Copper Schiff Base Complexes with Polyfunctional Amino Acids

L. G. MacDONALD, D. H. BROWN, J. H. MORRIS and W. E. SMITH*
Department of Pure and Applied Chemistry, University of Strathclyde, Cathedral Street, Glasgow, G1 1XL, U.K.
Received January 25, 1982

Amino acid Schiff base copper complexes of either salicylaldehyde or 2-hydroxy-1-naphthaldehyde with l-serine, l-histidine, histamine and l-tryptophan have been prepared by constituent combination. The amino acid part of the ligands coordinates to the copper through the carboxylate group and the other available functional group of the amino acids plays a more minor role as a blocking group or in intramolecular bonding. Schiff bases of salicylaldehyde or 2-hydroxy-1-naphthaldehyde with cysteine or penicillamine were prepared. In solution, NMR and IR evidence suggested thiazolidine ring formation in these bases and the presence of two conformers in the salicylidinecysteine base. Bases containing penicillamine produced a stoichiometric Schiffs base copper complex. Cysteine-containing ligands produced a stoichiometric compound with zinc but not with copper. However, with copper and not with zinc, constituent combination produced compounds of general formula, aldehyde (amino acid)$_2$ (copper)$_2$-xH_2O. These latter compounds are believed to contain either cystine or dipenicillamine.

Introduction

Studies of the chromophore in mono- and binucleating Schiff base complexes of copper and nickel, using complexes with ligands formed from either salicylaldehyde or 2-hydroxy-1-naphthaldehyde have demonstrated the value in these studies of incorporating an optically active centre in the molecule [1–3]. Amino acids provide a useful source of such centres but previous studies of Schiff base amino acid complexes have been concerned mainly with bidentate amino acids [4–8] or with complexes with transition metals other than copper [9–12]. Further, Schiff base amino acid complexes of copper(II) are believed to be key intermediates in non-oxidative transformation of pyridoxal dependent enzymes [13]. Consequently, a number of studies of the Schiff bases with amino acids have been reported using pyridoxal as the co-condensate. Emphasis is again on complexes of ligands containing bidentate amino acids [14–16].

The characterisation of Schiff base complexes with potentially tridentate amino acids would, therefore, extend the range of optically active Schiff base complexes available and would be useful in defining the role of the additional complexing groups. This latter point seems of interest biochemically, since the rate of reaction of pyridoxal Schiff base complexes is metal ion-dependent [14] and reactions of this type *in vivo* may well involve tridentate amino acids. In this study, the preparation and characterisation of copper Schiff base complexes of a range of potentially tridentate amino acids with salicylaldehyde and 2-hydroxy-1-naphthaldehyde is reported.

Experimental

There are several general methods of preparation of salicylaldiimine or 2-hydroxy-1-naphthaldiimine complexes [17], of which the following three were used,

1) Mixing copper acetate, aldehyde and amino acid in solution (constituent combination);

2) Mixing copper salicylaldehyde or 2-hydroxy-1-naphthaldehyde and amino acid in solution;

3) Mixing copper acetate and preformed Schiff base in solution (direct reaction)

No one method was found to be suitable for all the complexes. Where it was possible to prepare the Schiff base in pure form, method (3) was preferred, otherwise method (1) was generally used. The compounds prepared, contractions used to describe them and analytical data are given in Table I.

Infrared spectra were measured with a Perkin-Elmer 457 spectrometer, uv-visible spectra with Pye-Unicam SP1800 and Beckman Acta IV spectrometers and NMR data with a Perkin-Elmer 100 MHz R14 spectrometer. Magnetic measurements were by the Gouy technique and –SH measurements by Ellmans method [18]. Copper analysis was by atomic absorption spectrometry using carbon furnace atomisation.

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

TABLE I. Compounds Prepared and Analytical Data. Sal; Salicylaldehyde, Nap; 2-Hydroxy-1-naphthaldehyde; His, Histidine; Histam, Histamine; Try, Tryptophan; Ser, Serine; Pen, Penicillamine; Cys, Cysteine

Compound	Found %				Theoretical			
	C	N	H	S	C	N	H	S
salhisCu·$3H_2O$	41.72	11.35	3.38		41.66	11.21	4.54	
salhisCu·$\frac{1}{2}H_2O$	46.88	12.67	3.47		47.34	12.75	3.64	
salhistam1.$5H_2O$	44.49	12.38	4.34		44.89	12.08	4 03	
salhistam Cu3.$5H_2O$	45.38	12.11	4.95		44.79	13.06	4.97	
saltryCu2H_2O	53.62	6.72	4.40		53.26	6.90	4.19	
saltryCu·$\frac{1}{2}H_2O$	57.26	7.80	3.77		57.06	7.40	3.96	
salserCuH_2O	41.33	4.42	4.62		41.59	4.85	3 81	
salpen	55.09	5.45	6.10	12.53	56.92	5.53	5.93	12.65
nappen	63.38	4.55	5.61	10.55	63.37	4.62	5.61	10.56
Salcys	53.36	6.38	5.10	14.21	53.33	6.22	4.89	14.22
salpenCu1.$5H_2O$	42.06	3.88	4.28		42.29	4.11	4.40	
nappenCuH_2O	50.36	3.58	4.48	8.24	50.20	3.66	4.44	8.37
salpen$_2$Cu$_2$$\frac{1}{2}H_2O$	38.05	4.99	4.47		38.27	5.25	4.13	
salcys$_2$Cu$_2$2H_2O	30.93	5.50	3.20	12.64	30.95	5.55	3 37	12.70
nappenZn·$2H_2O$	48.27	3.28	4.05		47.95	3.50	4.50	
salcysZn·2.$5H_2O$	35.88	4.24	3.12	8.83	35.99	4.20	3.91	9.60

1) *N-salicylidine histidine copper and N-salicylidene histamine copper* was prepared in ethanol by method (1) with similar conditions to these of L. J. Theriot *et al.* [19]. A pH 8 phosphate buffer solution was added to ensure dissolution of the reactants. A pale green trihydrate and a dark green hemihydrate were obtained on separate occasions.

A histamine complex, which contained 1.5 moles of water, was prepared in a similar manner and a hemihydrate was prepared by heating this compound in an oven for 24 hours at 80 °C.

2) *N-salicylidine tryptophan copper* complexes were prepared as above using tryptophan in place of histidene. A blue complex, which could not be characterised definitely, precipitated out immediately. Evaporation of the solution to half volume produced a slower crystallization of a green hemihydrate. Addition of water to the resultant solution precipitated a dihydrate which reverted on standing to the hemihydrate.

3) *N-salicylidene serine copper* was prepared in a similar manner using serine. An immediate dark green precipitate was not characterised but solvent reduction produced a pale green monohydrate.

4) *N-salicylidine cysteine, N-salicylidine penicillamine and N-2-hydroxy-1-naphthilidine penicillamine.* Equimolar quantities of the appropriate amino acid dissolved in a minimum quantity of distilled water at 40 °C and the appropriate aldehyde in ethanol at 40 °C were mixed and the reaction was maintained at 40 °C for 15 mins. The product either precipitated almost immediately (sal cys) or recrystallized on volume reduction (sal pen, nap pen).

5) *N-salicylidine penicillamine copper and N-2-hydroxy-1-naphthilidine penicillamine* copper were prepared by general method (3) using ethanol or dioxan as solvent. After heating for 30 mins at 40 °C, and solvent removal, dark green products crystallized out. Equivalent reactions with cystine Schiff bases yielded impure or complex products.

6) *N-salicylidine (penicillamine)$_2$ copper$_2$* can be prepared by general method 1 or general method 2. With general method 1, ethanol was the preferred solvent with the amino acid dissolved in a minimum quantity of water. Solutions of the reactants were degassed with nitrogen for 30 mins, mixed and left standing at room temperature with nitrogen gas bubbling through the solution for 30 mins. A dark green tetrahydrate crystallized out almost immediately.

With general method 2, ethanol was again used as solvent with the amino acid dissolved in a minimum of water. The solutions were stripped with nitrogen and the reactions carried out under nitrogen as before. A dark green hemihydrate was obtained by solvent removal.

7) *N-salicylidine cysteine copper*

Equimolar quantities of cysteine dissolved in a minimum of distilled water at 40 °C and salicylaldehyde in ethanol at 40 °C were used. The ligand formed was precipitated and was kept partly in solution and partly in suspension with added ethanol

while an ethanolic solution of an equimolar quantity of copper acetate was added. The solution was maintained at 50 °C for 10 minutes during which time the brown dihydrate compound precipitated.

Zinc compounds. N-salicylidene cysteine and N-2-hydroxy-1-naphthilidenepenicillamine zinc were prepared by general method 3 using dioxan as solvent.

Results and Discussion

Schiff base ligands involving, serine, histidine, tryptophan and histamine were difficult to prepare. Refluxing a suspension of the components of the ligand in a solution of absolute ethanol as suggested by McIntyre for related ligands [20], produced insoluble materials which appeared to contain the ligand but were impure and difficult to recrystallize. Aqueous preparative methods produced hydrolysis reactions. With cysteine and penicillamine, however, hydrolysis reactions proved to be less of a problem and preparation of the ligands in aqueous ethanol was possible. The problem of competing reactions extends to the formation of the complexes by constituent combination methods. In the tryptophan and serine preparations, for example, an immediate precipitate formed on mixing the components but the precipitates contained a rather higher proportion of amino acids than expected for the Schiff base complex. Subsequent slower crystallization of the filtrate produced crystalline materials which analysed correctly for the Schiff bases. Further, the amino acids containing sulphur reacted in a complex manner if a constituent combination or direct reaction method of preparation of the complexes was attempted, possibly because copper ions are extremely efficient in catalysing the formation of disulphide bonds from thiols.

Consequently, no general method for the preparation of Schiff base amino acid complexes was found in this investigation, but preparative methods which give reproducible results have been found for quite a number of complexes of the amino acids investigated.

Complexes with Serine, Histidine, Histamine and Tryptophan

The serine complex would appear on the basis of the magnetic moment value (Table II) to be monomeric. The infrared spectrum indicates a coordinated azomethine (ν C=N at 1635 cm^{-1}) and a coordinated carboxylate (ν-COO^- at 1600 cm^{-1}). The relative assignment of these two bands to their respective groups is made on the basis of a comparison of peak intensities between the infrared and Raman spectra of the zinc complexes discussed later, since absorption of the laser beam by the copper complexes prevented a direct comparison. Analysis and thermal balance results suggest a monohydrate and it seems likely, in agreement with the related glycine and phenylalanine Schiff base copper complex structures [5, 8], that this water will be bonded in a fourth coordination position to make approximately planar four-coordinate copper.

TABLE II. Magnetic Moments of Copper Schiff Base Complexes Containing Histidine, Histamine, Tryptophan and Serine. See Table I for Explanations of Contractions Used.

	μ (B.M.)
SalhisCu3H_2O	2.25
SalhisCu½H_2O	2.00
SalhistamCu3.5H_2O	2.05
SalhistamCu	1 73
SaltryCu2H_2O	2.00
SaltryCu½H_2O	1.48
SalserCuH_2O	2.35

Trihydrate and hemihydrate salicylidene histidine Schiff base complexes have normal magnetic moments for d^1-complexes (Table II), and the infrared spectra again suggest coordinated azomethine (ν C=N– at 1630 and 1620 cm^{-1} respectively) and coordinated carboxylate (ν-COO^- at about 1600 cm^{-1} in each case). The hydrated histamine complex also has a normal magnetic moment and infrared evidence suggests coordinated azomethine and carboxylate groups. The i.r. spectrum of the imidazole was too complex to help in assessing its role in bonding. The anhydrous histamine compound has a lower magnetic moment, suggesting an exchange effect – possibly dimerisation [21].

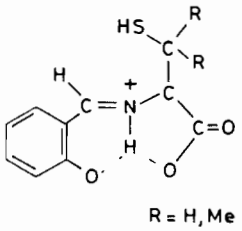
Martell and Abbott [13] have prepared a similar ligand to the histidine one using pyridoxal in place of salicylaldehyde. They indicate that a carbon in the imidazole ring is bonded to the azomethine carbon. In the present study, the complex was prepared *in situ* and there is no evidence that any group such as that found in solution by Martell and Abbott is present in the solid.

Several tryptophan Schiff base hydrates were prepared, of which the best defined were a dihydrate with a magnetic moment suggesting a monomeric complex and a hemihydrate with a low magnetic moment suggesting dimer or polymer formation (Table II). The i.r. spectra indicate that in both compounds carboxylate- is coordinated and there is little evidence that the indole ring is complexed to the metal. However, it would be expected to play at least a part as a blocking group in the crystal packing and there is an additional indole ring vibration at 3520–3580 cm^{-1}, possibly due to an interaction between the

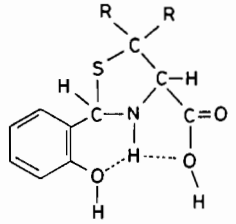
indole and a carboxylate group of a neighbouring molecule.

Complexes with Cysteine and Penicillamine

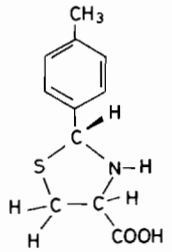
The ligands required for these complexes were quite simply prepared. In the solid-state, infrared measurements on the ligands indicated the presence of an –SH group, a hydroxide and an ionised carboxylate. The azomethine peak was slightly higher in energy than that of most complexes so far discussed and there was evidence of an N–H stretch at a rather low frequency – possibly due to hydrogen bonding [22]. These observations suggest that the nitrogen of the azomethine is protonated. Thus, the simplest structure for the ligand in the solid state would be structure I.



1



2

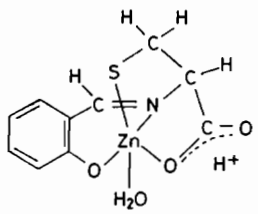


3

However, the infrared spectra of thin films of solutions of each of the ligands in DMSO indicated that there was a free carboxylate group at about 1720 cm^{-1}, that the N–H vibration was at a higher frequency than in the previous case and there was no –SH stretch. NMR spectra of Nappen, Salpen and Salcys show the characteristic proton signal of the azomethine carbon shifted downfield from its usual value of 9.5 ppm to a value of 5.53–6.50 ppm. A comparable result was obtained by Martell and Abbott [13]. They proposed the formation a thiazolidine ring (structure 2) to explain their results. Comparison of the spectra obtained for salcys with those obtained by Parathasarathy *et al.* [23] for 2(*p*-tolyl) thiazolidene-4-carboxylic acid (structure 3) indicated near identical chemical shifts for the two compounds for all similar protons, and confirm the existence of a thiazolidine ring in sal cys (structure 2). Nap pen and sal pen produce similar results for the 4–CH and 2-CH protons and consequently it would appear that thiazolidene ring formation occurs regularly for Schiffs base ligands of cysteine and penicillamine in solution.

Parathasarathy *et al.* [23] noted that the peaks in his compound were split into doublets which correlated with two different conformers found in the X-ray crystal structure of the N-acetyl derivative. This splitting was present in our compounds and is probably due to a similar cause. In the case of sal cys the conformers were present in an approximately equal amounts, but in sal-pen and nap-pen one conformer, that with its proton up field, was present in larger proportions.

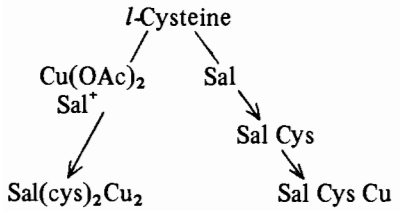
The copper complexes of these ligands were too insoluble in DMSO to permit NMR studies and consequently zinc complexes with sal cys and nap-pen were prepared. The infrared spectra indicated that both contained complexed carboxylate and coordinated azomethine. The NMR spectrum of the nap-pen complex was broad and there was no evidence of thiazolidene ring formation. For example, the 2-CH proton was observed at 8.9 ppm – indicative of a proton attached to the azomethine carbon. Free –SH bands in the nap-pen and sal cys ligands are observable in the infrared but there is no evidence of an –SH band in the infrared spectrum of the complexes. Tests with Ellman's reagent confirmed the absence of thiol groups in the complexes. Raman spectra taken on the anti Stokes side to overcome a fluorescence problem indicated probable M–S bonding in the zinc complexes. The disulphide stretch, expected at about 500 cm^{-1}, was not observed. It would appear therefore that the sulphur is coordinated to the zinc ion. Thermal balance studies indicate a discrete water loss at about 140 °C, probably indicating chemically bound water. Thus the most likely structure for the zinc complexes is 5 coordinate as,



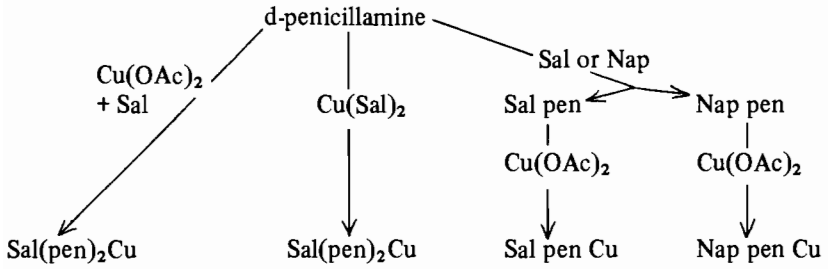
The possibility of polymer formation with the sulphur being attached to a neighbouring zinc ion cannot be ruled out.

Many different variations of the general methods of preparation were used in attempts to produce copper complexes with both cysteine and penicillamine but only a few produced unambiguous results. The successful reactions are outlined below for cysteine (Scheme 1) and penicillamine (Scheme 2).

Scheme 1



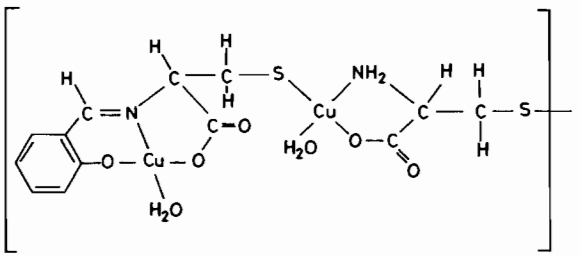
Scheme 2



The difference appears to be due to competitive reactions such as the formation of disulphide which tend to proceed more quickly for cysteine than for penicillamine.

No free thiol groups could be detected either by infrared or by the use of Ellman's reagent for the sal pen and nap-pen copper complexes. Raman spectra were not recorded due to self absorption of the sample. Infrared evidence again suggests coordinated carboxylate and azomethine. Therefore, a structure equivalent to the zinc one above (4) may apply in the copper case as well.

Complexes involving both cysteine and penicillamine and containing two formula units of each could be prepared quite simply. The number of copper atoms was confirmed in each case by atomic absorption spectrometry. The sal $cys_2Cu_2 2H_2O$ compound was considered in more detail. It had a magnetic moment of 2.11, B.M. Infrared evidence suggested coordinated azomethine and carboxylate groups. There was no evidence, chemical or spectroscopic of –SH groups. There were two consecutive weight losses on the thermal balance, each consisting of a weight loss equivalent to one water molecule and consequently it would appear that there are two chemically inequivalent water molecules present. Immediately following the second weight loss a further sharp loss occurred, suggesting ligand decomposition. This behaviour is similar to that observed for the penicillamine [24] compound and a similar structure is proposed (5). The dipenicillamine or cystine required for this formulation could be produced by the catalytic action of the copper ion on the thiol groups.



5

Thus, it would appear that the tridentate amino acids form Schiff base compounds similar to those of the bidentate ones but the extra complexing group does participate in the structure, for example, indole interactions, 5 coordinate sulphur-containing species and disulphide bond formation. The systems may, therefore, be of use in studies of the chromophore in Schiff base complexes, but the implications for biological models of the *in vivo* reactions of pyridoxal containing Schiff base intermediates are that further studies of the competitive effect of metal ions such as copper and zinc on these reactions would be required.

Acknowledgements

We thank the Science Research Council and CIBA/GEIGY Plastics and Additivies Co. (UK) Ltd. for a CASE award to one of us (L. G. Macdonald) and Dr. J. M. McCrae of CIBA/GEIGY for advice and encouragement throughout.

References

1 B. Bosnich, *J. Am Chem Soc , 90,* 627 (1968).
2 R. S. Downing and F. L. Urbach, *J Am. Chem Soc., 92,* 5861 (1970).
3 R. S. Downing and F. L. Urbach, *J. Am Chem. Soc., 91,* 5977 (1969).
4 P. Ray and A. K. Mukherjee, *J Ind Chem. Soc., 27,* 707 (1950).
5 A. Nakahara, M. Kıshita and M. Kubo, *Aust. J Chem , 17,* 810 (1964).
6 T. Veki, T. Ashida, Y. Sasada and M. Kakudo, *Acta Cryst., 22,* 870 (1967).
7 Y. Nakao, K. Sakurai and A. Nakahara, *Bull Chem Soc. Jap., 40,* 1536 (1967).
8 R. Hamalainen, U. Turpeinen, M. Ahlgren and M. Rantala, *Acta Chem. Scand., A32,* 549 (1978).
9 L. J. Theriot, G. O. Carlisle and H. J. Hu, *J Inorg Nucl. Chem., 31,* 2891 (1969).
10 R. C. Burrows and J. C. Bailar Jr., *J Am Chem Soc., 88,* 4150 (1966).
11 F. Baykut, A. Aydın and A. Uren, *Chim Acta Turkey, 3,* 105 (1975).
12 B. Hajek and F. Jursik, *Inorg. Chim Acta, 13,* 169 (1975).
13 A. E. Martell and E. H Abbott, *J. Am Chem. Soc., 92,* 1754 (1970).
14 E. E. Snell and S. J. Di Mari, in 'The Enzymes', Acad. Press, N.Y. (Ed. P. D. Boyer) Vol. II, p. 335, 1970 and references therein.
15 H. N. Christensen, *J Am. Chem. Soc , 81,* 6495 (1959) and references therein.
16 L. Davis, F. Roddy and D. E. Metzler, *J. Am Chem Soc., 83,* 127 (1961).
17 S. Yamuda, *Coord. Chem Revs., 1,* 415 (1966).
18 G. L. Ellman, *Arch Biochem Biophys., 82,* 70 (1959).
19 L. J. Theriot, G. O. Carlisle and H. J. Hu, *J Inorg Nucl. Chem , 31,* 2891 (1969).
20 F. C. McIntyre, *J. Am. Chem. Soc , 69,* 1377 (1947).
21 R. H. Holm, G. W. Everett and A. Chakravorty, *Prog Inorg Chem , 7,* 83 (1966).
22 L. J. Bellamy, 'The Infrared Spectra of Complex Molecules', Methuen, 1966.
23 R. Parathasathy, B. Paul and W. Korytryk, *J Am. Chem Soc., 98,* 6634 (1970).
24 L. G. Macdonald, D. H. Brown, J. H. Morris and W. E. Smith, *Inorg. Chim. Acta, 33,* L183 (1979).