

Investigation of Iron(III) Interaction with Alkylresorcinols in Aqueous Solutions: Oxidative Degradation of Microbial Autoregulators¹

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Abstract—Mössbauer spectroscopy was used to study iron(III) reduction by alkylresorcinols (ARs; i.e., chemical analogs of microbial autoregulators excreted by cells into the environment and involved in intercellular communication) in aqueous solutions simulating acidic soil conditions. The concomitant oxidation process for an AR (5-methylresorcinol) was monitored using UV spectrophotometry.

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INTRODUCTION

Alkylresorcinols (ARs; i.e., *m*-dihydroxybenzenes with alkyl substituents of various length and position in the aromatic ring; Fig. 1) are natural phenolic substances with a wide range of biological functions [1]. In particular, many microorganisms synthesize and excrete ARs into the environment, in which these substances implement autoregulatory and adaptogenic functions [2, 3]. It is also known that ARs, depending on the conditions and the nature of the alkyl substituent in the molecule, can influence the structure of DNA [1, 4] and its chemical stability [5, 6], along with the structural organization, permeability, and stability of membrane complexes [7]. They can also interact with proteins and other biopolymers [1] and stabilize enzymes in aqueous media, enhancing or inhibiting their activity [1, 8, 9]. One of the most important properties of ARs, and of many other phenolic substances as well, is their antioxidant and antiradical activity [1, 10].

Such diverse functionality of ARs, including their substantial role in microbial intercellular communication [2, 3], has aroused interest in physicochemical transformations of these chemically active substances involving environmental components. Nevertheless, information on these processes is evidently scarce. Besides the data on AR oxidation by oxygen catalyzed by special enzymes (laccases [11, 12]), which involves other phenolic substrates, there are only a few reports on the radiolysis of ARs in aqueous solutions [10]; their oxidation in alkaline media in the presence of O₂ and Cu²⁺ [6]; or the oxidation of AR analogs 2,4- and

2,6-dihydroxybenzoic acid and other substituted dihydroxybenzenes by iron(III) in weakly acidic media [13].

In this work, Mössbauer spectroscopy was used to study the process of iron(III) reduction in the presence of ARs in weakly acidic aqueous media. These conditions simulate the processes that can occur in relatively widely distributed acidic soils [14] and contribute to the oxidative degradation of various biologically active substances and signaling molecules excreted by many soil microorganisms [15–17]. The parallel process of AR oxidation (for 5-methylresorcinol) by iron(III) in aqueous solutions was spectrophotometrically monitored using UV absorption spectra of soluble organic products of the reaction.

EXPERIMENTAL

Chemical analogs of microbial autoregulators, 5-methylresorcinol (Fluka) and 4-*n*-hexylresorcinol (Sigma) with the substituents (*R*; see Fig. 1) CH₃ in position 5 and *n*-C₆H₁₃ in position 4, respectively, were used in the experiments. Aliquots (0.50 ml) of 0.06 M

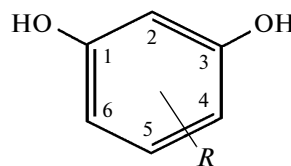


Fig. 1. Scheme of the molecular structure of alkylresorcinols (*R* is an alkyl substituent (C_{*n*}H_{2*n*+1}), where *n* ≥ 1; digits in the scheme show the numeration of carbon atoms in the aromatic ring).

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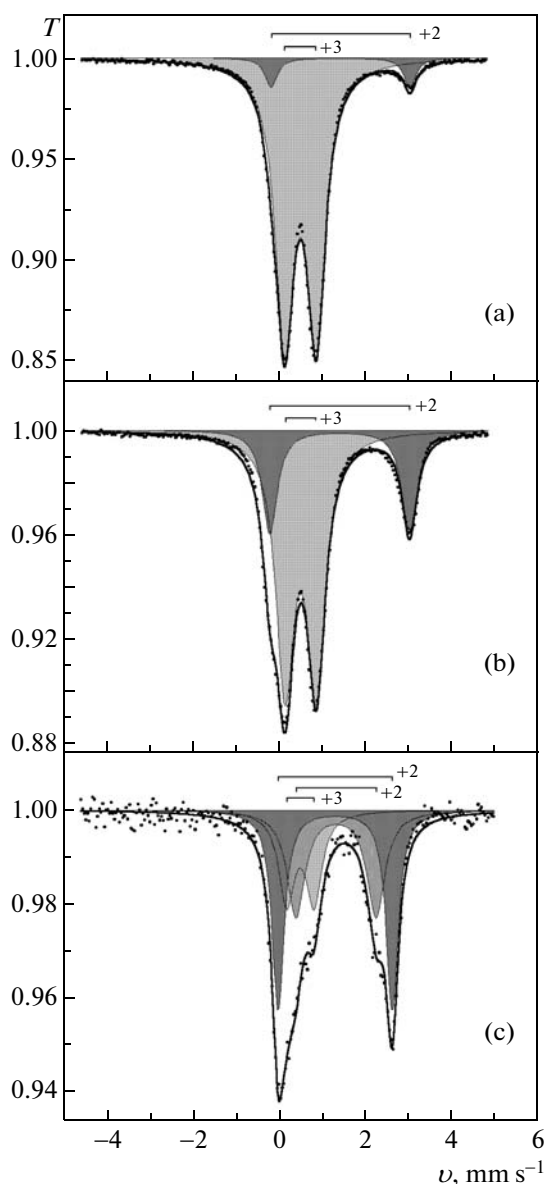


Fig. 2. Mössbauer spectra of aqueous $^{57}\text{Fe}^{\text{III}}$ -containing solutions of 5-methylresorcinol rapidly frozen at (a) 10 min and (b) 5.5 h after mixing the reagents (pH of mixture ~ 3), as well as (c) of the solid residue obtained by drying the mixture at room temperature (measurements performed at 80 K). Positions of doublets corresponding to different chemical forms of Fe are indicated above the spectra (digits on the right show the iron oxidation state of the corresponding form). Spectral components (quadrupole doublets) correspond to iron(III) and iron(II) (marked with a light and darker shading, respectively) contributing to the overall spectrum (solid envelope) obtained by fitting the experimental data (points) (see also table).

stock solutions of 5-methylresorcinol (in bidistilled water) and 4-*n*-hexylresorcinol (in water with 25 vol % of absolute ethanol to increase the solubility) were mixed with 0.100 ml of 0.1 M aqueous solution of $^{57}\text{FeCl}_3$ (enriched with the ^{57}Fe isotope to 90%), which corresponded to the molar ratio Fe : AR = 1 : 3 at a

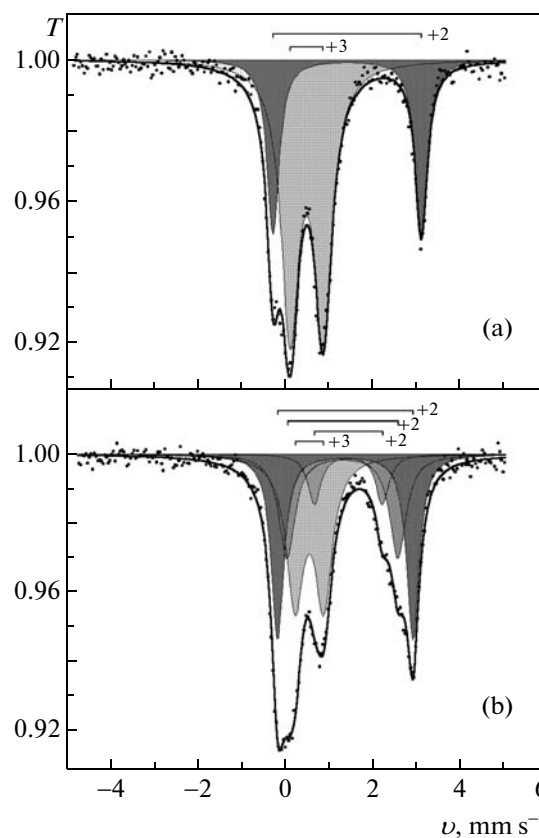


Fig. 3. Mössbauer spectra (a) of an aqueous $^{57}\text{Fe}^{\text{III}}$ -containing solution of 4-*n*-hexylresorcinol (with 20 vol % of ethanol) rapidly frozen 5 min after mixing the reagents (pH of mixture ~ 3), and (b) of the solid residue obtained by drying the mixture at room temperature (measurements performed at 80 K). Designations and shading are explained in the caption to Fig. 2 (see also table).

total Fe concentration of 0.016 M in the final mixture. The pH value of the mixture was immediately brought to pH ≈ 3 by adding 0.03 ml of 1 M KOH under stirring.

For Mössbauer spectroscopic measurements, the mixture (placed in a round flat polytetrafluoroethylene cup with an aqueous layer ~ 3 mm thick) was closed with foil to avoid evaporation and the effects of light, incubated for a certain period of time at room temperature, and then rapidly frozen in liquid nitrogen. Solid samples were prepared by drying the mixture in air (in the dark) at room temperature for 24 h. Spectra were measured in the transmission mode on a WISSEL spectrometer (Germany) working in the constant acceleration mode with a $^{57}\text{Co}(\text{Rh})$ source, by placing the frozen samples into a cryostat filled with liquid nitrogen (measurement temperature 80 K). The spectra obtained were fitted by the least squares method, using the MOSSWINN program [18]; all of the isomer shift values are reported relative to α -Fe at room temperature. We earlier described other methodological details of the measurements and equipment in [15–17].

UV absorption spectra of aqueous solutions of the initial 5-methylresorcinol (at concentration 0.12 mM, equivalent to those used in its mixtures with Fe^{III} at the same dilution; see below) and of soluble products of its oxidation in the presence of Fe^{III} (using FeCl₃ without ⁵⁷Fe enrichment) were measured on a Specord S-250 spectrophotometer (C. Zeiss, Germany) in a quartz cuvette of optical length 10 mm within the wavelength region 260–300 nm. Measurements were performed for mixtures with a composition similar to that mentioned above after their incubation for certain periods of time, from which solid particles had been removed by centrifugation in 2.0-ml Eppendorf tubes on a Microspin 12 microcentrifuge (United States) at 13400 rpm. Aliquots (0.100 ml each) of the obtained transparent supernatant liquid were diluted 400-fold with water in order to reduce the UV absorption to a level acceptable for measurements. Immediately after taking an aliquot, the remainder was stirred again, closed, and left until the next sampling.

RESULTS AND DISCUSSION

Mössbauer spectra of the AR mixtures with iron(III) in aqueous media, prepared as described above, and of their air-dried residues, measured at 80 K, are shown in Figs. 2 and 3. Mössbauer parameters calculated from the spectroscopic data are pre-

sented in the table. The obtained results provide evidence that iron(III) is gradually reduced in the presence of ARs in weakly acidic solutions. Note that while in the 5-methylresorcinol solution, about 7% of the iron(III) was reduced to iron(II) in 10 min and about 26% was reduced in 5.5 h (see Figs. 2a, 2b, table), reduction by 4-*n*-hexylresorcinol under similar conditions was much faster: within 5 min, around one-third of all the iron(III) was reduced (see Fig. 3a, table). It should be mentioned that, as follows from the results of special measurements, the presence of 20 vol % ethanol in aqueous solutions at pH ≈ 3 insignificantly influenced the rate of iron(III) reduction by 5-methylresorcinol (the data are not shown). It may therefore be assumed that the much more rapid reduction of iron(III) observed in the presence of 4-*n*-hexylresorcinol, as compared with that in the presence of 5-methylresorcinol (by virtually an order of magnitude at the initial steps of the process within the few first minutes), is related to the structure of the molecule, viz. to the length and/or the position of the alkyl substituent in the aromatic ring (see Fig. 1).

It should be considered that in aqueous media at pH ≈ 3, most of the iron(III) is in a hydrolyzed form (as polynuclear hydroxo complexes and colloidal particles) [19], which agrees with the Mössbauer parameters of the corresponding forms ($\delta \approx 0.48 \text{ mm s}^{-1}$ and $\Delta \approx 0.72\text{--}0.76 \text{ mm s}^{-1}$ at 80 K; see table). The Mössbauer parameters of the iron(II) species formed in

Mössbauer parameters* of aqueous ⁵⁷Fe^{III}-containing solutions of alkylresorcinols (ARs; see also Figs. 2 and 3) rapidly frozen to 80 K after certain periods of time and of their air-dried products (measurements performed at 80 K)

AR in mixture with ⁵⁷ Fe ^{III} in solution (state)	<i>t</i> ^{**} , h	Fe oxidation state (form)	δ	Δ	Γ_{exp}	<i>S_r</i> , %
			mm s ⁻¹			
5-methylresorcinol (solution)	0.17	+3	0.48(1)	0.74(1)	0.52(1)	93.3
		+2	1.41(1)	3.22(1)	0.34(1)	6.7
	5.5	+3	0.48(1)	0.72(1)	0.50(1)	74.1
		+2	1.38(1)	3.23(1)	0.42(1)	25.9
5-methylresorcinol (dry residue)	—	+3	0.48(1)	0.63(2)	0.43(2)	24.5
		+2 (form 1)	1.31(1)	1.86(3)	0.50(3)	33.3
		+2 (form 2)	1.29(1)	2.65(1)	0.34(1)	42.2
4- <i>n</i> -hexylresorcinol (solution)	0.08	+3	0.475(3)	0.757(4)	0.49(1)	68.8
		+2	1.402(3)	3.388(5)	0.34(1)	31.2
4- <i>n</i> -hexylresorcinol (dry residue)	—	+3	0.54(1)	0.65(1)	0.48(2)	34.9
		+2 (form 1)	1.43(1)	1.55(3)	0.35(3)	9.0
		+2 (form 2)	1.29(1)	2.54(2)	0.45(3)	23.7
		+2 (form 3)	1.359(3)	3.10(1)	0.34(1)	32.4

Notes: * δ , isomer shift (relative to α -Fe); Δ , quadrupole splitting; Γ_{exp} , experimentally measured full line width at half intensity (the values of the calculated errors in the last digit are given in brackets); *S_r*, the area of a spectral component (in percent of the total spectral area) corresponding to the content of iron in this form relative to the total iron content in the sample (provided that the Mössbauer effect probability is equal for all the forms); the relative error is ±4% for the *S_r* values.

** Period after mixing the reagents until freezing the sample.

aqueous media upon reduction ($\delta \approx 1.4 \text{ mm s}^{-1}$ and $\Delta \approx 3.2\text{--}3.4 \text{ mm s}^{-1}$ at 80 K; see table) show that their most probable state is the hydrated complexes $[\text{Fe}(\text{H}_2\text{O})_6]^{2+}$ [15, 19]. Nevertheless, for the dried solutions their spectra were distinguished by two iron(II) species in the case of 5-methylresorcinol and by three iron(II) species in the case of 4-*n*-hexylresorcinol (see Figs. 2c and 3b, respectively, and table). The parameters of these species point to the formation in the solid phase of different Fe^{II} complexes with organic ligands, with AR oxidation products apparently taking first place. Note that during AR oxidation, the first step is an additional hydroxylation of the aromatic ring [6]. For 5-methylresorcinol, therefore, two such isomers of the oxidized species are possible (1,2,4-trihydroxy-6-methylbenzene and 1,2,3-trihydroxy-5-methylbenzene), whereas for 4-*n*-hexylresorcinol, there are three such isomers (1,2,3-trihydroxy-4-*n*-hexyl-, 1,3,5-trihydroxy-6-*n*-hexyl- and 1,2,4-trihydroxy-5-*n*-hexylbenzene). This in particular could account for the larger number of species of iron(II) complexes in the dried sample in the case of 4-*n*-hexylresorcinol (see Fig. 3b and table). In relation to this, it is noteworthy that, according to the data reported in [20], resorcinol itself (nonalkylated *m*-dihydroxybenzene) did not reduce Fe^{III} in acidic aqueous solutions (in contrast to its *o*- and *p*-dihydroxy isomers).

It is also of importance that in air-dried samples, the iron(II) formed in the process of iron(III) reduction by ARs dominated, totaling as much as 75.5 and 65.1 mol % for 5-methylresorcinol and 4-*n*-hexylresorcinol, respectively (see table). For comparison, under similar conditions after total reduction of Fe^{III} to Fe^{II} by another important representative of extracellular signaling molecules produced by many microorganisms, indole-3-acetic acid (phytohormone of the auxin type [21]), drying the solution in air led to its total oxidation back to Fe^{III} [22, 23]. The AR oxidation products (e.g., the aforementioned alkyltrihydroxybenzenes and the corresponding hydroxylated quinones [1, 6]) evidently form relatively highly stable chelate complexes with iron(II), preventing its oxidation in air.

The dynamics of UV absorption alterations for soluble products of 5-methylresorcinol interaction with Fe^{III} (Fig. 4) was monitored by spectra measured after certain periods of time (see inset in Fig. 4). The data in Fig. 4 show that the initial period of the interaction is distinguished by a noticeable increase (by about 25%) in absorption at the maxima corresponding to 5-methylresorcinol ($\lambda_{\text{max}} = 273$ and 279 nm [10]), as well as in the region of the wings, at about 260 and 285–290 nm. A similar increase in absorption within the aforementioned regions was observed in [10] upon the radiation-induced oxidation of aerated aqueous solutions of 5-methylresorcinol (^{60}Co γ -radiation; doses of up to 30 kGy) with the appearance of broad, poorly resolved low-intensity bands in the visible spectral region with

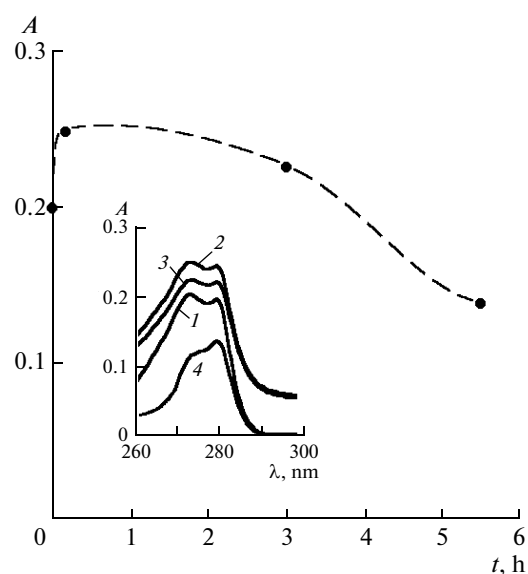


Fig. 4. Optical density A at a maximum ($\lambda_{\text{max}} = 279 \text{ nm}$) as a function of time t for the UV absorption spectra (inset) of an aqueous 0.12 mM solution of 5-methylresorcinol (1) and the products of its oxidation by iron(III) 10 min (2), 3 h (3) and 5.5 h (4) after mixing, equivalently diluted after centrifuging the mixture.

a maximum at about 500 nm. The UV absorption increase in the initial period of the oxidation process (see Fig. 4) may presumably be attributed to additional hydroxylation of the aromatic ring and (or) the formation of the corresponding hydroxylated quinone structures (see above; [1, 6]). A gradual decline in absorption over the UV region was observed within a few hours; this could be related to a partial destruction of chromophore groups (aromatic ring) in the course of ongoing oxidation and/or to the formation of insoluble iron complexes with oxidation products.

CONCLUSIONS

Thus, according to the data of Mössbauer spectroscopy and UV spectrophotometry, it has been shown that in weakly acidic aqueous solutions AR and iron(III) are involved in redox interactions. In real systems (soil, aquifers), such processes, which lead to oxidative degradation of ARs participating in the intercellular communication of microorganisms, can significantly affect the exchange of microbial molecular signals by lowering AR concentrations in the environment.

The marked differences in the iron(III) reduction rate for 5-methylresorcinol and 4-*n*-hexylresorcinol detected using Mössbauer spectroscopy testify to the role of the AR molecular structure (the length and position of the alkyl substituent in the aromatic ring) in the kinetics of the redox process.

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