Experimental. 3-Methoxy-6-dimethylaminopyridazine (I, $R^3=CH_3O$, $R^6=(CH_3)_2N$). 3-Chloro-6-dimethylaminopyridazine [6] (15.8 g) was refluxed with sodium methoxide (from 10 g of sodium) in methanol (120 ml) for two days. The conversion was 94 % after reflux for 20 h. Addition of water, extraction with chloroform, and distillation gave a colourless, hygroscopic oil (10.1 g, b.p. 88°/0.4 mm, m.p. *ca.* 25°), redistilled for analysis. (Found: C 54.22; H 7.41; N 27.32. Calc. for $C_7H_{11}N_3O$: C 54.89; H 7.24; N 27.43.)

3-Methoxy-4-t-butyl-6-dimethylamino-4,5-dihydropyridazine (IIc). A mixture of 3-methoxy-6-dimethylaminopyridazine (1.5 g), *t*-butylmagnesium chloride (*ca.* 30 mmol) and ether (40 ml) was stirred for 1 h at 25°. The product was poured onto ice, the ether decanted, and the aqueous layer extracted twice with chloroform. The emulsion was broken by adding hydrochlorid acid (pH ~ 9). The combined extracts were dried ($MgSO_4$) and concentrated *in vacuo* to give a semicrystalline residue (1.73 g). Recrystallisation from petroleum ether gave yellow crystals (1.33 g, m.p. 65–74°). Two additional recrystallisations gave light yellow crystals, m.p. 77–79°. (Found: C 62.70; H 9.95; N 19.77. Calc. for $C_{11}H_{21}N_3O$: C 62.53; H 10.02; N 19.89.)

3-Methoxy-4-t-butyl-6-dimethylaminopyridazine. Bromine (1.5 ml) was added dropwise to a stirred solution of the dihydropyridazine (IIc, crude, from 5.1 g of (I)) in water (30 ml). Sodium hydroxide was added to pH 6 and the product was extracted with ether. The solvent was evaporated and the residue refluxed with a solution of sodium methoxide (from 2.0 g of sodium) in methanol (20 ml) for 10 min. Addition of ice, extraction with chloroform, and distillation gave (according to NMR, see below) crude 3-methoxy-4-*t*-butyl-6-dimethylaminopyridazine (3.3 g, b.p. 96°/0.15 mm). The same product was obtained by treating 3-chloro-4-*t*-butyl-6-dimethylaminopyridazine [7] (500 mg) with sodium methoxide (from 200 mg of sodium) in methanol (4 ml) for 8 days at 100°. The crude reaction product consisted of a 1:3 mixture of the methoxylated pyridazine and the starting material; the identity of the former and the 3-methoxy-4-*t*-butyl-6-dimethylaminopyridazine prepared above was confirmed by the coincidence of peaks in their NMR-spectra.

1. Christensen, A. and Crossland, I. *Acta Chem. Scand.* **17** (1963) 1276.
2. Crossland, I. *Acta Chem. Scand.* **16** (1962) 1877.
3. Crossland, I. *Acta Chem. Scand.* **26** (1972) 3257.
4. Kofod, H. *Ph. D. Dissertation 1968*, The Technical University, Denmark, in Danish.
5. Kelstrup, E. *Acta Chem. Scand.* **23** (1969) 1797.
6. Crossland, I. and Kofod, H. *Acta Chem. Scand.* **21** (1967) 2131.
7. Crossland, I. and Kofod, H. *Acta Chem. Scand.* **24** (1970) 751.

Received October 22, 1972.

Models of Copper-Protein Interaction: The Crystal Structure of (Glycyl-L-histidylglycinato)-copper(II) Sodium Perchlorate Hydrate

R. ÖSTERBERG,[a] B. SJÖBERG[a] and R. SÖDERQUIST[b](†)

[a] *Department of Medical Chemistry, University of Göteborg, Fack, S-400 33 Göteborg 33, Sweden and* [b] *Research Institute of National Defence (FOA), S-172 04 Sundbyberg 4, Sweden*

It is indicated from recent studies on copper proteins that nitrogen ligand atoms are important for binding copper.[1,2] Thus, attention is focused on histidine and lysine side chains as well as α-amino and amide groups. Of these, the histidine side chain is known to be involved in the labile copper(II) interaction of both myoglobin [3] and serum albumin.[4] Therefore, in order to construct proper models for the co-ordination structures in copper proteins, it seems important to ask what copper ion complexes will form with imidazole groups when they are present within a peptide chain. The smallest possible molecule of this kind, glycylhistidylglycine (HA), was chosen as a model in this study.

Violet crystals, $CuH_{-1}A(NaClO_4)H_2O$, were prepared from solutions of copper(II), glycylhistidylglycine (HA) and sodium perchlorate in the pH range 4.5 to 10. At pH

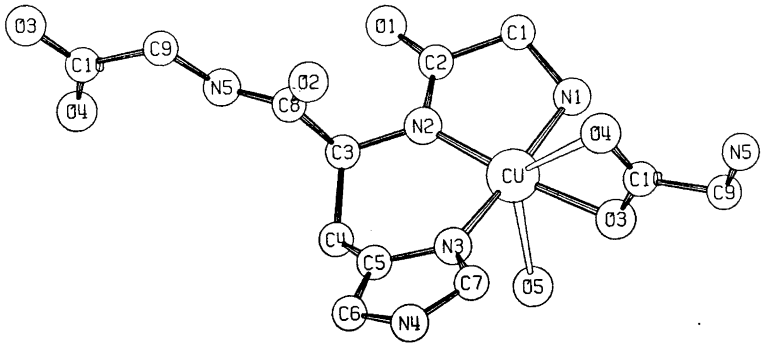

Fig. 1.

4.5 these violet crystals developed in the mother liquor of the light blue triclinic crystals, $CuA(ClO_4)2H_2O$, where both phases coexisted. However, the violet phase appears to be thermodynamically more stable since the light blue phase recrystallized into the violet one.

The violet crystals are orthorhombic with $a = 12.92$, $b = 19.34$, $c = 6.62$, $D_m = 1.86$, $D_c = 1.89$ g cm^{-3}, $Z = 4$ for $CuH_{-1}A(NaClO_4)H_2O$, space group $P2_12_12_1$. Intensity data were recorded using equi-inclination Weissenberg photographs and Cu$K\alpha$ radiation; the reflections were estimated visually. At the present stage of refinement, for 1449 independent reflections, R is 0.075. The structure is shown in Fig. 1.

Within the crystal, each tripeptide binds one copper atom at its amino N(1), peptide N(2), and imidazole N(3) nitrogen atoms, and a second copper atom at the carboxylate atom O(3). The Cu–N distances are 2.01, 1.93, and 1.93 Å, and the Cu–O(3) distance is 2.03 Å. The second carboxy-oxygen O(4) and a water molecule O(5) interact weakly with the copper atom at 2.76 and 2.74 Å. The structure contains two kinds of infinite chains consisting of -copper-peptide- and -sodium-perchlorate- that are parallel to the b- and a-axes, respectively.

There are important similarities among the structures of the present violet crystals, that of the blue-violet crystals,[5] $CuH_{-1}A.xH_2O$ (x *ca.* 12), and that of the crystals of copper(II)-glycyl-L-histidine.[6] In these three structures, copper(II) is co-ordinated *via* the 1-nitrogen of the imidazole ring and the nitrogen atoms of the peptide and amide groups. The structures [5] of the two tripeptides (Ref. 5 and Fig. 1) demonstrate that copper(II) only forms chelates with a histidyl residue on the NH_2-terminal side of the peptide chain (*cf.* Ref. 6). Thus, a stable Cu^{2+} site exists in a protein, if a histidyl residue is present in the second or third position of the peptide chain; one example is found in human serum albumin.[4]

This work was supported by grants from the *Swedish Natural Science Research Council* and the *Magnus Bergwall Fund*. We thank Mrs. G. Sterner for skilful technical assistance.

1. Malmström, B. G., Reinhammar, B. and Vänngård, T. *Biochim. Biophys. Acta* **67** (1968) 156.
2. Gould, D. C. and Ehrenberg, A. In Ghiretti, F., Ed., *Physiology and Biochemistry of Hemocyanines*, Academic, New York 1968, p. 95.
3. Banaszak, L. J., Watson, H. C. and Kendrew, J. C. *J. Mol. Biol.* **12** (1965) 130.
4. Bradshaw, R. A., Shearer, W. T. and Gurd, F. R. N. *J. Biol. Chem.* **243** (1968) 3817.
5. Österberg, R., Sjöberg, B. and Söderquist, R. *Chem. Commun.* **1972** 983.
6. Blount, J. F., Frazer, K. A., Freeman, H. C., Szymanski, J. T. and Wang, C. H. *Acta Cryst.* **22** (1967) 396.

Received November 22, 1972.