65 %. For $C_{72}H_{60}O_{16}Cl_3As_4Fe$ (M_r = 1643.16) w_i(calc.): 3.40 % Fe, 52.63 % C, 3.68 % H; w_i(found): 3.35 % Fe, 52.25 % C, 3.80 % H.

Infrared spectra of the compounds studied were taken on a Philips analytical PU 9800 FTIR spectrometer for Nujol mulls over the range 200—4000 cm^{-1}. Electronic spectra in the region 4000—15000 cm^{-1} were measured with Nicolet Magma 750 FTIR spectrometer for Nujol mulls.

The antibacterial activity of the iron perchlorate complexes and ligands $OEPh_3$ was evaluated by a microdilution method using G^+ bacteria *Bacillus subtilis*, *Staphylococcus aureus* and G^- bacterial strains *Escherichia coli* and *Pseudomonas fluorescens* [6].

The effect of these compounds on the yeasts *Candida albicans* and *Candida parapsilosis* was determined by the macrodilution method in L-shape tubes [7]. The cultures of bacteria and yeasts were incubated under vigorous shaking. The effect of prepared derivatives on filamentous fungi *Rhizopus nigricans, Aspergillus flavus, Alternaria alternata, Botrytis cinerea, Fusarium nivale, Microsporum gypseum,* and *Trichophyton terrestre* was investigated by macrodilution technique on solidified broth medium during static culturing [7].

The tested compounds were dissolved in dimethyl sulfoxide; its final concentration never exceeded 1.0 vol. % in either control or treated samples. Concentration of the compounds ranging from 10 to 500 μg cm^{-3} for bacteria and yeasts and from 50 to 1000 μg cm^{-3} for filamentous fungi was used in all experiments.

The antimicrobial activity was characterized by the IC_{50} values (concentration of a derivative which in comparison to the control inhibits the growth of microorganisms to 50 %) and MIC values (minimal inhibitory concentration of a derivative which inhibits microbial growth by 100 %). The IC_{50} and MIC values were read from toxicity curves.

MIC experiments on subculture dishes were used to assess the minimal microbicidal concentration (MMC). Subcultures were prepared separately in Petri dishes containing competent agar medium and incubated at 30 °C for 48 h (bacteria, yeasts) and at 25 °C for 96 h for filamentous fungi. The MMC value was taken at the lowest concentration which showed no visible growth of microbial colonies in the subculture dishes.

RESULTS AND DISCUSSION

Complexes $Fe(OAsPh_3)_4(ClO_4)_2$ (*II*) and $Fe(OAsPh_3)_4(ClO_4)_3$ (*IV*) were investigated by the IR (Table 1) and electronic spectroscopy. The IR spectrum of *II* showed bands corresponding to an ion-band ClO_4 group, *viz.* symmetric bending vibration at $\tilde{\nu}(\nu_2)$ = 467 cm^{-1}, asymmetric stretching vibration at $\tilde{\nu}(\nu_3)$ = 1088 cm^{-1}, and asymmetric bending vibration at $\tilde{\nu}(\nu_4)$ = 625 cm^{-1}. Also in the IR spectrum of *IV* are the bands at $\tilde{\nu}(\nu_2)$ = 467 cm^{-1}, $\tilde{\nu}(\nu_3)$ = 1088 cm^{-1}, and $\tilde{\nu}(\nu_4)$ = 623 cm^{-1}, which points to ionic band of perchlorate group. For the free ClO_4 anion vibrations ν_2, ν_3, and ν_4 have the values of wavenumbers in the region of 455—480 cm^{-1}, 1070—1100 cm^{-1}, and 615—635 cm^{-1}, respectively [8]. Vibrations of ν(As—O) are shifted towards lower energies as a result of $OAsPh_3$ coordination [9] with respect to the central atom Fe(II) or Fe(III) (through oxygen atom), *i.e.* to the value of $\tilde{\nu}(\nu(\text{As—O}))$ = 864 cm^{-1} and 839 cm^{-1} for iron(II) complex *II* and to $\tilde{\nu}(\nu(\text{As—O}))$ = 837 cm^{-1} for Fe(III) complex *IV*. Stretching vibrations ν(Fe(II)—O) and ν(Fe(III)—O) are in the region of wavenumbers 428 cm^{-1} and 364 cm^{-1}, and 428 cm^{-1} and 337 cm^{-1}, respectively.

Electronic spectrum of *II* in the visible region shows two bands. One band, very broad and asymmetrically centred around 460 nm, can be characterized as charge-transfer [10]. The second band at λ = 1028 nm can characterize the symmetry of the $[Fe(OAsPh_3)_4]^{2+}$ cation between $\mathbf{T}_d$ and $\mathbf{D}_{4h}$ [11]. Electronic spectra of *I* and *Ia* are different. While the electronic spectrum of β-form shows two bands, one at λ = 335 nm and the second at λ = 520 nm, α-form gives three bands: at λ = 335, 570, and 925 nm [1]. Conspicuous band in the visible part of spectrum, with maximum at about 520 and 570 nm observed for *I* and *Ia* was characterized as charge-transfer [10]. Presence of another band at λ = 925 nm (α-form) characterizes the symmetry of the $[Fe(OPPh_3)_4]^{2+}$ cation between $\mathbf{T}_d$ and $\mathbf{D}_{4h}$[1, 11]. In the visible region of spectrum of *IV*, as well as of spectrum of *III* [1], there is no band. Electronic spectra of *III* and *IV* in the UV region show one remarkable maximum at λ = 335 nm.

On the basis of the study of spectral properties of compounds *I*—*IV* (Table 1) we assume a tetrahedral arrangement around the iron atom. The structure of this coordination is formed by four molecules of $OEPh_3$ (E = As or P) linked to the iron(II) or iron(III) atom through oxygen donor atoms. All the complexes contain an ion-bonded ClO_4 group. A close structural similarity is seen between $OPPh_3$ and $OAsPh_3$ iron(II) and iron(III) complexes.

Since some iron—$OEPh_3$ complexes (E = P or As) were proved to exhibit biological effects [12], the complexes presented in this paper were tested with the aim to compare their biological effects and evaluate how the perchlorate anions and oxidation state of iron affect the biological activity.

The assessment of bioactivity of the prepared compounds by their IC_{50}, MIC, and MMC val-

Table 1. Some Characteristic IR Wavenumbers of the Complexes ($\tilde{\nu}_i$/cm^{-1})

Complex	$\nu_2(ClO_4)$	$\nu_3(ClO_4)$	$\nu_4(ClO_4)$	ν(Fe—O)	ν(E—O)[a]	Ref.
I		1080	618	433, 340	1148	[1]
II	467	1088	625	428, 364		This paper
III		1088	620	426, 350	1146	[1]
IV	467	1088	623	428, 337		This paper

a) For *I* and *III* E = P; for *II* and *IV* E = As.

Table 2. Antimicrobial Activity ($IC_{50}/(\mu g\ cm^{-3})$ and $MIC/(\mu g\ cm^{-3})$) of Iron Complexes and Ligands $OEPh_3$

Compound	Bacteria[a]		Filamentous fungi													
	1		*2*		*3*		*4*		*5*		*6*		*7*		*8*	
	{IC_{50}}	{MIC}	{IC_{50}}	{MIC}	{IC_{50}}	{MIC}	{IC_{50}}	{MIC}	{IC_{50}}	{MIC}	{IC_{50}}	{MIC}	{IC_{50}}	{MIC}	{IC_{50}}	{MIC}
I	30	500[c]	450	>1000	100	>1000	200	>1000	500	>1000	>1000	>1000	220	1000[e]	250	1000[d]
II	20	100[b]	>1000	>1000	300	>1000	500	>1000	1000	>1000	>1000	>1000	900	>1000	200	500[b]
III	>500	>500	300	>1000	300	>1000	500	>1000	800	>1000	>1000	>1000	200	1000[e]	155	500[b]
IV	>500	>500	>1000	>1000	400	>1000	1000	>1000	>1000	1000[d]	>1000	>1000	>1000	>1000	>1000	>1000
V	200	500[c]	600	1000[e]	80	>1000	500	1000[d]	120	>1000	500	>1000	100	1000[d]	100	500[b]
VI	500	>500	>1000	>1000	600	1000[d]	>1000	>1000	>1000	>1000	>1000	>1000	1000	>1000	500	>1000

1. B. subtilis, 2. R. nigricans, 3. A. alternata, 4. B. cinerea, 5. F. nivale, 6. A. flavus, 7. M. gypseum, 8. T. terrestre.
a) All tested compounds were inactive against bacteria *S. aureus, E. coli, P. fluorescens* and against yeasts *C. albicans, C. parapsilosis*; *b*) MMC = 500 $\mu g\ cm^{-3}$; *c*) MMC > 500 $\mu g\ cm^{-3}$; *d*) MMC = 1000 $\mu g\ cm^{-3}$; *e*) MMC > 1000 $\mu g\ cm^{-3}$.

ues was concentrated primarily at the determination of antibacterial activity against both pathogenic and nonpathogenic G^+ and G^- bacteria; determination of antiyeasts activity on model pathogenic and nonpathogenic yeasts; determination of activity against filamentous representatives of toxinogenic, phytopathogenic, and dermatophytic fungi; assessment of activities.

Antimicrobial activity of the iron perchlorate complexes and ligands $OEPh_3$ characterized by IC_{50} and MIC values is summarized in Table 2. All tested compounds were inactive towards G^+ *Staphylococcus aureus,* and G^- *Escherichia coli, Pseudomonas fluorescens* and pathogenic yeasts *Candida albicans* and *Candida parapsilosis.* Their IC_{50} values were higher than 500 $\mu g\ cm^{-3}$.

The antibacterial effect against G^+ *Bacillus subtilis* was found with ligands $OEPh_3$ *V* and *VI* (IC_{50} = 200 $\mu g\ cm^{-3}$ and 500 $\mu g\ cm^{-3}$, respectively) and their Fe(II) complexes *I* and *II* (IC_{50} = 30 $\mu g\ cm^{-3}$ and 20 $\mu g\ cm^{-3}$, respectively), which are more effective than the ligands. The iron(II) perchlorate (IC_{50} = 500 $\mu g\ cm^{-3}$), which was used as starting compound, was much less effective against *B. subtilis* than the complexes *I* and *II.* Iron(III) complexes *III* and *IV*, and iron(III) perchlorate were inactive (IC_{50} > 500 $\mu g\ cm^{-3}$) (Table 2).

Antifungal activity of the compounds is limited to filamentous fungi (Table 2). In the case of *A. alternata* the $OPPh_3$ complexes *I* and *III* were less active than their free ligand *V.* On the other hand, the $OAsPh_3$ complexes *II* and *IV* were more active than their free ligand *VI.* The effect against *B. cinerea* was increased with Fe(II) complexes *I* and *II*, and Fe(III) complex *IV* compared with competent free ligands *V* and *VI.* The efficiency of Fe(III) complex *III* was unchanged towards *V.* The effect against *F. nivale* was decreased with complexes *I* and *III* compared with the ligand *V*, however, it is almost unchanged with complexes *II* and *IV* compared with the ligand *VI.* In the case of these phytopathogenic fungi the Fe(II) complexes *I* and *II* were generally more active than the Fe(III) complexes *III* and *IV.* Antifungal activity in particular against dermatophytic fungi *M. gypseum* decreased in the order: *V, III, I, II, VI, IV* and that against *T. terrestre* in the order: *V, III, II, I, VI, IV.* Only a weak activity against toxinogenic *A. flavus* was observed with the free ligand *V* (IC_{50} = 500 $\mu g\ cm^{-3}$). The effect against *R. nigricans* is higher with iron—$OPPh_3$ complexes *I* and *III* than with the free ligand *V.* Uncoordinated ligand *VI* and its complexes do not influence the growth of *R. nigricans.* Antifungal activity of the Fe(II) complexes *I* and *II* in particular against *A. alternata, B. cinerea, M. gypseum,* and *T. terrestre* was much higher than the activity of iron(II) perchlorate (IC_{50} = 1000 $\mu g\ cm^{-3}$). The Fe(III) complexes *III* and *IV* were more effective against *A. alternata* than iron(III) perchlorate (IC_{50} > 1000 $\mu g\ cm^{-3}$). However, the increased efficiency against *R. nigricans, B. cinerea, M. gypseum,* and *T. terrestre* was observed only with the Fe(III)—$OPPh_3$ complex *III.* The activity of iron(III) perchlorate was comparable with the Fe(III)—$OAsPh_3$ complex *IV.* The phosphine compounds *I, III,* and *V* were generally more effective against filamentous fungi than the arsine compounds *II, IV,* and *VI*, which is in a good agreement with the antifungal activity of other iron—$OEPh_3$ complexes studied previously [12]. However, the perchlorate iron(II) complexes showed a higher antimicrobial activity than the perchlorate and other iron(III) complexes [12]. We assumed that the observed higher antimicrobial activity of iron(II) compounds is connected with easy oxidizability of Fe(II) to Fe(III).

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