Structural Studies of Fe(III) and Cu(II) Complexes of Salicylaldehyde Benzoyl Hydrazone, a Synthetic Chelating Agent Exhibiting Diverse Biological Properties

ALEJANDRO A. ARUFFO*, TERRANCE B. MURPHY*, DAVID K. JOHNSON**, NORMAN J. ROSE* and VERNER SCHOMAKER*

*Departments of *Chemistry and **Medicinal Chemistry, University of Washington, Seattle, Wash. 98195, U.S.A.*

Received May 26, 1982

Introduction

Salicylaldehyde benzoyl hydrazone (SBH) is a Schiff base that can function as a tridentate chelating agent [1–3]. Some thirty years ago, it was shown that this compound has modest bacteriostatic properties when tested *in vitro* against microorganisms such as *Mycobacterium tuberculosis, Mycobacterium smegmatis, Candida albicans* and *Aspergillus niger,* although these effects were not sufficiently marked to encourage further study at that time [4, 5]. Recently, however, interest in the biological properties of SBH has been renewed with the recognition that aroylhydrazones of this type are able to induce iron excretion in mammals and thus are potentially of use in the treatment of iron overload on man [6, 7]. SBH itself can mobilize iron from iron-loaded reticulocytes *in vitro* [8] and produces high levels of iron excretion when administered to rats [9]. Preliminary studies have also shown that SBH is an unusually potent inhibitor of DNA synthesis in a variety of cultured human and rodent cells and that the complex [CuCl(SBH)]·H_2O produces significant inhibition of tumor growth when given to mice bearing a transplanted fibrosarcoma [10–12].

The common mechanism underlying these various biological effects of SBH appears to be an ability to penetrate cell membranes and disrupt the intracellular metabolism of essential metal ions. The exact nature of such disruptions, and the extent to which they may be exploited for therapeutic purposes, require much additional study, including detailed elucidation of the chemical properties of complexes formed between SBH and physiologically-important transition metals. This paper reports single crystal X-ray diffraction studies of two such complexes, [$FeCl_2$(SBH)(CH_3OH)] and [CuCl(SBH)]·H_2O.

TABLE I. Selected Bond Distances, in Å.

	[$FeCl_2$(SBH)(CH_3OH)] (compound *A*)	[CuCl(SBH)]·H_2O (compound *B*)
M–O1	2.068(2)	1.975(9)
M–O2	1.874(2)	1.888(8)
M–O3	2.171(3)	–
M–N2	2.119(2)	1.936(7)
M–Cl1	2.303(1)	2.208(4)
M–Cl2	2.345(1)	–

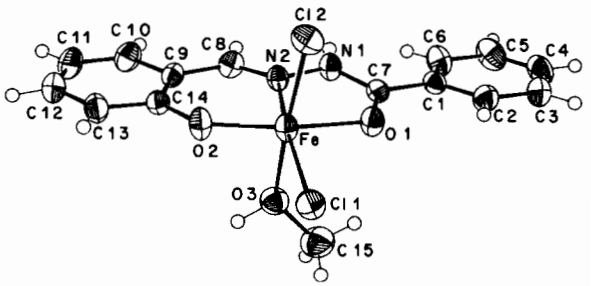

Fig. 1. ORTEP drawing of [$FeCl_2$(SBH)(CH_3OH)] (compound *A*).

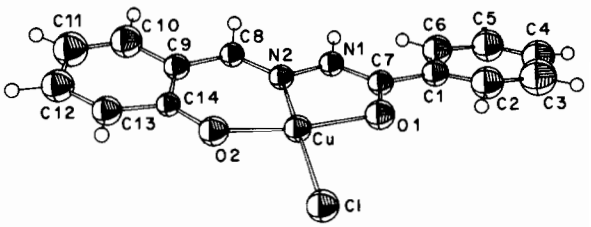

Fig. 2. ORTEP drawing of [CuCl(SBH)]·H_2O (compound *B*), generated with isotropic temperature factors. The water molecule, which is not coordinated to the Cu(II) atom, has been omitted for clarity.

Experimental

Crystals of [$FeCl_2(C_{14}H_{11}N_2O_2)(CH_3OH)$] (hereafter *A*) were obtained by mixing equimolar quantities of SBH and $FeCl_3 \cdot 6H_2O$ in methanol. The resulting solution was filtered and, on standing for 4 days at room temperature then for 3 days at 5 °C, yielded dark green crystals of the product. MW = 398.0, space group $P\bar{1}$, $Z = 2$, $a = 6.665(2)$, $b = 13.818(6)$, $c = 10.122(4)$ Å, $\alpha = 108.40(2)°$, $\beta = 73.23(2)°$, $\gamma = 103.32(2)°$, $V = 837$ Å^3. For 3740 reflections with $|F_c|$ or $F_o \geq 3\sigma(F_o)$, $R = 0.043$, $R_w = 0.036$. Green-gray crystals of [$CuCl(C_{14}H_{11}$-

0020-1693/82/0000-0000/$02.75

$N_2O_2)]\cdot H_2O$ (hereafter *B*) were prepared as previously described [1], by mixing refluxing ethanolic solutions containing equimolar amounts of SBH and $CuCl_2\cdot 2H_2O$ and allowing the resulting solution to stand overnight at room temperature. MW = 356.3, space group $P2_1/a$, $Z = 4$, $a = 16.201(21)$, $b = 7.107(10)$, $c = 12.540(18)$ Å, $\beta = 89.9(1)^\circ$, $V = 1444$ Å. For 1330 reflections with $|F_c|$ or $F_o \geqslant 3\sigma(F_o)$, $R = 0.094$, $R_w = 0.061$. Intensity data out to 2θ values of 57.5° for *A* and 45° for *B* were obtained by $\theta/2\theta$ scans on a FACS-1 Picker automatic diffractometer with Nb-filtered Mo Kα radiation. Full details of the structural analyses will be published elsewhere.

Discussion

In both crystals, SBH functions as a planar, tridentate, uninegative ligand, the coordination polyhedron in the equatorial plane comprising O1, O2, N2 and a chloride ion (Figs. 1 and 2). *A* is 6-coordinate, the axial positions being occupied by a chloride ion (Cl2) and the oxygen atom of a methanol molecule (O3) (Table I). Although Iskander *et al.* proposed a 5-coordinate structure for *B* on the basis of infrared evidence [1], the oxygen atom of the water molecule present in crystals of *B* is more than 3.5 Å distant from the copper atom and the coordination polyhedron is unequivocally 4-coordinate and square planar. Intermolecular hydrogen bonds are formed in A between the O3 and Cl 1 atoms of one molecule and the Cl 1 and O3 atoms of an adjacent molecule and, in *B*, between the water molecule and both N1 of one adjacent molecule and O2 of a second adjacent molecule.

Comparison of the structures of *A* and *B* with those of the closely-analogous complexes $[FeCl_2(PIH)]Cl$ [13], $[FeCl_2(PIH)(H_2O)]Cl\cdot H_2O$ [13] and $[CuCl(PSH)]\cdot H_2O$ [14] (PIH = pyridoxal isonicotinoyl hydrazone, PSH = pyridoxal salicyloyl hydrazone) reveals the influence of the heterocyclic nitrogen atoms, which are present in the latter ligands but absent in SBH. These nitrogen atoms accept protons released from O2 and N1 such that the ligands in all three of the above complexes are bound in the form of zwitterions containing the conjugated linkage C8=N2–N1=C7–O1$^-$. SBH lacks any such mechanism for redistributing protons and, in both *A* and *B*, only the phenolic proton is lost on complexation and the hydrazidic linkage is more nearly described as C8–N2–N1H–C7=O.

Some tentative inferences can be drawn concerning the importance of various structural features in determining the biological properties of ligands such as SBH and PIH. The absence of protonated (and therefore charged and hydrophilic) heterocyclic nitrogen atoms in SBH and its complexes should enable these species to pass more readily across cell membranes than do the PIH analogs. This is seen in practice, where there is an apparent correlation between ligand lipophilicity and both cytotoxicity toward cultured cells [12] and ability to remove iron from hepatocytes *in vivo* [9]. While the presence or absence of hydrophilic sites in the molecule may determine the extent of cellular uptake and efflux, the planar, tridentate mode of metal ion binding common to SBH, PIH and related ligands may play a central role in their behaviour once inside a cell. Unlike clinically used chelators such as EDTA and desferrioxamine, which form hexadentate complexes with essential metal ions, tridentate binding of an aroylhydrazone such as SBH leaves up to three coordination sites available for binding to endogenous ligands. This ability to form covalent aroylhydrazone-metal-substrate complexes may be responsible for the cytotoxicity of these chelators, if the covalently-bound substrate is a nucleic acid or an enzyme involved in DNA replication. Similarly, aroylhydrazone-mediated transport of metals out of hepatocytes may entail intracellular covalent binding of an aroylhydrazone-metal moiety, of the type present in *A* and *B*, to endogenous ligands such as cholic acid which are excreted from the cell in the course of normal enterohepatic cycling. Thus, although ligands such as SBH presumably have a lower thermodynamic affinity for essential metals than do hexadentate chelators, their hydrophobic character and the coordinative unsaturation of their metal chelates may prove advantageous for some biological applications.

Acknowledgements

This research was supported in part by a Biomedical Research Support Grant from the Graduate School of the University of Washington and by Institutional Cancer Grants IN-26U and IN-26W from the American Cancer Society.

References

1 M. F. Iskander, A. M. El-Aggan, L. S. Refaat and L. El Sayed, *Inorg. Chim. Acta, 14,* 167 (1975).
2 N. S. Biradar and B. R. Havindale, *Inorg Chim. Acta, 17,* 157 (1976).
3 K. K. Narang and A. Aggarwal, *Transition Met. Chem., 2,* 29 (1977).
4 H. A. Offe, W. Siefken and G. Domagk, *Z. Naturforsch., 7b,* 446 (1952).
5 J. R. Dimmock, G. B. Baker and W. G. Taylor, *Can. J. Pharm. Sci , 7,* 100 (1972).
6 A. Jacobs, *Brit. J. Haematol., 43,* 1 (1979).

7 C. Hershko, S. Avramovici-Grisaru, G. Link, L. Gelfand and S. Sarel, *J. Lab. Clin. Med., 98,* 99 (1981).
8 P. Ponka, J. Borova, J. Neuwirt, O. Fuchs and E. Necas, *Biochim. Biophys. Acta, 586,* 278 (1979).
9 D. K. Johnson, M. J. Pippard, T. B. Murphy and N. J. Rose, *J. Pharmacol. Exp. Ther., 221,* in press (1982).
10 L. Pickart, W. H. Goodwin, T. B. Murphy and D. K. Johnson, *J. Cell. Biochem. Suppl. 6,* L482 (1982).
11 L. Pickart, W. H. Goodwin, W. Burgua, T. B. Murphy and D. K. Johnson, unpublished data.
12 D. K. Johnson, T. B. Murphy, N. J. Rose, W. H. Goodwin and L. Pickart, submitted to *Inorg. Chim. Acta.*
13 T. B. Murphy, D. K. Johnson, N. J. Rose, A. Aruffo and V. Schomaker, *Inorg. Chim. Acta, 66,* L67 (1982).
14 P. Domiano, A. Musatti, M. Nardelli, C. Pelizzi and G. Predieri, *Transition Met. Chem., 4,* 351 (1979).