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Complexation of Indole-3-acetic Acid with Iron(III): Influence of Coordination on the π-Electronic System of the Ligand

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Summary. The iron(III) complex of indole-3-acetic acid (**1**) was prepared, and its physicochemical properties, mode of iron(III) coordination, and electronic structure were studied using UV/Vis, diffuse reflectance infrared *Fourier* transform (DRIFT), and transmission ^{57}Fe *Mössbauer* spectroscopic techniques. The data obtained provide evidence that iron(III) is not only coordinated by the carboxylic O-donor atom, but also *via* the conjugated π-electronic system of the pyrrole moiety involving both the non-shared electronic pair of the heteroatom and the C(2)–C(3) double bond. Considering the well-known increased sensitivity of the pyrrole residue in indole derivatives to oxidation as compared to the benzene ring, as well as the formation of a triple complex (peroxidase-**1**-O_2) proposed for the enzymatic **1** oxidative degradation mechanism involving as a key step the $Fe^{3+} \rightarrow Fe^{2+}$ transition in the enzyme form as discussed in literature, it is concluded that iron(III) coordination with **1** can influence the redox properties of the pyrrole ring by affecting its π-electronic system.

Keywords. Indole-3-acetic acid; Iron(III) complexes; Diffuse reflectance infrared *Fourier* transform spectroscopy (DRIFT); *Mössbauer* spectroscopy.

Introduction

Indole-3-acetic acid (**1**), a phytohormone of the auxin series, is a substance of essential and multifunctional biological significance [1]. Its biosynthesis is an intrinsic feature of higher plants and is also well documented to be performed by a number of soil microorganisms which excrete it into the environment [2]. This feature, widespread among plant growth promoting rhizobacteria [3], has been established as an essential component of associative plant-microbe interactions

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[3, 4]. As a result, plant growth regulation is thus induced *via* the influence of **1** on plant root growth, morphology, development, and nutrient accumulation [4–6]. However, expression of **1** in excessive amounts by deleterious rhizobacteria has been documented to suppress plant growth [7]. The induction of a bacterial gene by **1** has been demonstrated recently for the first time for auxin [8]. It should be noted that the latter function was found to be structurally specific, as neither tryptophan, which has a similar 3-substituted indole moiety, nor conjugates of **1** appeared to be active [8].

The level of **1** is thought to be controlled *in vivo* by its oxidative degradation involving plant peroxidases and/or photo-oxidation giving similar products [9–11]. One of the key steps in the kinetic mechanism, assuming a specific interaction of peroxidase with **1** leading to the formation of a triple complex (enzyme-**1**-oxygen), is the reduction of the Fe(III) form of the enzyme to its Fe(II) modification [11].

Being a chemically active compound, **1** can interact with certain soil components including metal cations when excreted into soil in noticeable quantities [1–3, 7], influencing their chemical and biological availability. Iron, one of biologically essential metals, is usually abundant in soil largely in the form of ferric hydroxo compounds [12]. However, the latter exhibit an extremely low bioavailability owing to their negligible solubility in the near-physiological *pH* range, which is overcome by specific iron(III)-chelating agents (siderophores) excreted by many soil microorganisms under iron deficiency [12]. Previously, it has been shown that **1** [13] as well as some of its metabolically related precursors including anthranilic acid and *L*-tryptophan [14–16] are capable of reductive solubilization of iron(III) under certain conditions, which is of ecological significance [15–17].

In the present work, the iron(III) complex of **1** was prepared, and some of its physicochemical properties and structural characteristics were studied using UV/Vis, diffuse reflectance infrared *Fourier* transform (DRIFT), and transmission ^{57}Fe *Mössbauer* spectroscopic techniques. A possible influence of iron(III) coordination on the redox properties of the ligand is also considered from the viewpoint of the nature of the bonding system involved.

Results and Discussion

The results of the elemental analysis (see Experimental) show that the composition of the complex corresponds to *tris*-(indole-3-acetato)-iron(III) (**2**; $Fe[(C_8H_6N)\text{-}CH_2COO]_3$). The complex was found to be poorly soluble in water and well soluble in oxygen-containing organic solvents (ethanol, isopropanol, acetone) in contrast to *e.g.* tetrachloromethane. The molecular formula was not, in our opinion, *a priori* evident. For instance, the iron(III) anthranilate (*o*-aminobenzoate) complex, which can be obtained under conditions similar to those applied in the present work and which is also well soluble in oxygen-containing organic solvents in contrast to water, $CHCl_3$, or CCl_4 [18], has the composition $Fe[(anthr)_2(H_2O)OH]$ (*anthr* = anthranilate). The later complex is evidently a chelate in which iron(III) is coordinated by both the N- and O-donor atoms of the anthranilate ions as well as by additionally coordinated water and hydroxyl, with the evident coordination number 6 typical for iron(III). In that case, *Dinsel* and *Sweet* [18] have hypothesized that the solubility of the complex in O-containing organics may well be related to the

replacement of the coordinated water molecule by an organic solvent molecule, the latter being also coordinated with iron(III) through the oxygen donor atom [19].

In the case of **1**, the above formula implies that commonly hexa-coordinated iron(III) has to be bound not only to the oxygen atom of the side-chain carboxylate. As the N heteroatom of the pyrrole ring cannot be involved in iron(III) chelation through its non-shared electron pair owing to the planar conjugation of the latter with the π-electrons of the double bond [20], it can be reasoned that the whole π-electronic system is involved in iron(III) chelation. The variable relative orientations of the side chain towards the indole plane and of the carboxylic group [21] can account for the lack of appreciable steric hindrances upon chelation.

One can expect that the involvement of the heteroaromatic ligand in coordination with iron(III) would influence the π-electron density of the ring. This could be verified using electronic absorption spectroscopy. The electronic absorption spectrum of **1** in ethanol is featured by intensive π-π^*-transition bands with $\lambda_{max} = 219\,nm$ $(\varepsilon = 6.2 \times 10^4\,M^{-1} \cdot cm^{-1})$ and $\lambda_{max} = 280\,nm$ $(\varepsilon = 1.3 \times 10^4\,M^{-1} \cdot cm^{-1})$ (Fig. 1). For **2**, the former of the π-π^*-transition bands undergoes a bathochromic shift with a hyperchromic effect ($\lambda_{max} = 223\,nm; \varepsilon = 8.6 \times 10^4\,M^{-1} \cdot cm^{-1}$), whereas the position of the band at 280 nm remains unchanged; nevertheless, its intensity is higher as well $(\varepsilon = 2.7 \times 10^4\,M^{-1} \cdot cm^{-1})$. These changes evidence the involvement of the π-electronic system in coordination.

In the spectrum of **2** (Fig. 1), there appears a new band in the UV region with $\lambda_{max} = 337\,nm$ related to charge transfer which corresponds to its relatively high intensity $(\varepsilon = 5.9 \times 10^3\,M^{-1} \cdot cm^{-1})$, as well as two *d*-*d**-transition bands related to the coordinated iron(III) cation with $\lambda_{max} = 410\,nm$ $(\varepsilon = 8.7 \times 10^2\,M^{-1} \cdot cm^{-1})$ and $\lambda_{max} = 516\,nm$ $(\varepsilon = 5.4 \times 10^2\,M^{-1} \cdot cm^{-1})$. The shape of the spectrum of **2** is similar at different concentrations, and *Beer*'s law is observed (not shown), which provides evidence for the stability of the complex.

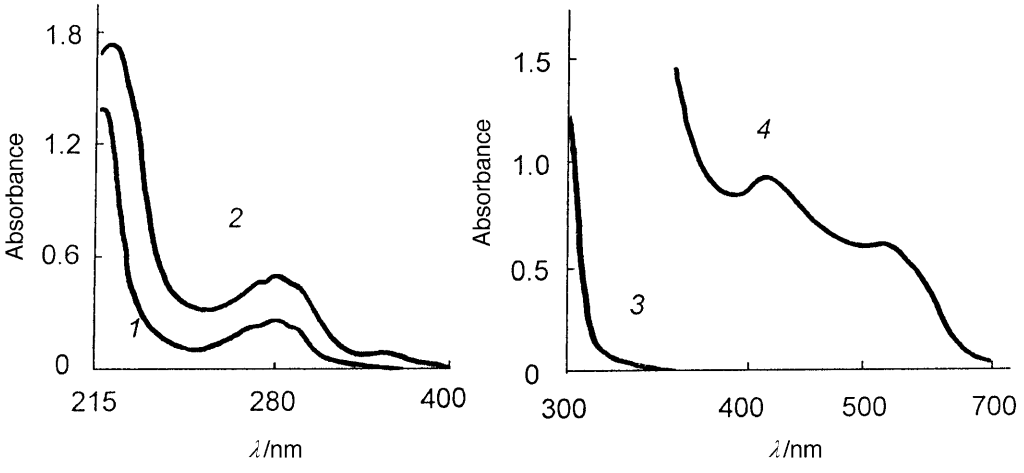

Fig. 1. UV/Vis spectra of indole-3-acetic acid (**1**; *1, 3*) and its complex with iron(III) (**2**; *2, 4*) in ethanol; concentrations: $2 \times 10^{-5}\,M$ (*1, 2*), $1 \times 10^{-3}\,M$ (*3, 4*)

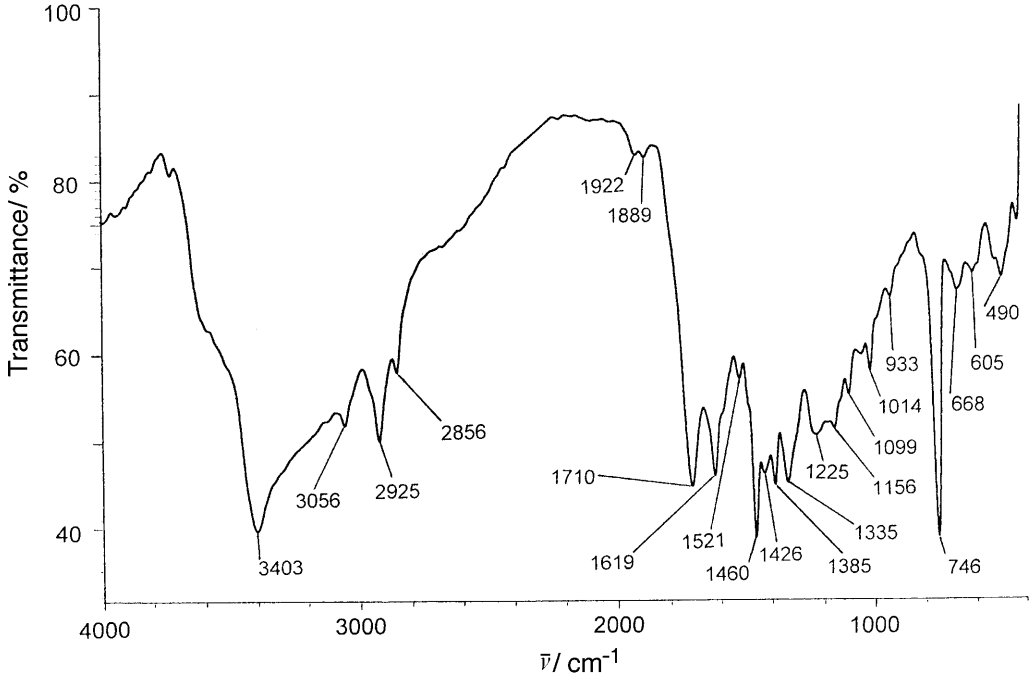

Fig. 2. Diffuse reflectance infrared *Fourier* transform (DRIFT) spectrum of **2**

The DRIFT spectrum of **2** is shown in Fig. 2. It can be seen that the characteristic stretching N-H vibration of the indole moiety, which is featured by a very intensive sharp band at 3390 cm^{-1} in **1** [22], gives a broadened asymmetric band centred at 3403 cm^{-1} in **2** (see Fig. 2). Also, the very intensive band assigned to the out-of-plane γ(N–H) wagging mode, which gives a strong sharp band at 517 cm^{-1} in **1** [22] and is typical for indole (500 cm^{-1} in the solid state) and pyrrole as well [23], broadens and noticeably decreases in intensity in **2** (see Fig. 2). This provides evidence for the involvement of the pyrrole moiety of the ligand in coordination. However, the typical vibration bands of the benzene ring in **1** (*e.g.* out-of-plane γ(C–H) at 740 cm^{-1}; in-plane δ(C=C) at 1457 cm^{-1}; ν(C=C) at *ca.* 1620 cm^{-1} [22]) in going to **2** remain practically unchanged both in position and intensity (see the corresponding bands at 746, 1460, and 1619 cm^{-1} in Fig. 2). This effect shows that the benzene ring of **1** is not involved in coordination with iron(III) in **2**, which, as proposed earlier, involves π-electrons of the pyrrole ring conjugated with the free electron pair at the heteroatom [24].

Though iron(III) coordination with the carboxylic group is undoubted, it should be mentioned that the very typical strong ν(C=O) band in **1** at 1703 cm^{-1} [22] does not disappear as observed in many carboxylates featured by the formation of COO^--metal electrostatic bonds [16,19], but undergoes a slight hypsochromic shift (1710 cm^{-1}; see Fig. 2) and noticeably decreases in intensity. Thus, there is an inequality between the oxygen atoms, as iron(III) is coordinated through one O-donor atom. However, the weaker band at 1521 cm^{-1} (see Fig. 2) obviously features the asymmetric stretching mode of the (O–C=O) fragment. It can thus be reasoned that indole-3-acetate is a bidentate ligand which chelates iron(III) *via* its side-chain

carboxyl and the π-electronic system of the pyrrole ring, forming a *quasi*-six-membered ring for each ligand.

The *Mössbauer* spectrum of **2** (not shown) consists of a symmetric well-resolved quadrupole doublet with the following parameters: $IS = 0.38 \pm 0.01\,\mathrm{mm/s}$ (relative to α-Fe), $QS = 0.78 \pm 0.01\,\mathrm{mm/s}$, $FWHM = 0.49 \pm 0.02\,\mathrm{mm/s}$ (resonant absorption 13%, $T = 298\,\mathrm{K}$). These parameters confirm that iron(III) in **2** is in the high-spin state and in the distorted octahedral coordination [25,26]. The latter is demonstrated by the relatively large *QS* value somewhat exceeding that of *e.g.* the iron(III)-tryptophan complex ($QS = 0.69 \pm 0.05\,\mathrm{mm/s}$ at 295 K [27]), reflecting the evident inequality of coordination bonds. However, the *IS* values which are related to the electron density at the iron nucleus [25,26] are similar for **1** (see above) and tryptophan ($IS = 0.41 \pm 0.05\,\mathrm{mm/s}$ at 295 K [27]). This corresponds to the involvement of the pyrrole ring, along with the side-chain carboxyl group, in coordination with iron both for **1** (see above) and for tryptophan with charge transfer [28].

It should be mentioned that the type of coordination of iron(III) with **1** considered here in which the pyrrole ring is generally more prone to oxidation as compared to the benzene moiety [29–31] may affect the redox properties of the pyrrole residue and thus influence the kinetics and/or mechanism of (photo)-chemical and enzymatic processes of the oxidative degradation of **1** [10, 11]. This corresponds to the fact that aerobic photo-oxidation of the double bond in the pyrrole ring of indole derivatives is catalyzed by iron(III) ions [29]. Such processes may well occur abiotically in soil where **1** and its derivatives may be in direct contact with iron(III) cations. This is essential for plant root-microbe interactions in which **1** has recently been proposed to be involved as a reciprocal signalling molecule [32]. It should also be noted that several complexes of **1** with lanthanides (Ln^{3+}) of the formula $LnL_3 \times 3H_2O$ have been found to exert an even stronger auxin effect than **1** (or $LnCl_3$) [33], which is obviously a result of the influence of metal coordination on the physiological activity of **1**.

Experimental

Tris-(indole-3-acetato)iron(III) (**2**; $FeC_{30}H_{24}N_3O_6$)

An $Fe(NO_3)_3$ solution of known concentration was prepared by dissolving Fe metal powder (pharmacological purity, reduced in H_2 atmosphere) in a slight excess of 50% HNO_3 (analytical purity) as described earlier [13]. 0.15 g (0.84 mmol) of **1** (Eastman Kodak, chemical purity) was dissolved in bidistilled H_2O at 60°C under permanent stirring with a magnetic stirrer and then slowly gradually mixed with 0.03 *M* iron(III) nitrate solution up to a final Fe-to-**1** molar ratio of 1:3 (final *pH*: 4.5). The solution quickly got coloured, and in a few minutes a cocoa-reddish precipitate of the iron(III)-**1** complex (**2**) was formed. It was filtered, washed with a dilute solution of $NaHCO_3$ to remove possible residues of free **1** and then with bidistilled H_2O, and dried under vacuum (3 mm Hg) for 25 min. Elemental analysis: found: C 64.71, H 4.82, N 7.63; calcd.: C 62.22, H 4.14, N 7.25.

Spectroscopic techniques and data acquisition

UV/Vis spectra of **1** and **2** were measured in 95% ethanolic solutions within the concentration range of 2×10^{-5} (UV region) to $1 \times 10^{-3}\,M$ (visible region) using a UV/Vis Specord M40 spectrophotometer (C. Zeiss, Germany) in quartz cuvettes (1 cm).

The infrared spectrum was obtained in the diffuse reflectance infrared *Fourier* transform (DRIFT) mode using a Nicolet spectrophotometer (model Magna IR 750, USA; DTGS detector, Nichrome source, beamsplitter: KBr) with a total of 100 scans; resolution: up to 4 cm^{-1}. A small portion of **2** (*ca.* 0.2 mg) was carefully ground with *ca.* 15 mg of spectroscopically pure KBr (Merck) and placed into a Micro sampling cup (Spectra-Tech Inc., USA). Spectra were recorded using a Spectra-Tech Diffuse Reflectance accessory against a KBr background. Treatment of the spectra was performed using the OMNIC 3.1 software (Nicolet) accompanying the equipment.

The *Mössbauer* spectrum was obtained using a constant-acceleration electrodynamic spectrometer (Ranger Electronics MS-700, USA) in the absorption mode with a ^{57}Co[Rh] source kept at room temperature. Experimental data were registered by a PC-based multichannel analyzer. Standard PC-based statistical analysis included fitting the experimental data obtained to a sum of *Lorentzian*-shaped component lines with a least squares fit which enabled the calculation of the values of isomer shift (*IS*; relative to α-Fe), quadrupole splitting (*QS*), and linewidth (*i.e.* full width at half maximum, *FWHM*). Other details of the experimental procedure have been reported elsewhere [16, 17]. If not indicated otherwise, all measurements were performed at room temperature (295$\pm$3 K).

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