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AN Fe(II) COMPLEX OF PYRIDINE-2,6-DI-(MONOTHIOCARBOXYLIC ACID) - A NOVEL BACTERIAL METABOLIC PRODUCT[1]

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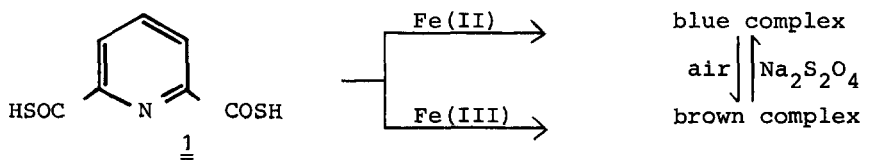
The culture medium of a yet unidentified strain of Pseudomonas turns dark blue upon addition of ferric citrate; when shaken with air the color changes to reddish brown. Extraction with isopropanol yields an amorphous powder the main component of which can be isolated by chromatography (silicagel 60, Merck, Darmstadt; ethyl acetate/methanol 4:1) and subsequent purification on an ion exchange resin (Lewatit S 100, Bayer, Leverkusen). The pigment contains a redox system: in solution its brown form can be reduced by addition of $Na_2S_2O_4$ to give a blue product which is stable only in the presence of an excess of reducing agent and which is reoxidized easily (e.g., by air) to the starting material.

Electron, IR (strong structured band with a maximum at 1580 cm^{-1}: carboxylate?, aromatic system?), NMR (presence of Fe!) and electron impact mass spectra did not give any structural information. A field desorption (FD) mass spectrum if measured after admixture of Na_2CO_3 or K_2CO_3 yielded ions of mass 450 plus one, two or three alkali metal atoms[2], a value in agreement with an osmometric determination of the molecular weight (~350). At p_H 6,9 (phosphate buffer) and 8,0 (triethyl ammonium bicarbonate buffer) migration to the anode is observed in electrophoresis.

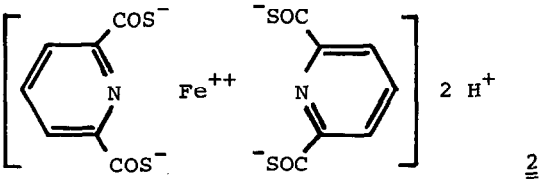
A methanolic solution of the brown pigment reacts slowly with diazomethane. Analysis of the reaction product by gas chromatography yielded, *i.a.*, pyridine-2,6-di-(monothiocarboxylic acid) di-S-methyl ester ($C_9H_9NO_2S_2$ by high resolution mass spectrometry; NMR 2,47 ppm, 6H, s: SCH_3, and 8,0-8,5 ppm, 4H, m: pyridine β and γ H)[3] the structure of which could be confirmed by synthesis[4]. Treatment of the free acid (1) with Fe salts results in blue or brown complexes according to the following scheme:



1

The brown complex thus obtained is identical (IR-, ESCA-, FD-spectrum, chromatographic behavior) with the natural product.

The Mössbauer spectrum of the brown complex (chemical shift + 0,244 mm/sec rel. to $Fe_{met.}$, quadrupole splitting 2,36 mm/sec) most probably indicates the presence of Fe(II)[5]. Since the ESCA[5] spectrum does not show signals of any metal but Fe, all the evidence discussed points towards the formulation 2 for the brown complex (free acid)[6] although higher aggregates exhibiting the same Fe-to-ligand ratio cannot be excluded rigorously.



2

The complex 2 (as well as 1) show antibiotic activity[7]. Since iron sulfur proteins participate in biological redox systems[8] the activity of 2 may well be the results of an interference with the active sites of such proteins.

Acknowledgements

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References

1) Bacterial constituents, II. Part I: H.Korth, A.Römer, H.Budzikiewicz, and G.Pulverer, *J. Gen. Microbiol.* 104, 299 (1978).

2) A behavior typical for acids. The ion of highest mass in the M^+ cluster of a dibasic acid YH_2 in the presence of alkali is usually due to an ion formed by attachment of an alkali metal ion to the dialkali salt, *viz.* $YMet_3^+$. Cf., e.g., H.Budzikiewicz and M.Linscheid, *Biomed. Mass Spectrom.* 4, 103 (1977), and H.-R.Schulten and F.W.Röllgen, *Org. Mass Spectrom.* 10, 649 (1975).

3) D.H.Williams and I.Fleming, Spektroskopische Methoden zur Strukturaufklärung, Thieme, Stuttgart, 1975; The Aldrich Library of NMR Spectra, Vol.IX, No.50 C.

4) Pyridine 2,6-dicarboxylic acid chloride treated with Na_2S in pyridine and subsequently with CH_3I. The free acid (1) can be obtained by reaction of the acid chloride with H_2S (orange crystals, m.p. 116° (decomp.)).

5) We wish to thank Prof. U. Hauser and Dipl.-Phys. F. Mbescherubusa, I. Physikalisches Institut der Universität Köln, for measurement and interpretation of the Mössbauer, and Dipl.-Chem. H.J. Freund, Lehrstuhl für Theoretische Chemie der Universität Köln, for the ESCA spectrum.

6) Elemental analysis is in agreement with the 1:2 ratio Fe/ligand.

7) Test data will be reported elsewhere.

8) R.H.Holm, *Accounts of Chem. Res.* 10, 427 (1977).

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