Preparation, characterization, and biologic evaluation of copper(II) – Schiff base complexes derived from anthranilic acid and aldoses

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Received April 6, 1992[2]

MOHAMMAD S. IQBAL, SYED J. KHURSHID, and MOHAMMAD Z. IQBAL. Can. J. Chem. **71**, 629 (1993).

Cu(II) – Schiff base complexes containing anthranilic acid and aldoses (sugars) as part of the base were prepared and characterized by microanalytical, thermogravimetric, magnetic, and spectroscopic data. The complexes are four-coordinate, anhydrous, and ML_2 type. The electron paramagnetic resonance spectral lines exhibited rhombic distortion from axial symmetry in the square-planar Cu(II) complexes with $g_{\parallel} > g_{\perp} > g_e$. The complexes were found to be active against kaolin paw oedema and standard strains of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

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On a préparé des complexes du Cu(II)/bases de Schiff contenant de l'acide anthranilique et des aldoses (sucres) dans la base et on les a caractérisés par des méthodes microanalytique, thermogravimétrique, magnétique et spectroscopique. Les complexes sont tétracoordinés, anhydre et du type ML_2. Les raies des spectres de résonance paramagnétique électronique des complexes Cu(II) plan carrés présentent une distorsion rhombique par rapport à la symétrie axiale avec $g_{\parallel} > g_{\perp} > g_e$. On a trouvé que les complexes sont actifs contre l'oedème et certaines souches d'*Escherichia coli*, *Staphylococcus aureus* et *Pseudomonas aeruginosa*.

[Traduit par la rédaction]

Introduction

Schiff bases and transition metal – Schiff base complexes are important intermediates in certain biologic processes, such as non-enzymatically controlled transamination reactions (1). In these reactions amino acids lend their amino group for Schiff base formation whereas the carbonyl group can be provided by a host of carbonyl compounds, such as pyridoxal compounds, aldoses, and certain hormones, occurring naturally in biological systems. The chemistry and the biochemistry of Schiff bases and transition metal – Schiff base complexes derived from amino acids and pyridoxal compounds, and some other carbonyl compounds that do not occur in biological systems, have been extensively studied (2, 3). Although they occur abundantly in biological systems, no attempt has been made to study the possibility of Schiff base formation in vitro utilizing the carbonyl group of aldoses. However, Horecker *et al.* (see refs. 4–6 in ref. 4) suggest Schiff base formation, in vivo, between an aldose and a terminal amino group associated with an amino acid of an enzyme. In the present work we prepared and characterized some copper(II) – Schiff base complexes in which the Schiff bases were derived from anthranilic acid and naturally occurring sugars.

Since the claim (5) of enhanced anti-inflammatory (AI) activity of Cu(II) complexes of AI drugs, a variety of Cu(II) complexes of both non-AI and AI drugs have been prepared and studied for their AI activity (6–10). The present work includes a study of the effect of the complexes under investigation on kaolin paw oedema in rat. Taking into consideration the possible role of pathogens in rheumatic diseases (11, 12), the antibacterial activity of these complexes is also presented. The combination of the sugar components in these complexes is of significance because sugar containing substances have been used since ancient time for wound treatment (13–15) and in antimicrobial effects (16).

Experimental

Materials

L-Arabinose, D-xylose, D-glucose, and D-galactose were obtained from Sigma Chemical Co. and anthranilic acid and copper acetate dihydrate from E. Merck. The standard strains used for antibacterial activity were *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Pseudomonas aeruginosa* (ATCC 27853).

Preparation of complexes

Anthralinic acid (0.1 mol) and the appropriate sugar (0.1 mol) were mixed together in ethanol (200 mL), and potassium hydroxide (0.1 mol) was added. The mixture was refluxed for 15 min. To the lemon-yellow solution thus obtained a solution of copper acetate dihydrate (0.05 mol in 50 mL ethanol) was added slowly and the reaction mixture was refluxed for a further 15 min. The resulting green precipitate was isolated by filtration, washed with ethanol and ether, and dried under vacuum. The complexes thus obtained are listed in Table 1.

Characterization

Microanalysis was carried out by the usual techniques. Copper was estimated by the Hitachi Z-8000 atomic absorption spectrophotometer. Molecular weights were determined mass spectrometrically. Thermal analysis was carried out on a Netzsch simultaneous thermal analyzer on TGA and DTA modes. Conductivity measurements were carried out using a Wescan 212 conductivity meter in *N*,*N*-dimethylformamide (DMF) at room temperature. Magnetic moments were determined by Gouy's

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[2]Revision received December 8, 1992.

TABLE 1. Microanalytical data of the complexes: found (calculated)[a]

Complex	C	H	N	Cu
1. Bis(*N*-L-arabinose-imine-anthranilate) Cu(II), (arab anth)$_2$Cu, ($C_{12}H_{14}NO_6$)$_2$Cu MW 600.006	47.88 (48.03)	4.80 (4.67)	4.73 (4.67)	10.61 (10.59)
2. Bis(*N*-D-xylose-imine-anthranilate) Cu(II), (xyl anth)$_2$Cu, ($C_{12}H_{14}NO_6$)$_2$Cu MW 600.006	47.98 (48.03)	4.88 (4.67)	4.45 (4.67)	10.56 (10.59)
3. Bis(*N*-D-glucose-imine-anthranilate) Cu(II), (glu anth)$_2$Cu, ($C_{13}H_{16}NO_7$)$_2$Cu MW 660.054	47.26 (47.30)	5.01 (4.85)	4.39 (4.24)	9.49 (9.63)
4. Bis(*N*-D-galactose-imine-anthranilate) Cu(II), (gal anth)$_2$Cu, ($C_{13}H_{16}NO_7$)$_2$Cu MW 660.054	47.44 (47.30)	5.00 (4.85)	4.41 (4.24)	9.58 (9.63)

[a]Abbreviations: arab = arabinose; xyl = xylose; glu = glucose; gal = galactose; anth = anthranilic acid.

TABLE 2. Observed infrared bands (cm^{-1}) and assignments

Complex	ν(OH)	ν(C=N)	ν(COO)	ν(COO)	δ(CO)	π(CO)	ν(MN)	ν(MO)
1. (arab anth)$_2$Cu	3405	1610	1540	1325	750	500	410	300
2. (xyl anth)$_2$Cu	3375	1620	1540	1325	750	500	410	320
3. (glu anth)$_2$Cu	3388	1618	1545	1325	755	525	425	340
4. (gal anth)$_2$Cu	3405	1618	1540	1325	720	550	420	340

technique and diamagnetic corrections were calculated from Pascal's constants (17). Infrared spectra were recorded on a Perkin–Elmer 882 IR spectrophotometer using KBr disc and Nujol mull techniques. Electronic absorption spectra were obtained with a Hitachi 220S spectrophotometer using DMF as solvent. Electron paramagnetic resonance (EPR) spectra were recorded in powder and solution (in DMF) form, both at room temperature, on a Jeol JES-FE 1XG machine, in the X-band, operating at a microwave frequency of 9.44 GHz. The g values were determined by use of the Kneubühl approximation (18). The spectra were calibrated using α,α-diphenyl-β-picrylhydrazyl radical (dpph, g = 2.0036) as a field marker.

Anti-inflammatory activity

Kaolin paw oedema was induced, according to a reported method (19), in male Wistar rats (90–100 g) in groups of five. The Cu(II) complexes under investigation were administered orally in 5% Mulgophen (GAF Co. Manchester) in distilled water (0.2 mL/100 g body weight) 1 h before the kaolin. The rats were dosed on a weight of drug (mg) per body weight (kg) of animal basis. Oedema was evaluated 4 h after the subplantar administration of kaolin in 0.9% w/v sodium chloride solution. Inhibition of oedema was evaluated by comparing the swelling obtained in treated animals with the controls, and was expressed as percentage inhibition. The statistical significance was evaluated by using the Student t-test.

Antibacterial activity

The antibacterial activity against the standard strains of *E. coli*, *S. aureus*, and *P. aeruginosa* of the complexes was determined using the plate method (20). The inhibitory zones were measured in mm.

Results and discussion

Characterization

Microanalytical data (Table 1) agree with a ML_2 composition of the complexes, where M = Cu(II) and L = the Schiff base ligand. Thermal analysis indicated the absence of any lattice or coordinated water. The molecular weights as determined mass spectrometrically also confirm the ML_2 composition. The infrared (IR) spectra of the complexes contained all the bands due to the ligands with additional bands indicative of the coordination of the ligands with copper through N and O. Some important bands along with their assignments are listed in Table 2. The assignments were made by comparison with related Schiff base complexes (21). It is seen that ν(C=N) frequencies are slightly higher due to coordination and lie in the 1610–1620 cm^{-1} range, which is in agreement with the literature values for such compounds (22). The C=N coordination was confirmed by the appearance of a new ν(M—N) mode around 420 cm^{-1}. In the complexes the ν(CO_2) were observed to be on the lower side as a result of coordination of the carboxylate ion.

Imine formation occurred when a sugar and anthranilic acid were mixed, and was indicated by a yellowing of the solution and development of an absorption band with a maximum at about 400 nm (23). The Schiff base ligands could not be isolated due to their instability. It is known that, normally, the Schiff bases obtained from aliphatic carbonyl compounds hydrolyze readily and are, therefore, not very stable; however, they can be isolated as metal chelates. On addition of the metal salt the yellow color was replaced by

TABLE 3. Electronic absorption spectra [nm (ε, mol^{-1} cm^2)] in DMF

Complex	Ligand bands	*d–d* bands
1. (arab anth)$_2$Cu	280(40 005), 370(7090), 410(1700)	650(566)
2. (xyl anth)$_2$Cu	280(40 006), 370(7003)	655(509)
3. (glu anth)$_2$Cu	280(40 006), 329(7100)	655(550)
4. (gal anth)$_2$Cu	280(40 003), 370(6881)	655(560)

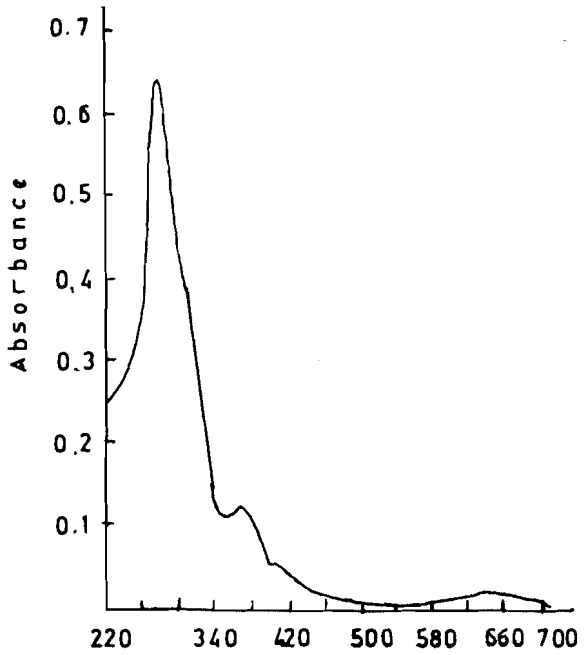

FIG. 1. Electronic absorption spectrum of (arab anth)$_2$Cu in DMF.

TABLE 4. Magnetic moments of the complexes

Complex	μ (BM)
1. (arab anth)$_2$Cu	1.92
2. (xyl anth)$_2$Cu	1.85
3. (glu anth)$_2$Cu	1.90
4. (gal anth)$_2$Cu	2.00

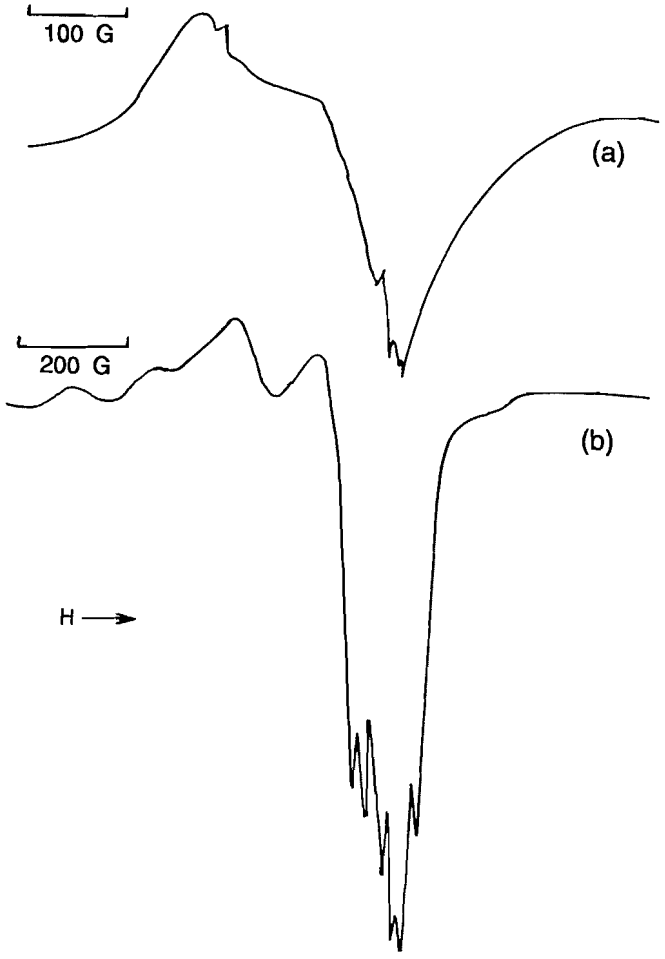

FIG. 2. EPR spectra of (arab anth)$_2$Cu: (a) powder; (b) in DMF.

a green color and complexation occurred. The electronic absorption maxima of the complexes under investigation are listed in Table 3. The representative spectrum of (arab anth)$_2$Cu is reproduced in Fig. 1. The intense band at 280 nm was assigned to a phenyl ring π–π^* transition (24). The bands in the 329–370 nm range, by analogy with (Sal)(−)pn–Zn (25), were attributed to a π–π^* transition originating in the -CH=N- chromophore, while the lower energy bands were due to *d–d* transitions. The electronic spectra of Cu(II) complexes are generally poor indicators of geometry. However, the values of extinction coefficient and position of the absorption bands of the Cu(II) complexes under investigation are consistent with a distorted square-planar environment (26).

The magnetic moments of the complexes (Table 4) are normal values as expected for mononuclear Cu(II) complexes. From the microanalytical and the spectroscopic data all the complexes reported in this work appear to be four-coordinate. The coordinating groups as indicated by the IR spectra are C=N and -COO^- present in the Schiff base ligands. Lower molar concentration values (15.23–17.19 ohm^{-1} mol^{-1} cm^2) were indicative of the nonelectrolytic nature of the complexes. Neutral four-coordinate Cu(II) complexes with chelating agents are usually planar.

The polycrystalline and solution EPR spectra of the (arab anth)$_2$Cu are shown in Fig. 2. The spectra produced by other complexes were similar and hence are not shown. There was general correspondence between the powder and solution spectra, however; the $g_\parallel$ region was well resolved in solution. The spectra exhibited rhombic distortion from axial symmetry in the square-planar geometry because of two different kinds of atoms (O and N) coordinating directly to the metal ion. The spectra were characteristic of magnetically dilute systems with Cu(II) ions in the $d_{x^2-y^2}$ ground state ($g_\parallel > g_\perp > g_e$ (Table 5)). Hyperfines due to nitrogen ($I = 1$)

TABLE 5. EPR parameters of the complexes

Complex	Solid	Solution		
	g_0[a]	$g_\parallel$[b]	$g_\perp$[b]	$\lvert A_\parallel(\mathrm{Cu})\rvert$[c]
1. (arab anth)$_2$Cu	2.17	2.256	2.028	16.00
2. (xyl anth)$_2$Cu	2.20	2.310	2.030	16.51
3. (glu anth)$_2$Cu	2.19	2.298	2.029	16.23
4. (gal anth)$_2$Cu	2.14	2.200	2.028	16.00

[a]±0.02.
[b]±0.001.
[c]$\times 10^3$ cm^{-1}, ±0.08.

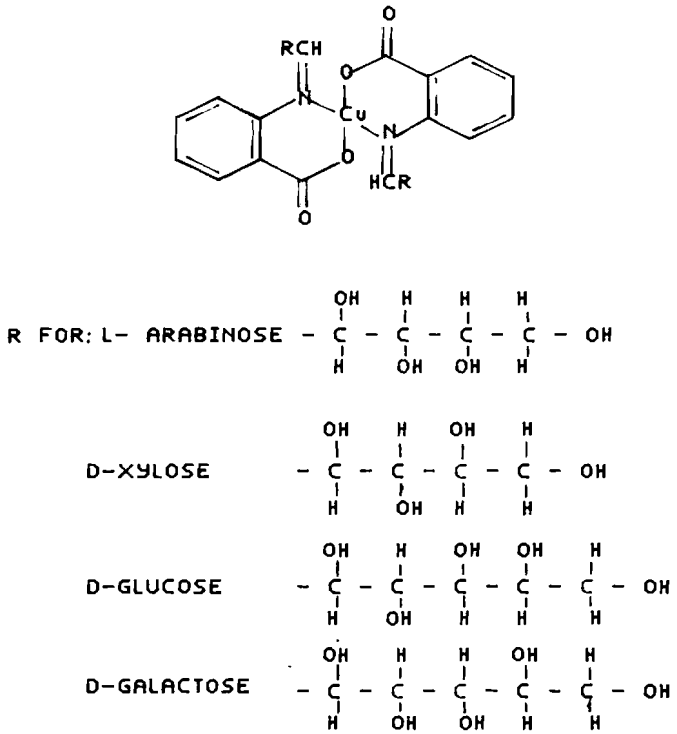

FIG. 3. Proposed structures of the complexes.

TABLE 6. AI effect of the complexes in kaolin paw oedema

Complex	Dose (mg/kg)	% Inhibition of odema	LD_{50} (mg/kg)
1. (arab anth)$_2$Cu	50	30[a]	1750
2. (xyl anth)$_2$Cu	50	25[a]	1750
3. (glu anth)$_2$Cu	50	28[a]	1800
4. (gal anth)$_2$Cu	50	25[a]	1700

[a]$p < 0.05$.

are visible on the main absorption line, $g_\perp$ (Fig. 2), confirming the coordination through N.

Sugars usually exhibit hemiacetal structure in solution, which results from cyclization of the carbonyl group. Apparently there is no free carbonyl group available. From the results obtained it appears that the hemiacetal linkage of the reducing sugars is labile and the carbonyl group becomes available for formation of the Schiff base. This phenomenon is not unusual as the reactivity of the carbonyl group in cyclic or acyclic form (which are in equilibrium) has been demonstrated in some other reactions as well (27). We have included two hexsoses and two pentoses in the preparation of the ligands because the hexsoses are by far the most abundant, while the pentoses are important components of nucleic acids and various polysaccharides.

The present study clearly indicates that the ligands form stable coordinate complexes with Cu(II) as evidenced by microanalytical, magnetic, and spectroscopic data. Unsuccessful attempts to isolate crystals suitable for X-ray studies prevented further structure elucidation. The structures of the complexes under investigation, proposed on the basis of the above experimental evidence, are shown in Fig. 3.

Anti-inflammatory activity

All of the complexes inhibited kaolin paw oedema (Table 6). They were found to be equipotent at the dose tested. The toxicity data (LD_{50}, Table 6) indicate that these compounds can become potential AI drugs because they possess comparable AI activity to that of Cu(II)-aspirinate and far less toxicity (ca. $LD_{50} = 760 \pm 100$ for Cu(II)-aspirinate (5). The low toxicity of these complexes can be attributed to greater biocompatibility of the Schiff base ligands and also to the presence of sugar as one of the components in the complexes.

Antibacterial activity

All the complexes under investigation were found to be active against the strains tested. The observed antibacterial activity of the complexes is of interest and supports the speculated role of pathogens in inflammatory processes, as previously indicated by the antibacterial activity of a variety of AI drugs including Cu(II) complexes (12).

1. E. E. Snell and D. E. Metzler. Pyridoxal catalysis, enzymes and model systems. Interscience, New York. 1968.
2. R. H. Holm, G. W. Everett, and A. Chakravarty. Prog. Inorg. Chem. **7**, 83 (1966).
3. G. N. Weinstein, M. J. O'Connor, and R. H. Holm. Inorg. Chem. **9**, 2104 (1970).
4. M. L. Wolform. Adv. Carbohydr. Chem. **10**, 170 (1955).
5. J. R. J. Sorenson. J. Med. Chem. **19**, 135 (1976).
6. G. E. Jackson, P. M. May, and D. H. Williams. J. Inorg. Nucl. Chem. **40**, 1189 (1978).
7. D. H. Brown, A. J. Lewis, W. E. Smith, and J. W. Teape. J. Med. Chem. **23**, 729 (1980).
8. J. R. J. Sorenson. Inflammatory diseases and copper. Humana Press, N.J. 1982.
9. R. Nagir and G. Mohan. J. Inorg. Biochem. **42**, 9 (1991).
10. S. Oga, S. F. Tanguchi, R. Najjar, and A. R. Souza. J. Inorg. Biochem. **41**, 45, (1991).
11. T. Pullar, J. A. Hunter, and H. A. Capell. Br. Med. J. **290**, 1535 (1985).
12. M. S. Iqbal, S. J. Khurshid, and M. Z. Iqbal. J. Pak. Med. Assoc. **40**, 221 (1990).
13. R. D. Forrest. J. R. Soc. Med. **75**, 268 (1982).
14. L. Herszage, J. R. Montenegro, and A. L. Joseph. Bol. Trab. Soc. Cir. Buenos Aires, **41**, 315 (1980).
15. J. L. Trouillet, J. Y. Fagoon, Y. Domart, J. Chastre, J. Pierre, and C. Gibert. Lancet, 180 (1985).
16. J. Chirife, L. Herszage, A. Joseph, and E. S. Kohn. Antimicrob. Agents Chemother. **23**, 766 (1983).
17. A. Earnshaw. Introduction to magnetochemistry. Academic Press, New York. 1968. p. 6.
18. F. K. Kneubühl. J. Chem. Phys. **33**, 1074 (1960).
19. A. J. Lewis, J. Cottney, and M. F. Sugrue. J. Pharm. Pharmacol. **27**, 375 (1975).
20. P. Gerhardt. *In* Manual of methods for general bacteriology.

Edited by R. N. Castello. American Society for Microbiology. 1981. pp. 65–208.
21. S. T. Chow, D. M. Jones, and C. A. MacAuliffe. Inorg. Chim. Acta, **22**, 1 (1977).
22. G. H. Rist, J. S. Hyde, and T. Vangard. Proc. Natl. Acad. Sci. U.S.A. **77**, 1339 (1977).
23. D. E. Metzler. J. Am. Chem. Soc. **91**, 5977 (1962).
24. B. Bosnich. J. Am. Chem. Soc. **90**, 627 (1968).
25. R. S. Dowing and F. L. Urbach. J. Am. Chem. Soc. **91**, 5977 (1966).
26. L. Sacconi and M. Ciampolini. J. Chem. Soc. 276 (1964).
27. F. Shafizada. Adv. Carbohydr. Chem. **13**, 9 (1958).