TO MY MOTHER

AND ANNE

PREFACE

Amino acids are amongst the most important basic components of organic life. Proteins, which have so many different roles in maintaining metabolism and genetic reproduction either alone or as enzymes, are built from amino acids. At this time, when the structure of proteins and enzymes is receiving such widespread attention, the structures of the individual amino acids are also seen to be of importance, not only because they are important compounds in themselves, but also because their structures can be useful in protein structure analysis indicating typical conformation and bonding arrangements. Further, the metal coordination compounds of the amino acids have many interesting, yet often unexplained properties, and they can often serve as models for the active centres of enzymes.

CONTENTS

* In this thesis 'histidino' and 'histidinato' have equivalent meanings.

PART I

THE CRYSTAL STRUCTURE DETERMINATION OF BIS-L-HISTIDINATO-COBALT(II) MONOHYDRATE

PART I

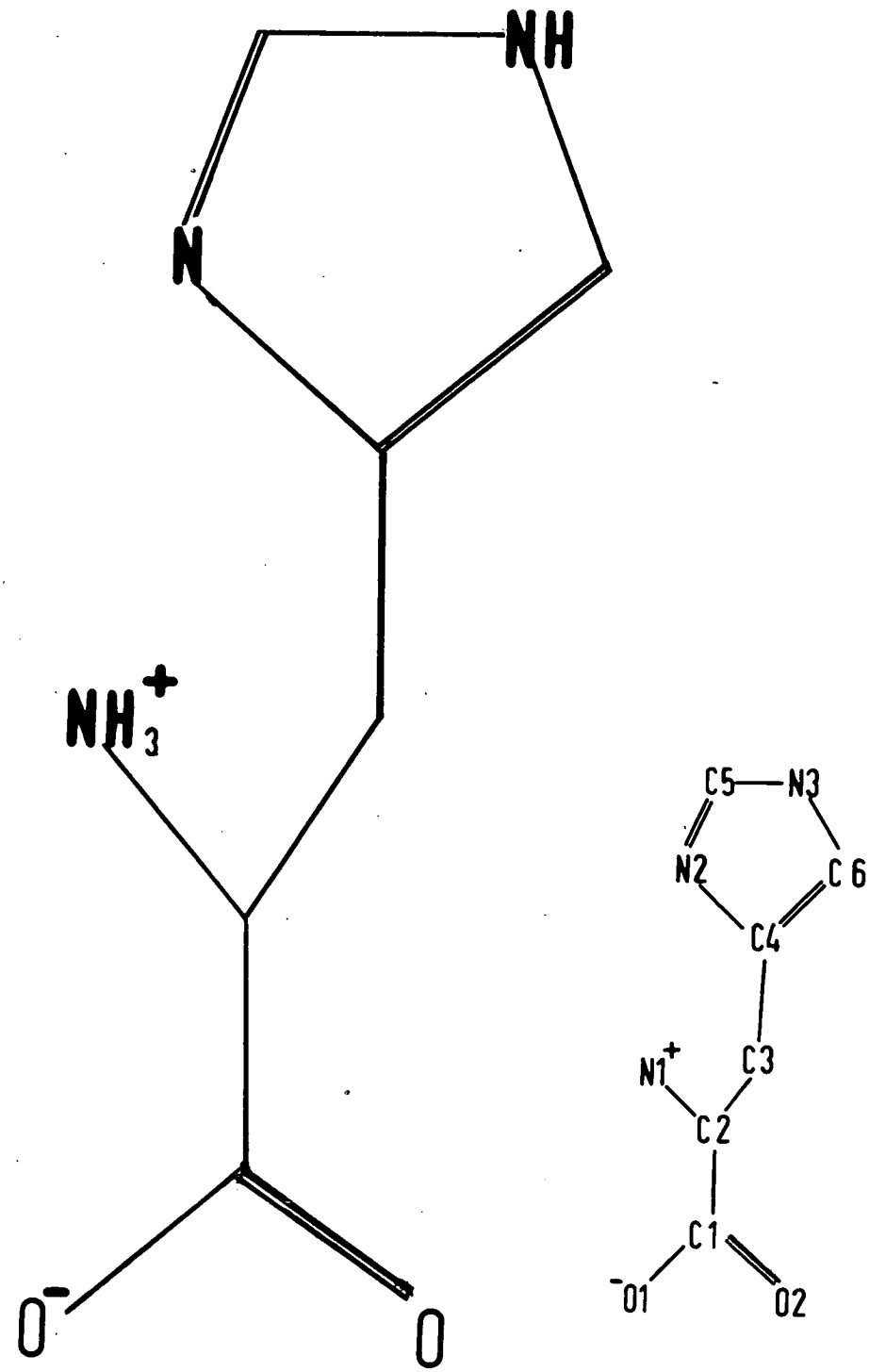
SECTION I

INTRODUCTION

Amino acids are able to form complexes with transition metals instead of simple salts since they have the ability to form chelate rings, preferably five-membered for greatest stability. There are three coordinating atoms commonly found in amino acid coordination: the amino nitrogen, an oxygen of the carboxyl group, and sulphur in sulphur-containing amino acids. Further, there are other possible coordinating points, such as the imidazole ring nitrogens in histidine. The most stable complexes are formed by α-amino acids, less stable complexes being formed by β-amino acids, and only normal salts being obtained from γ-, δ-, and ε- amino acids (1).

Histidine

Histidine ($C_3H_3N_2CH_2CH(NH_2)COOH$), α-amino-5-imidazole-propionic acid (Fig.I), is a commonly occurring, natural α-amino acid, and was first isolated by Kossel (2) and Hedin(3) in 1896. The most distinguishing feature of histidine is an imidazole ring which, as mentioned above, allows it two further possible coordination sites, making



HISTIDINE $C_3H_3N_2CH_2CH(NH_2)COOH$

FIG I

/five in all. With the one exception of cysteine ($SH.CH_2CH(NH_2)COOH$) histidine forms the most stable complexes of all natural amino acids with transition metals. The crystal structure of histidine hydrochloride monohydrate has been determined (4) and refined (5). This thesis is concerned with the structure of a complex formed between histidine and divalent cobalt, and a brief review of the stereochemistry of complexes of divalent cobalt is given below.

The Stereochemistry of Divalent Cobalt Complexes

Cobalt is a transition metal of atomic number 27, and can lose the two 4s electrons to give divalent cobalt Co^{2+}, with $3d^7$ configuration. Cobalt(II) can show several coordination numbers, including four, five, six and eight, and for each coordination number, spin-free and spin-paired complexes are possible, because of the incomplete d shell. Only a little structural work has been carried out on divalent cobalt complexes, but examples of each coordination number have been examined by X-ray methods and various stereochemical arrangements have been found to exist.

One would expect spin-free complexes of Co(II) with coordination number four to have sp^3 hybridisation, giving tetrahedral coordination, and this is indeed found with the more easily polarised ligands such as the halides, other ligands such as water giving coordination number six. The three unpaired electrons of Co^{2+} give a calculated magnetic moment of 3·88 B.M. and this can be used as a distinguishing criterion for these tetrahedral complexes, although, because of spin-orbital coupling, the magnetic moment can be as high as 4·9B.M., as in the $Co(I_4)^{2-}$ ion. This value is taken as the upper limit since the I^- ion has the weakest ligand field. An example of a tetrahedral Co(II) complex is cobalt dipivaloylmethane (6),

$$Co(II)[(CH_3)_3C-\overset{O}{\overset{\|}{C}}-\overset{H}{\overset{|}{C}}=\overset{O^-}{\overset{|}{C}}-C(CH_3)_3]_2$$

which has three unpaired electrons and an approximately tetrahedral structure.

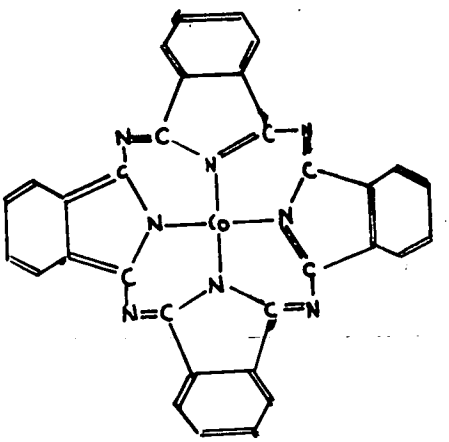
Spin-paired four-coordinate complexes of Co(II) have a magnetic moment of about 2B.M. and are square-planar. There is no evidence for spin-paired tetrahedral complexes in the first transition series. The square-planar arrangement seems to occur only when the Co(II) ion is forced/

/forced into this position by the nature of the ligand and examples are the cobaltous phthalocyanine and porphyrin complexes. Nickel phthalocyanine has been determined by X-ray methods (7) and the cobaltous complex shown to be isomorphous. (Fig IIa)

Five coordination of cobalt(II) is also possible in the spin-paired configuration (dsp^3 hybridisation) and either a trigonal-bipyramidal or square-pyramidal arrangement might be expected. However the structure of Co(II) bis--(2-dimethylaminoethyl)methylamine dichloride (Fig.IIb) recently investigated (8) cannot be described easily in terms of either of these geometries, even allowing for distortion.

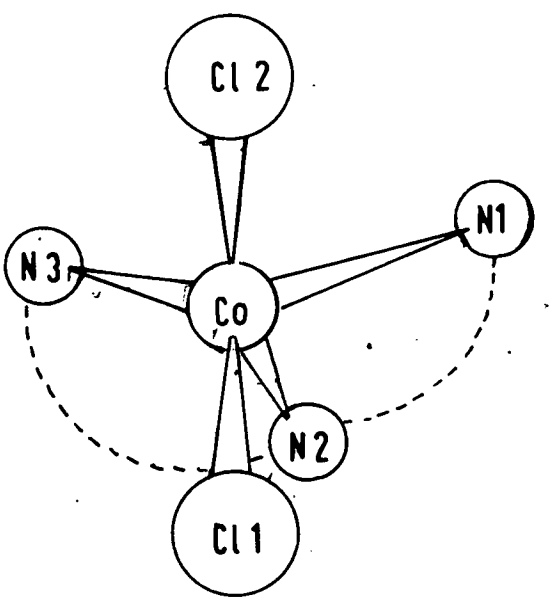
In the six-coordinate complexes of cobalt(II) sp^3d^2 and d^2sp^3 hybridisations are possible for the spin-free and spin-paired configurations respectively. The latter is unstable as it involves the promotion of a 3d electron to the 4d shell and the existence of complexes of this nature is uncertain.

The spin-free six-coordinate complexes are the most common and the most studied. Octahedral coordination is expected/



COBALT PHTHALOCYANINE

(a)

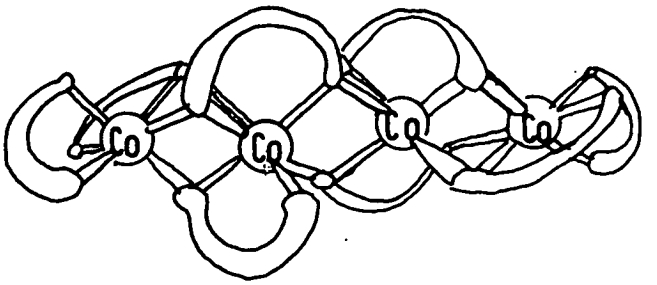


$Co(denMe)Cl_2$

CH_2-CH_2 chains represented by broken lines

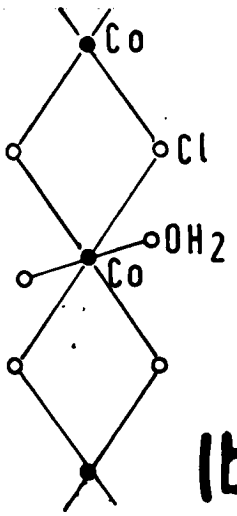
(b)

FIG I



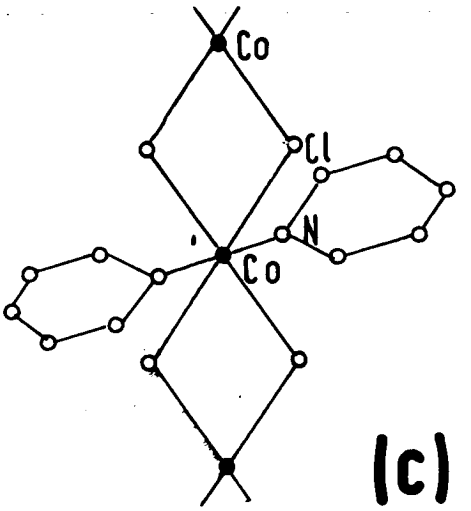
COBALT ACETYLACETONATE (a)

O-C-C-C-O rings indicated by curved lines



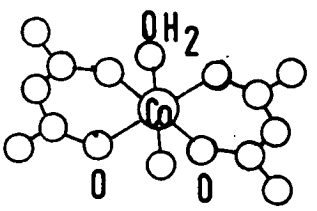
(b)

COBALT DICHLORIDE DIHYDRATE



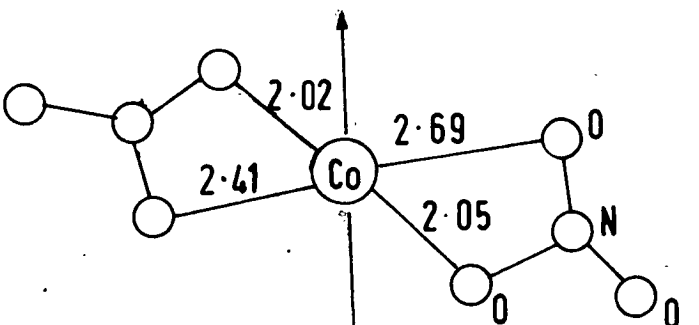
(c)

α-COBALT DIPYRIDINE DICHLORIDE



(d)

COBALT BISACETYLACETONE DIHYDRATE



$[As(C_6H_5)_4]_2[Co(NO_3)_4]$ (e)

Rest of molecule generated by rotation through 180° about axis

FIG III

/expected from the sp^3d^2 hybridisation and is indeed found. Although the octahedral ligand field, according to ligand field theory, splits the orbitals into an antisymmetrical low energy group of $3d\epsilon$ orbitals and two $d\gamma$ orbitals of higher energy, thus:

only small deviations from octahedral symmetry are expected as the $d\epsilon$ orbitals are directed between the octahedral bonds and not along them.

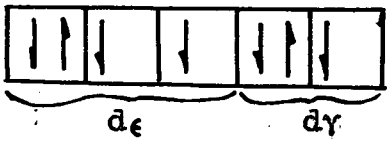
Cobalt dichloride dihydrate (9,10)(Fig.IIIb) is one example of this type of compound, consisting of polymeric chains of $CoCl_2$ held together in a near square-planar arrangement, with the two water molecules completing the octahedron. There are, however, some inequalities in the bond lengths between the cobalt and chlorine atoms and slight distortions from the square-planar arrangement of these atoms. This compound can be compared with violet α-cobalt(II) dipyridine dichloride (11)(Fig.IIIc), which also has polymeric chains, in which each metal ion is surrounded by four chlorine atoms and two nitrogen atoms. The chlorines are equidistant from pairs of metal atoms, the angles are near right angles. It would seem that the difference between the violet and blue forms/

/forms of this complex is not one of cis-trans isomerism, for the blue form can be compared with blue cobalt di-p-toluidine dichloride (12), which has a structure composed of discrete tetrahedral molecules. Cobaltous diammines occur regularly in two isomorphous forms and it is probable that the two structural types hold throughout the series: pink/octahedral, blue/tetrahedral.

Another complex which has been structurally investigated (13) is cobaltous chloraluminate $Co(AlCl_4)_2$ which is a blue octahedral complex. The cobalt has octahedral complexing in the form of $CoCl_6$, but is distorted. It is one of the exceptions to the rule that octahedral complexes of divalent cobalt are pink; but this is explained by the symmetry of the structure being sufficiently low to remove degeneracies of the excited energy levels of Co^{2+}, thus allowing large shifts in absorption bands to occur.

Other complexes studied are cobalt sulphate hexahydrate (14), which consists of discrete sulphate tetrahedra and $Co(H_2O)_6^{++}$ octahedra; cobalt titanate $CoTiO_3$ (15), which has both titanium and cobalt octahedrally coordinated; cobalt acetate tetrahydrate $Co(CH_3COO)_2.4H_2O$ (16), in which the cobalt atom is surrounded octahedrally by four water molecules and two oxygen atoms from different acetate groups; and cobalt molybdate $NaCo_{2.34}(MoO_4)_3$ which contains both Co^{2+} and Co^{3+} in octahedral dispositions. (17)

Cobalt(II) bisacetylacetone dihydrate $Co(C_5H_7O_2)_2.2H_2O$ (Fig.IIId) has a coordination polyhedron of oxygens around cobalt in the form of a tetragonally distorted octahedron (18), the cobalt-oxygen bond lengths being 2·05 - 2·06Å, and the cobalt-water bond lengths 2·23Å. The formation of a weaker longer bond with a water molecule may be interpreted in terms of the lower electronegativity of its oxygen atom, or in terms of ligand field theory. Bullen (18) has suggested that the asymmetry of the d_ϵ subshell may not be enough to account for this deviation and that there is asymmetry of the $d\gamma$ subshell through mixing in of the configuration $d_\epsilon^4 d\gamma^3$



since this would be consistent with the large spin-orbital coupling in this and other cobalt(II) complexes. A comparable complex is that of the tetrameric, anhydrous crystalline cobalt(II) acetylacetonate (19)(Fig.IIIa), in which each cobalt atom has octahedral coordination through a system of oxygen bridging.

Recently, single crystal work has been performed on cobalt(II) hexammine dichloride $Co(NH_3)_6.Cl_2$ (20) to obtain an accurate determination of the cobalt(II)-nitrogen bond length. This complex crystallises in the cubic space group Fm3m and must therefore have pure octahedral symmetry.

Finally, before moving on to consider the complex between divalent cobalt and histidine, mention must be made of one complex which shows coordination number eight. The crystal structure determination of $[As(C_6H_5)_4]_2[Co(NO_3)_4]$ (Fig.IIIe) by Cotton and Bergman (21) showed that each nitrate ion was bidentate and the cobalt(II) eight-coordinate in a dodecahedral complex. The cobalt-oxygen bonds of the $[Co(NO_3)_4]^{2-}$ ion can be divided into two sets, one bond of each NO_3^- being in the range 2·02 - 2·03Å, the other between 2·41 and 2·69Å, this latter being short enough to give definite bonding. If a mean position for each of these bonding oxygens is calculated it is discovered that the coordination is very nearly tetrahedral in accordance with the implications of the magnetic and spectroscopic results.

A table of relevant bond lengths, determined in some of the above structural analyses is given in Table I.

TABLE I

Compound	Co - N Å	Co - O Å	Ref.
Cobalt-bis(2-dimethyl-aminoethyl)methylamine dichloride	2·11 2·15 2·30		(8)
Cobalt dichloride dihydrate		2·04 (H_2O)	(10)
Cobalt dipyridine dichloride	2·14		(11)
Cobalt di-p-toluidine dichloride	1·92		(12)
Cobalt sulphate hexahydrate		2·11 2·05 2·14 2·13 (H_2O) 2·12 2·11	(14)
Cobalt titanate		2·08 2·16	(15)
Cobalt acetate tetrahydrate		2·12 2·11 (H_2O) 2·06 (H_2O)	(16)
Cobalt molybdate		2·03 2·03 2·07 2·17 2·01 2·06 2·16	(17)
Cobalt bisacetylacetone dihydrate		2·05 2·06 2·23 (H_2O)	(18)
Cobalt hexammine dichloride	2·11		(20)
$As(C_6H_5)_4$ 2 $Co(NO_3)_4$		(2·02 - 2·04) (2·41 - 2·69)	(21)
Cobalt histidine monohydrate	2·19 2·19 2·11 2·17	2·12 2·12	This Thesis

The Cobalt(II) - Histidine Complex

Cobalt(II) forms a complex with histidine, in aqueous solution, which can be written as:

$$Co(II)[C_3H_3N_2CH_2CH(NH_2)COO^-]_2$$

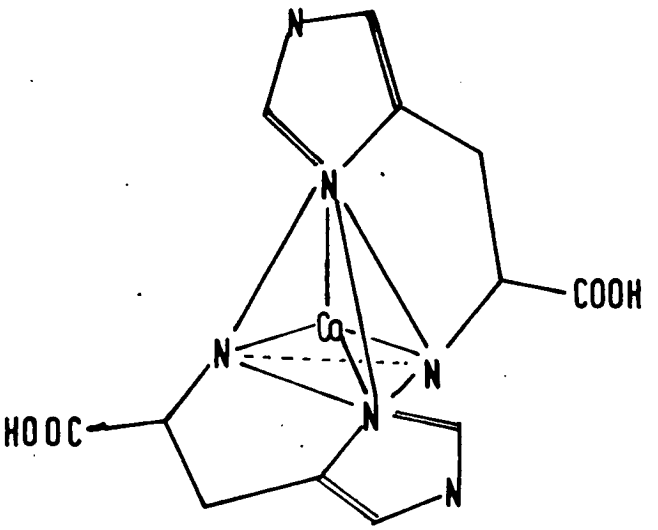
In future references to this complex and corresponding complexes of histidine with other divalent transition metals, the formula within the square brackets which is histidine less one hydrogen ion, will be referred to as "hist", where appropriate, to distinguish it from uncomplexed histidine and from the histidyl residue found in proteins. Thus the above complex will be written as $Co(II)(hist)_2$, and, if necessary, the optical isomeric form of the histidine residues will be signified by the appropriate prefix such as L or DL.

The cobalt complex with histidine is unusual in that it combines reversibly with oxygen (22,23,24). Though, in fact the complex bis-salicylaldehyde ethylenediimine cobalt(II) was the first example (25,26) of a cobaltous chelate which absorbed molecular oxygen, bis-histidino cobalt(II) was the first example of a synthetic cobalt chelate which could act as a reversible oxygen carrier in aqueous solution. The chelates of Mn^{2+}, Fe^{2+}, Ni^{2+} and Cu^{2+} with histidine do not have/

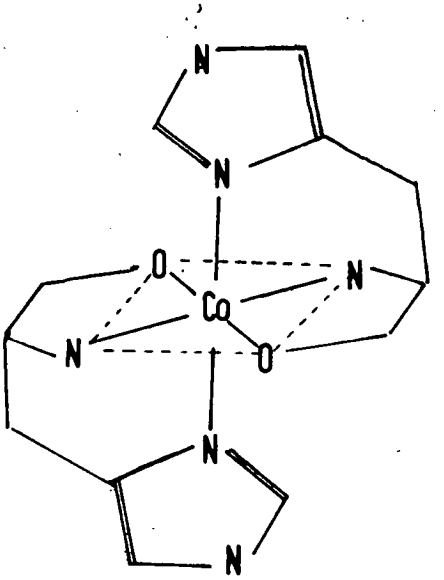
/have this property (27). The ferrous ion is immediately and irreversibly oxidised to the ferric state, whereas Ni^{2+} and Cu^{2+} are stable in the presence of histidine and oxygen.

By virtue of this unusual property of bis-histidino cobalt(II), there has been much interest in its structure, since one can draw an analogy between this complex and the naturally occurring oxygen carriers the haemoglobins and the haemocyanins. It has, in fact, been shown (28) that sperm whale metmyoglobin, a haem protein which occurs as an intercellular pigment and whose function is oxygen storage, has the iron atom of the iron(II) protoporphyrin group (haem) coordinated to the imidazole nitrogen of an histidyl residue. In addition, although the structural analysis of haemoglobin has only been done in part (29), it has been shown that both horse and human haemoglobin contain histidine linked to haem as in sperm whale myoglobin and that the structures are quite comparable (30).

Bis-histidino cobalt(II) absorbs oxygen reversibly in the ratio of one mole of oxygen to two moles of cobalt. Hearon (27) proposed possible structures for both the unoxygenated and the oxygenated complexes. It is the structure of the former which has been derived in this thesis. Hearon obtained a value for the magnetic moment of/



(a) HEARON'S PROPOSED TETRAHEDRAL STRUCTURE



(b) HEARON'S PROPOSED OCTAHEDRAL STRUCTURE

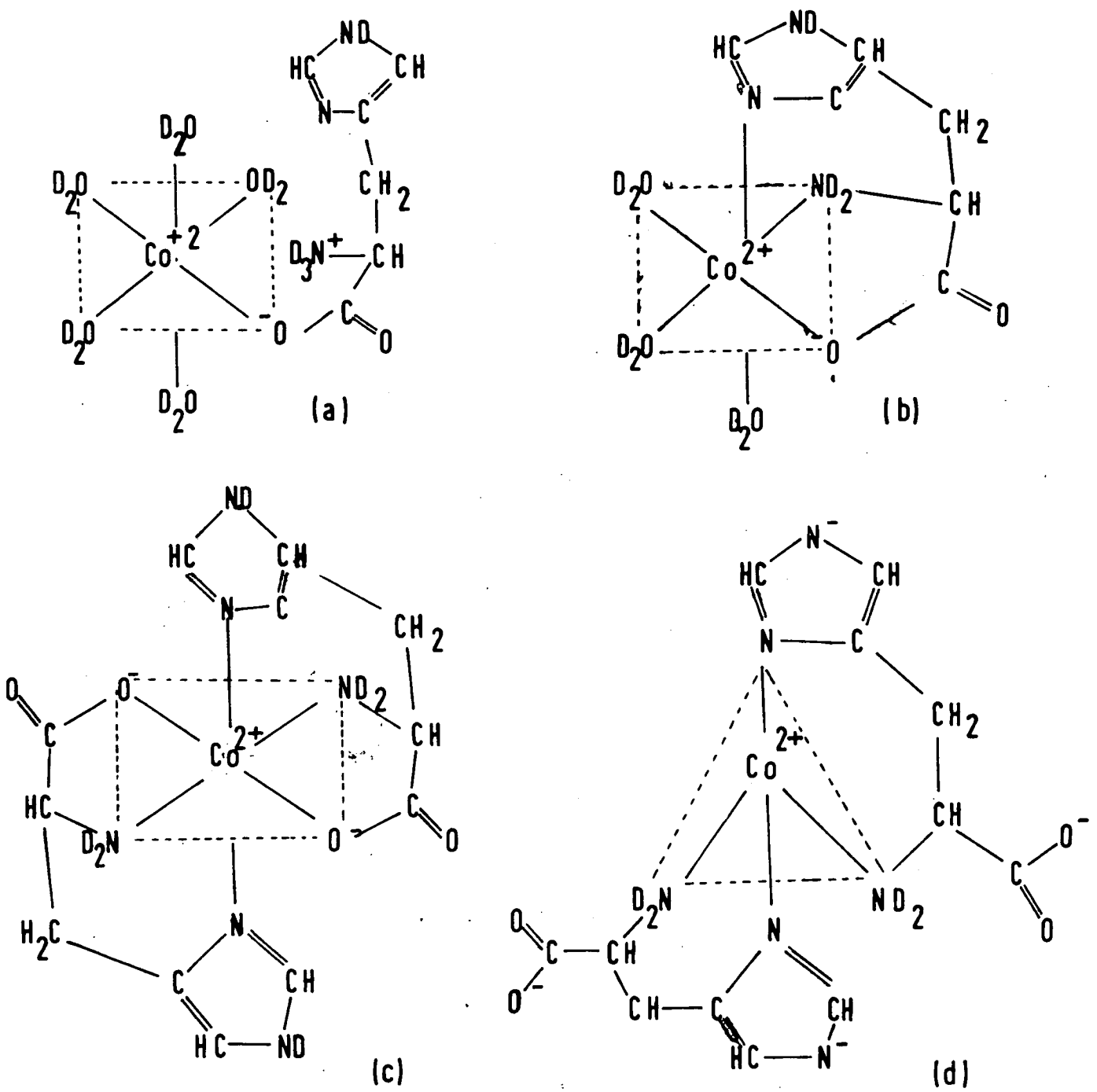
FIG IV

/of the complex in aqueous solution of 4·4B.M. corresponding to three unpaired electrons. He suggested a tetrahedral structure (Fig.IVa) for the complex, stabilised by various resonant forms, including double-bond formation between the cobalt atom and the imidazole nitrogens, but also gave an alternative octahedral structure. (Fig.IVb)

Earnshaw and Larkworthy (31), however, by the more rigorous exclusion of oxygen, obtained a value of 5·13B.M. for the magnetic moment agreeing well with the value obtained by Michaelis (24) of 4·97B.M., this corresponding to the value expected from an octahedral spin-free cobalt(II) complex. They also noted (31) that the pink solution obtained was characteristic of octahedral cobalt complexes, although a few exceptions have been found, e.g. cobaltous chloraluminate (13). Investigation of the infra-red spectrum showed vibration of ionized and probably coordinated carboxyl groups at 1,587cm^{-1}, and the visible absorption spectrum obtained was very similar to that of other octahedral cobalt(II) complexes. This work indicated that the complex was in fact octahedral and was thus in agreement with the work of Leberman and Rabin (32), who deduced by potentiometric titration that all the three available donor groups of the histidine molecules were coordinated to the cobalt ion.

The complex appears to have been first crystallised by Minoshima (33), who obtained long, brown crystals in vacuo, though one would have expected them to be pink. Minoshima, however, obtained a maximum visible absorption peak at 480mμ, comparing with Earnshaw and Larkworthy's value of 486mμ (31). Later the complex was crystallised by Jaselskis (34), who studied the oxidation of the complex by polarography, and more recently by Sano and Tanabe (35), who obtained a pale pink crystalline powder, and recorded the infra-red spectrum and X-ray diffraction powder pattern of the complex.

McDonald and Phillips (36) have conducted a nuclear magnetic resonance study on the complexes of histidine with divalent cobalt. By first of all recording and interpreting the proton resonance spectra of histidine and cobalt(II) separately in aqueous solution, and comparing these spectra with a series of spectra of histidine in the presence of cobalt(II) at varying pH and various concentrations, they were able to distinguish four different complexes: a weak 1:1 histidine-Co(II) complex at pH4 and below (Fig.Va); strong 1:1 and 2:1 complexes between pH4 and pH10 (Figs.Vb and Vc); and a tetrahedral 2:1 complex at pH values greater than 11. By blocking various coordinating points of the histidine molecule they obtained complexes of Co(II) with the following compounds: L-3-methylhistidine, L-1-methylhistidine, histamine, histidine methyl ester and N-acetylhistidine. Interpretation of/



STRUCTURES PROPOSED BY McDONALD & PHILLIPS
(deuterated forms)

FIG V

/of the N.M.R. spectra of these compounds taken in conjunction with the previous N.M.R. spectra led them to deduce certain schematic structures for the four complexes formed. Figure V shows the deuterated forms of these structures. They also found, by comparison of the contact shifts observed when Co(II) was complexed with L-histidine and with racemic DL-histidine that the complex of Co(II) with racemic histidine was more stable than the complex with optically-active histidine in either the L or D form, and, as might be expected, there was no difference in stability between the DD- and LL-forms of the complex.

McDonald and Phillips were thus able to confirm the octahedral structure of the 1:2 Co(II)-histidine complex. This thesis will give the final proof of the octahedral structure and show the true conformation of the molecule of bis-L-histidino cobalt(II) monohydrate, in the crystalline state.

Structural Studies on Transition Metal - Histidine Complexes

The series of complexes of histidine with Zn(II), Ni(II) Cd(II) and Co(II) has recently been the subject of intensive study by X-ray diffraction (37,38,39,40).

The first two structures determined were those of zinc(II)-DL-$(hist)_2$ pentahydrate (37) and zinc(II)-L-$(hist)_2$ dihydrate (38). Cadmium(II)-DL-$(hist)_2$ pentahydrate, cadmium(II)-L-$(hist)_2$ dihydrate, nickel(II)-L-$(hist)_2$ monohydrate and nickel(II)-DL-$(hist)_2$ monohydrate were prepared by the author (page 16) and preliminary work initiated. A more complete study of the two cadmium complexes has been carried out by Candlin (39). The structural work on nickel(II)-DL-$(hist)_2$ monohydrate has been completed by Fraser (39,40). Nickel(II)-L-$(hist)_2$ monohydrate is isomorphous with the cobalt-L-histidine complex which is the subject of this thesis.

When the X-ray investigation of bishistidino-cobalt monohydrate was begun, the only previous X-ray work done on histidine complexes was that concerned with the zinc complexes (37,38). These were both found to have distorted tetrahedral/

/tetrahedral coordination, with coordination by the amino and imidazole nitrogens and weak association of an oxygen of each carboxyl group.

A fuller discussion of the structures of the complexes so far investigated will be given in a comparative study with the structure of bishistidino-cobalt(II) monohydrate in section VIII.

SECTION II

The Preparation of Complexes of Histidine with Divalent Transition Metal Ions

A series of preparations was carried out involving optically active L-histidine and the divalent transition metal ions Cu^{2+}, Ni^{2+}, Cd^{2+} and Co^{2+}; a corresponding series of preparations, using racemic DL-histidine in place of L-histidine was also performed.

Pure histidine of both forms was obtained from L-histidine monohydrochloride and DL-histidine monohydrochloride respectively, the hydrochloride salts being supplied by B.D.H. In both cases the free base was obtained by neutralisation with a strong solution of sodium hydroxide to pH 8, followed by precipitation of the histidine with ethanol. The histidine was filtered, washed with a 1:1 water/ethanol mixture and then pure ethanol. Finally the histidine was recrystallised by dissolving in the minimum of hot water, adding ethanol until the solution was turbid and cooling slowly. Filtration and washing were carried out as before and the histidine dried over calcium chloride *in vacuo*.

Cadmium, copper and nickel complexes

The preparation and crystallisation procedure for the cadmium, copper and nickel complexes was as follows and was the same for both L and DL-histidine. Histidine (4m.moles) was dissolved in 20mls. distilled water and warmed to about 60°C. The appropriate metal carbonate (2m.moles) was then added with evolution of carbon dioxide. When this had ceased, the warm solution was filtered into a porcelain evaporating basin, removing any excess metal carbonate. The solution was set aside and allowed to evaporate at room temperature.

Both forms of the nickel complex crystallised after a few hours giving blue crystals. The cadmium complexes crystallised after standing for about three days giving colourless crystals. Copper, however, did not give crystalline complexes, but instead, a blue glass was formed in each case on evaporation to dryness. Further preparations of the copper complexes were tried using 1:1 ratios of Cu(II) and histidine, but nothing other than a green glass was obtained in any experiment, again on evaporation to dryness. The general equation for the reaction of histidine with Ni(II), Cd(II), and also Co(II) carbonates is:

$$MCO_3 + 2C_3H_3N_2CH_2CH(NH_2)COOH \longrightarrow M[C_3H_3N_2CH_2CH(NH_2)COO]_2 + CO_2 + H_2O$$

where M = Ni(II), Cd(II) or Co(II).

Cobalt complexes

The fact that cobalt-histidine complexes absorb oxygen in solution necessitated the exclusion of air from preparations and crystallisations. At first preparations were attempted following the above procedure for the other complexes, by reacting cobaltous carbonate with histidine in a glove-box under an atmosphere of nitrogen previously washed in alkaline pyrogallol. After reaction and filtration, clear pink solutions were obtained, but as the solutions had to be left to crystallise for some days it was found that trace quantities of oxygen were being absorbed and no crystals were obtained. The next approach, which was successful, was to react cobaltous carbonate (0·238g.) with histidine (o·620g.) in about 20mls. of boiling water in air, no oxygen being absorbed. For this a 100ml. conical flask was used. After twice decanting the boiling solution and concentrating to half volume by boiling, approximately 50mls. of boiling ethanol were added to the solution, which was once more concentrated by boiling. When about 25mls. of the solution remained, the flask was sealed and allowed to cool slowly overnight in a hot water bath. Crystals appeared during cooling, and, after the solution had stood for a day, the flask was transferred to a glove-box containing a nitrogen atmosphere. The flask was then opened and the/

/the pink crystalline complex was quickly filtered off and washed with a small quantity of boiled out 1:1 water/ethanol mixture and then a little boiled out ethanol. Finally, the crystals were dried over calcium chloride *in vacuo* for several hours.

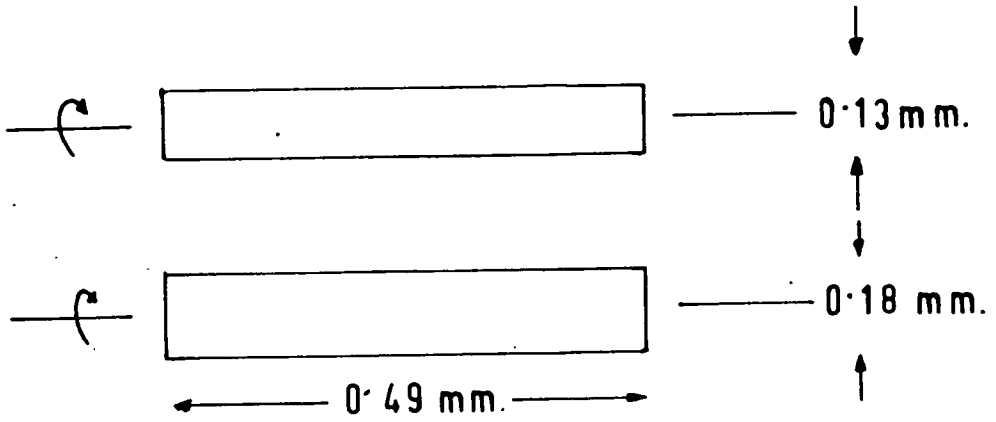
Both 1:2 complexes of divalent cobalt with histidine were obtained in this manner as pink crystals, those obtained from DL-histidine being much larger than those obtained from L-histidine. The crystals, when dry, appeared to be stable to oxygen, and infrared spectra of both complexes in Nujol mull were recorded, no precautions being taken to exclude oxygen from the specimens. The spectrum of the L-histidine complex corresponded to the infrared spectrum of unoxygenated bishistidino cobalt(II) obtained by Sano and Tanabe (35), where the form of histidine was unspecified. An infrared spectrum of the L-histidine complex taken twelve months later proved to be identical in every respect to the original, confirming that no oxygen had been absorbed.

SECTION III

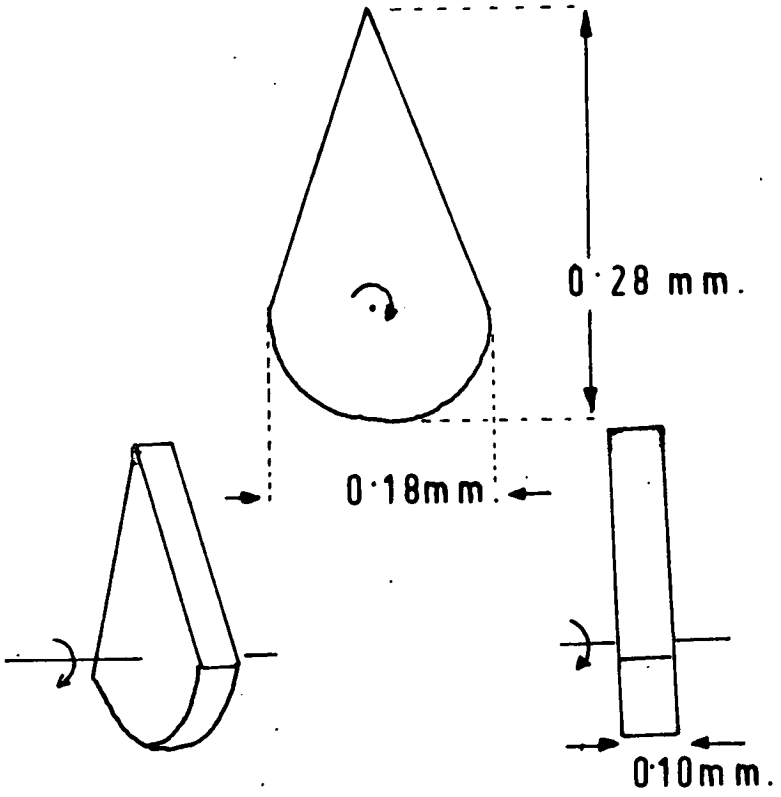
Preliminary Work in The Determination of The Structure of Bis L-histidino Cobalt(II) Monohydrate

The crystals of the complex, prepared several times, were rather small, pink needles, showing extinction of polarised light parallel to the needle axis. One of the largest single crystals (Fig.VIa) was mounted on a glass fibre along the needle axis.

Preliminary oscillation and Weissenberg photographs were taken of this crystal using a Philips PW1010 generator with sealed vacuum X-ray tube and a Unicam non-integrating, equi-inclination Weissenberg camera. The radiation used was copper Kα, the working voltage being 40Kv and tube current 20mA. After the crystal had been set accurately about the needle axis, an oscillation photograph was taken and from the layer lines a reciprocal spacing $d^* = 0{\cdot}185$ was derived. The photograph showed a mirror plane perpendicular to b such that $I(hkl) = I(\bar{h}kl)$. Zero-layer and first-layer photographs about this axis showed further that $I(hkl) \neq I(\bar{h}kl)$. From this evidence it was deduced that the cell was monoclinic. On plotting the reciprocal lattice of the h0l and h1l reflections it was observed that there was face-centring present.



CRYSTAL A FOR b AXIS
(side elevations)



CRYSTAL B FOR c AXIS

FIG VI

Approximate cell dimensions were then calculated by means of Weissenberg and Bernal charts. Values for a^* and c^* were obtained from the zero-layer Weissenberg photograph and b^* from the oscillation photograph about the b axis. A value for β^* was obtained from the zero-layer photograph, by measuring the separation of the axial lines and calibrating this distance by measuring the separation between equivalent reflections 180° apart. The approximate dimensions found thus were:

a^*	=	0·052		a	= 29·6 Å
b^*	=	0·186	giving	b	= 8·3 Å
c^*	=	0·242		c	= 6·37Å
β^*	=	90°		β	= 90°

$\alpha = \gamma = 90°$

The cell volume was therefore found to be 1565Å^3.

The systematic absences were noted and the conditions limiting possible reflections determined. These were:

hkl	reflections	$h + k = 2n$
h0l	••	($h = 2n$)
0k0	••	($k = 2n$)

Thus three possible space groups were indicated: C2, Cm or C2/m. This ambiguity was easily resolved by the nature of/

/of the complex. For either of the space groups Cm or C2/m to be possible, the mirror image of bis L-histidino cobalt(II) would have had to be present. The mirror image, however, is bis D-histidino cobalt(II). As no D-histidine was present in the preparation of the complex and no procedure followed in preparation whereby racemisation could have taken place it was concluded that the space group was in fact C2.

Space group C2 has four equivalent positions $(x,y,z;\ \bar{x},y,\bar{z};\ {}^1/_2+x,{}^1/_2+y,z;\ {}^1/_2+\bar{x},{}^1/_2+y,\bar{z})$ and requires therefore a minimum of four molecules per unit cell, unless special positions are occupied. The density of the complex was determined to be 1·655g/cc by flotation in a mixture of carbon tetrachloride and iodomethane. Assuming therefore that there were four isostructural units in the unit cell, the molecular weight of each was calculated as 390·0. Now the molecular weight of bis-histidino cobalt(II) was calculated as 367·3. It was concluded therefore, that each molecule of the complex included one molecule of water of crystallisation giving a molecular weight of 385·3. This was confirmed by weighing a quantity of the complex and then heating to constant weight at 120°C. The loss in weight found corresponded to a loss of one molecule of water per cobalt ion, confirming that the complex was a monohydrate.

Accurate Cell Dimensions

Accurate cell dimensions were obtained by taking zero-layer Weissenberg photographs about the b axis and the c axis. At either end of each film a powder photograph of copper wire was superimposed. A powder diffractometer trace of the copper wire was recorded on a Philips PW1051 diffractometer to check the purity of the wire, and the d-spacings were found to correspond to those calculated from a cell edge of 3·615Å (41). By measuring the distances between equivalent copper lines on the top and bottom of the films using a travelling microscope and then calculating θ values for each line, assuming a cell edge of 3·615Å, an accurate calibration of each film was obtained.

The copper powder lines recorded were: 111, 200, 220, 311, 222, 400(α_1,α_2), 331(α_1,α_2), 420(α_1,α_2). On plotting d versus θ, where d was the separation of equivalent lines on the film a straight line graph was obtained in each case. Thus the films showed uniform shrinkage and it was decided to interpolate θ values for high-angle reflections from the crystal against the copper line with the nearest θ value. The separations of high angle reflections on the a, b and c axes were then measured with a travelling microscope, θ values deduced by interpolation and their appropriate reciprocal dimensions calculated from

$$d^* = \frac{2\sin\theta}{n}$$

From the h0l Weissenberg β^* was calculated accurately using the new values of a^* and c^* converted to copper $K\alpha_1$ radiation. The θ values of various reflections which were high order with respect to both h and l were calculated as above and the monoclinic formula

$$\cos\beta^* = \frac{(2\sin\theta)^2 - (ha^*)^2 - (lc^*)^2}{2hla^*c^*}$$

was used to calculate β^*:

The cell dimensions thus obtained at room temperature, which was approximately 20°C were as follows:

a	=	29·44	±	0·02Å
b	=	6·347	±	0·005Å
c	=	8·324	±	0·005Å
β	=	90°0′	±	5′
α	=	γ	=	90°

The accurate cell volume was thus calculated as 1555Å^3, giving a value of 1·646g/cc for the density, which compares reasonably well with the measured density of 1·655g/cc.

Linear Absorption Coefficient

The linear absorption coefficient μ was calculated for copper Kα radiation using:

$$\mu = G\Sigma p\mu_m$$

where G = density of the crystal, p = fraction by weight of element, μ_m = mass-absorption coefficient for wavelength under consideration. The value obtained was $89 \cdot 1 cm^{-1}$

Chemical Analysis

A commercial analysis of the L-histidine complex was obtained and the results for cobalt, carbon, nitrogen and hydrogen were as follows.

	Calculated	Observed
Cobalt	15·30%	18·5%
Carbon	37·40%	38·2%
Nitrogen	21·82%	20·3%
Hydrogen	4·67%	4·7%

SECTION IV

Study of The Structure in Two-dimensional Projection

Data Collection and Intensity Measurement

For projection work it was decided that zero-layer photographs about the b and c axes of the crystal would be most informative. The crystal used in the preliminary work (Fig.VIa) was selected for the b-axis zero-layer photographs, and another crystal (Fig.VIb) which did not show the characteristic needle shape but was more suitable than a needle was chosen for the c-axis zero-layer photographs.

For each layer a multiple pack of five Ilford Industrial G films was given a 48 hour exposure with nickel-filtered CuKα radiation at 40Kv and 20mA. A calibrated intensity strip was then prepared using the needle crystal oscillating about the b axis to obtain a series of oscillation photographs of known ratio recorded along a film. This strip was then used to estimate visually, by comparison, the intensities for the h0l and hk0 layers. In cases where high-order reflections were split into their α_1 and α_2 components the intensity of the α_1 reflection was estimated and then increased by 50% to allow for the α_2 reflection. For the c axis intensities both the hk0 and $\overline{hk}0$ intensities were/

/were measured and then averaged to eliminate anomalous dispersion effects (see page40).

Each set of films was scaled internally by calculating the ratio of common reflections in adjacent pairs of films, reflections being chosen for comparison which did not have intensities near the upper or lower limits of the intensity strip on either film. Graphs of these ~~ratios~~ intensities were plotted for each adjacent pair of films and the scaling factor determined by drawing the best straight line through the points. The two sets of films were then scaled to the same relative value by comparison of the h00 reflections which were common to each film.

Intensity Corrections and Absolute Scaling

The intensities were then corrected for the Lorentz and polarisation factors by the chart method of Cochran(4 2). No absorption correction was made at this stage, for, although the linear absorption coefficient of the complex was high, it had not been decided whether or not final refinement would be made using two-dimensional or three-dimensional data.

An absolute scale factor for the corrected intensities was then obtained by Wilsons's statistical method (43). By plotting $\ln(|\overline{F_{obs}}|^2/\Sigma_j^o f^2{}_j)$ calculated for several zones of $\sin^2\theta$, against $\sin^2\theta$, for both the hOl reflections and the hkO reflections separately, it was possible to determine values for the absolute scale factor and the overall temperature factor from the relationship:

$$\ln(|\overline{F_{obs}}|^2/\Sigma_j^o f_j^2) = \ln K - 2B\frac{\sin^2\theta}{\lambda^2}$$

where f_j is the atomic scattering factor for atom type j. This is the equation of a straight line with intercept lnK and gradient $(-2B/\lambda^2)$. K relates the observed, corrected intensities to the absolute intensities thus:

$$|F_{obs}|^2 = K|F_{abs}|^2$$

Hence 1/K was the scale factor to be applied to the corrected intensities to place them on an absolute scale. The following results were obtained:

	hOl reflctns.	hkO reflctns.
1/K	49·5	36·6
B	2·9	1·9

As the plot for the hOl reflections gave a much closer approximation to a straight line than the hkO reflections it was/

/was decided to take the values of 1/K and B as 49 and 3 repectively. Thus the corrected intensities which are better described now as the squared structure amplitudes were obtained on an absolute scale and were then used for the projection work to be described in the next sections.

SECTION V

Determination of The Structure

The Patterson Projection

The first step in the determination of the structure was the computation of a P(x,z) Patterson projection and a sharpened $P^1(x,z)$ projection by means of Beevers-Lipson strips, the summation formula being:

$$P(x,z) = P(U,W) = \frac{2}{A_c} \sum_{o}^{\infty} \sum_{o}^{\infty} [|F(hl)|^2 \cos 2\pi(hU + lW) + |F(\bar{h}l)|^2 \cos 2\pi(hU - lW)]$$

The squared structure amplitudes were sharpened by drawing a curve of $1/\bar{F}^2$ for ranges of $\sin^2\theta$ against $2\sin\theta$ and then multiplying each F^2 value by the value of $1/\bar{F}^2$ read off the curve at the appropriate value of $2\sin\theta$. Space group C2 has a vector set corresponding to C2/m (44) and thus the plane group for the P(x,z) projection is P2, with $c^1 = c$, $a^1 = a/2$, and the projections were therefore calculated over the asymmetric unit ($^1/_4 a \times c$) at intervals of 120ths and 30ths respectively. These projections are shown in Figs VII and VIII.

(010) PATTERSON PROJECTION

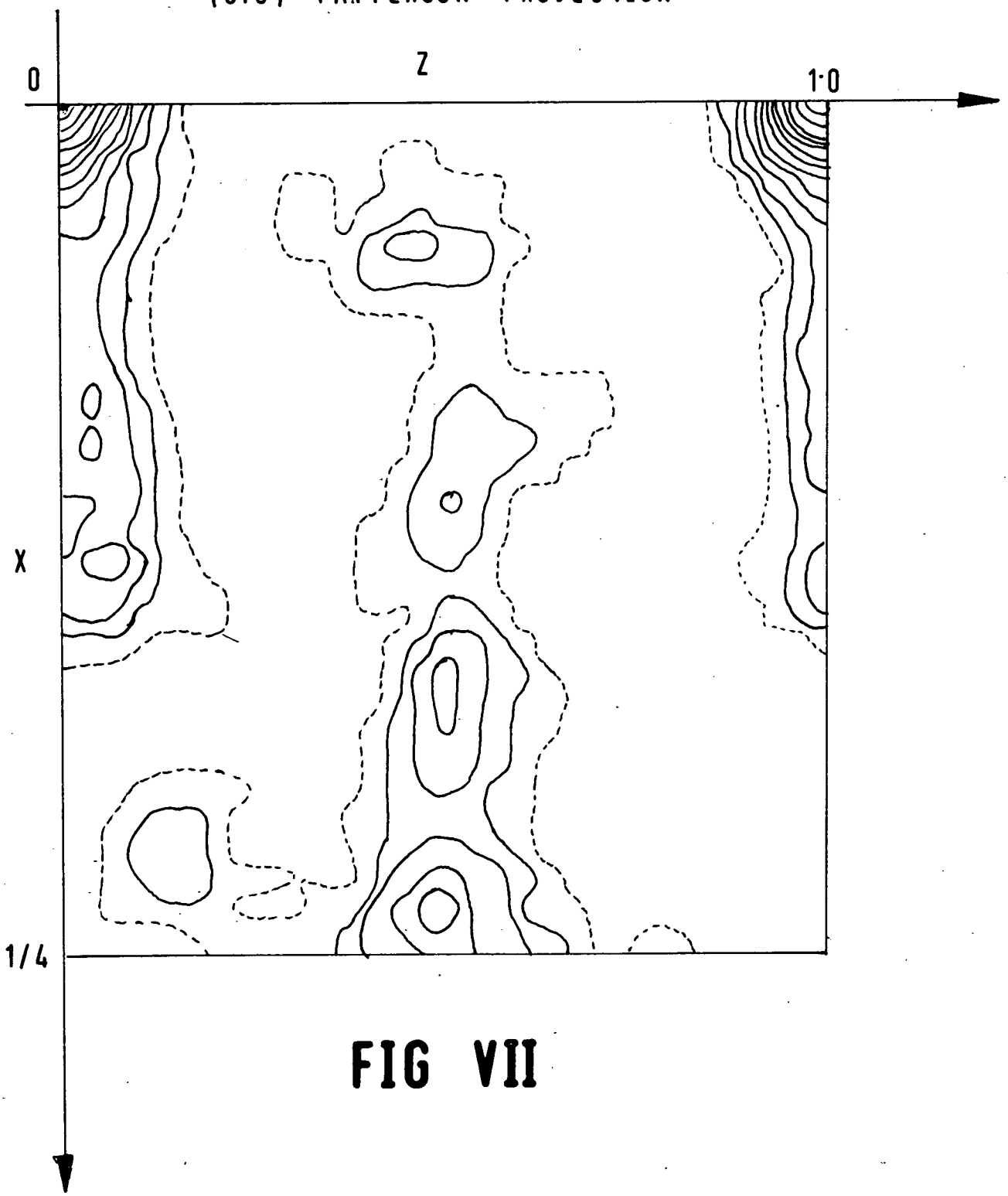


FIG VII

(010) SHARPENED PATTERSON PROJECTION

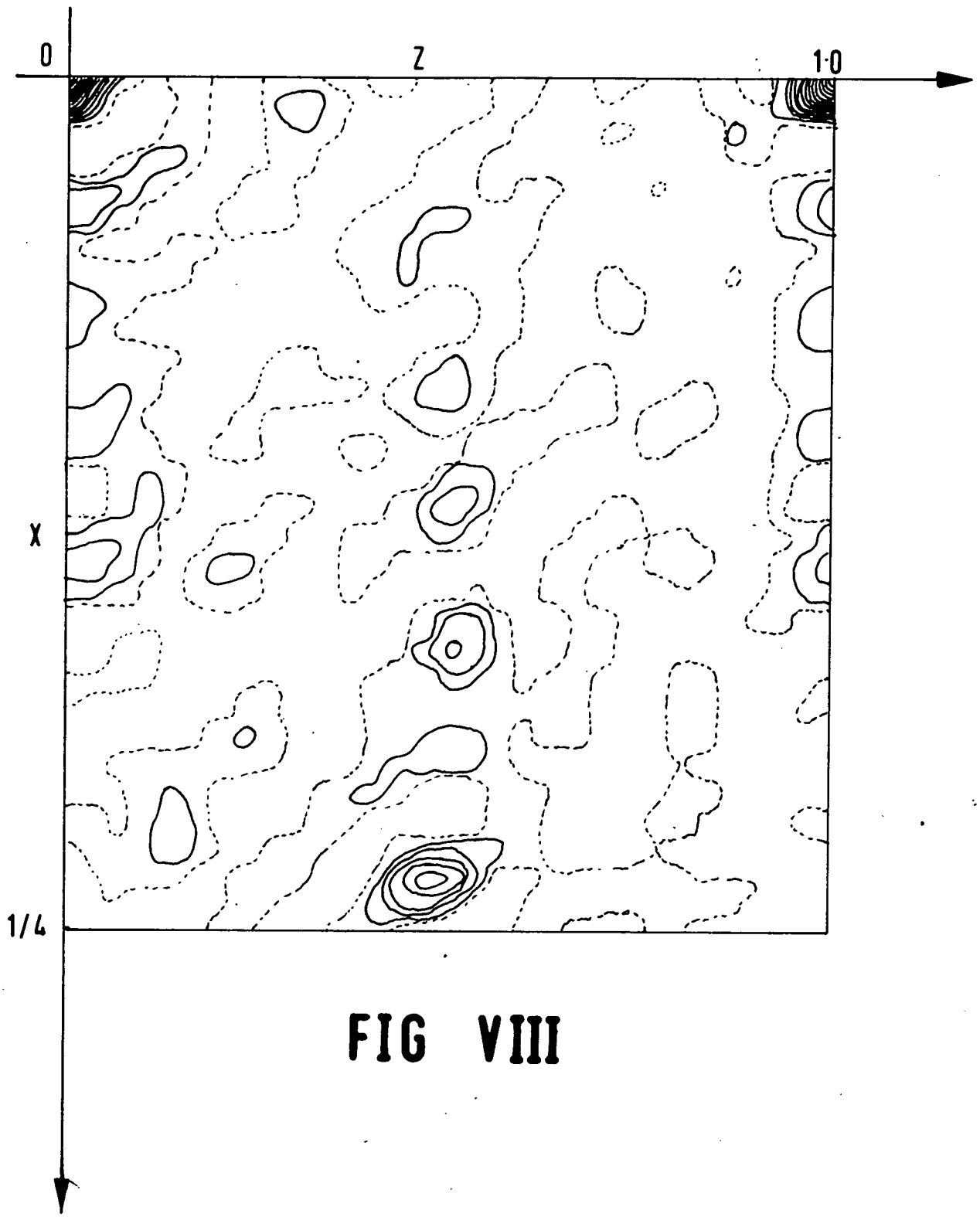


FIG VIII

The largest single vector peak expected on the Patterson projection is that from the cobalt-cobalt vector at (2x,2z) where x and z are the coordinates of the cobalt atom. As well as the peak at (2x,2z) the cobalt-cobalt vector gives the symmetrically related peak at (-2x, -2z) in the P(x,z) projection, corresponding to ($^1/_2$-2x, -2z) for the non-primitive cell. This gives two possible solutions of the cobalt position, there being no means of distinguishing the peaks. In the centrosymmetric projection the two solutions correspond merely to a change of origin but this is not true for the non-centrosymmetric (001) projection. The cobalt-cobalt vector peak was not well resolved on the unsharpened projection, but the sharpened projection showed it very clearly at (28/120, 14/30). From the peak at (28/120, 14/30) the cobalt position was calculated as (14/120, 7/30), the symmetrically related vector peak being (32/120, 16/30) giving an alternative position of (16/120, 8/30). The former position was used in the first two electron density syntheses.

Patterson Superposition

At this point a graphical superposition of the sharpened Patterson projection (Fig.IX) was made by superposing the origin of the projection to the cobalt-cobalt vector peak and thus determining vector peak coincidences. This indicated that the light atoms of the coordinated ligands lay, for the most part parallel to the a axis and at approximately the same z value as the cobalt atom.

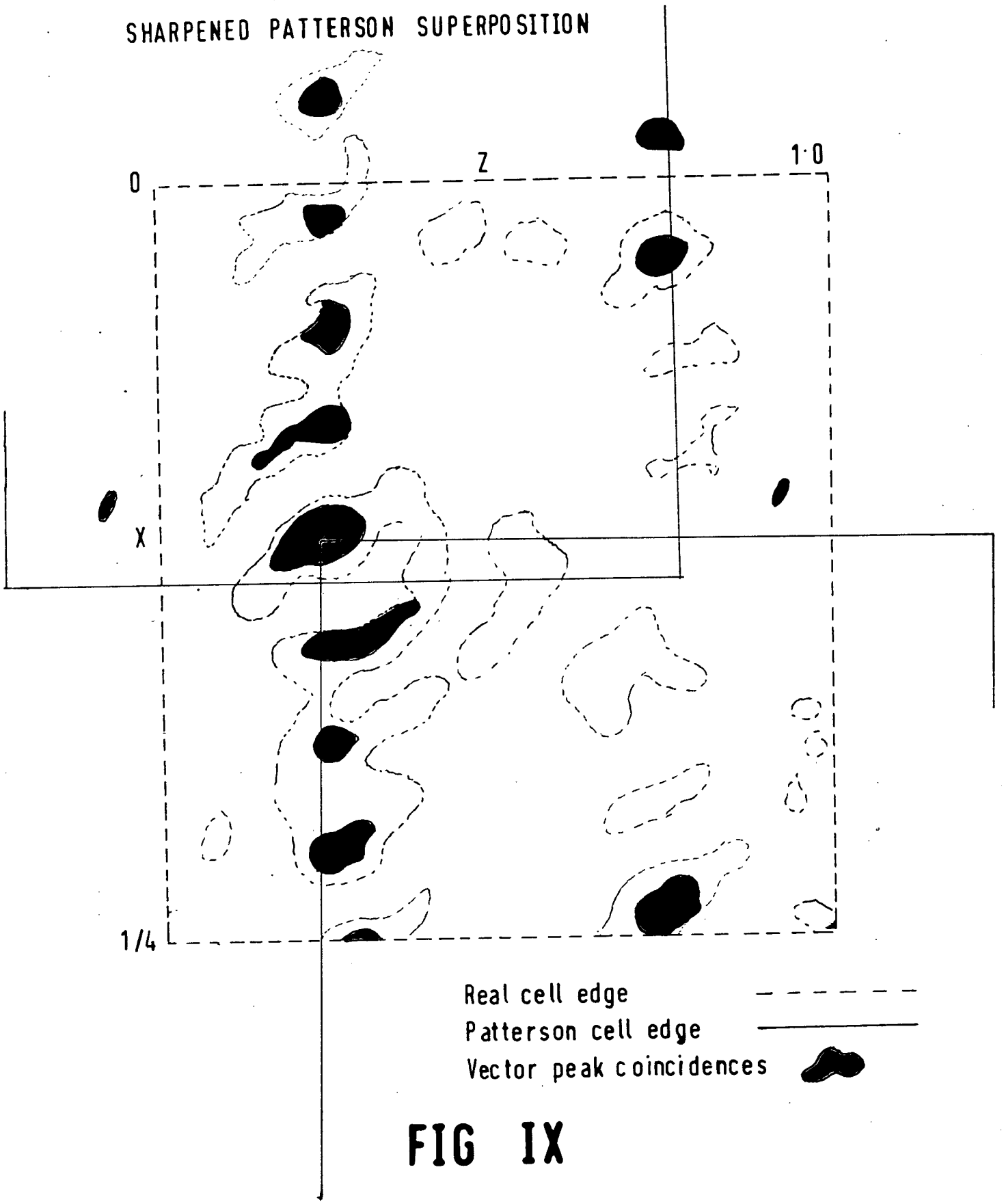

SHARPENED PATTERSON SUPERPOSITION
0
Z
1·0
X
1/4
Real cell edge
Patterson cell edge
Vector peak coincidences

FIG IX

Two-dimensional Electron Density Projections

Structure factors for the hOl reflections were calculated for the cobalt atom at (14/120, 7/30) and the observed structure amplitudes phased accordingly, the structure factor formula being:

$$F_{hOl} = 2\sum_{j=1}^{n} f_j(hOl)\cos 2\pi(hx_j + lz_j)$$

These phased structure amplitudes were then used in the (010) electron density synthesis, using Beevers-Lipson strips and a Hagg-Laurent electro-mechanical analogue machine (45) the synthesis being:

$$\rho(XZ) = \frac{2}{A_c}\sum_{0}^{\infty}\sum_{0}^{\infty}[F(hOl)\cos 2\pi(hX + lZ) + F(\bar{h}Ol)\cos 2\pi(-hX + lZ)]$$

The asymmetric unit ($^1/_2$a x c) was calculated in 120ths and 30ths respectively. The origin in the y direction was set on the cobalt atom and structure factors and thence the (001) projection phased on the cobalt atom was computed on the Manchester Atlas computer by means of a Fourier program (7/5) written by M.M. Harding. The formulæ involved in these calculations are as follows.

$$A = \Sigma f_{j(hk0)} 4\cos^2 2\pi \frac{h+k}{4} \cos 2\pi hx \cos 2\pi ky$$

$$B = \Sigma f_{j(hk0)} 4\cos^2 2\pi \frac{h+k}{4} \cos 2\pi hx \sin 2\pi ky$$

$$|F_{(hk0)}| = \sqrt{(A^2_{(hk0)} + B^2_{(hk0)})} \qquad \tan \alpha_{hk} = B_{hk}/A_{hk}$$

$$\rho(XY) = \frac{2}{A_c}\left\{ \sum_1^{h+2n} |F(h0)| \cos 2\pi hX + \sum_2^{k+2n} |F(0k)| \cos[2\pi kY - \alpha(0k)] + 2\sum_1^{h+2n} \sum_1^{k+2n} |F(hk)| \cos 2\pi hX \cos[2\pi kY - \alpha(hk)] \right\}$$

This projection was calculated over the assymetric unit ($^1/_4a \times {}^1/_2b$) in 120ths and 60ths respectively. Since the observed structure amplitudes were phased on the cobalt atom alone at y = 0, a false mirror plane parallel to a and cutting b at $^1/_2$ was obtained, this giving an ambiguous mirror image for every Fourier peak. (Fig.XI)

On examination of the (001) electron density projection it was discovered that the cobalt peak occurred at (16/120, 0) and it was deduced that the original choice of position for this atom had been incorrect and that the true position was (16/120,y,8/30). The (010) projection (Fig.X) was recontoured with change of origin to give the projection corresponding to the new position and comparable with the (001) projection. (Fig.XII).

(010) ELECTRON DENSITY PROJECTION No. 1

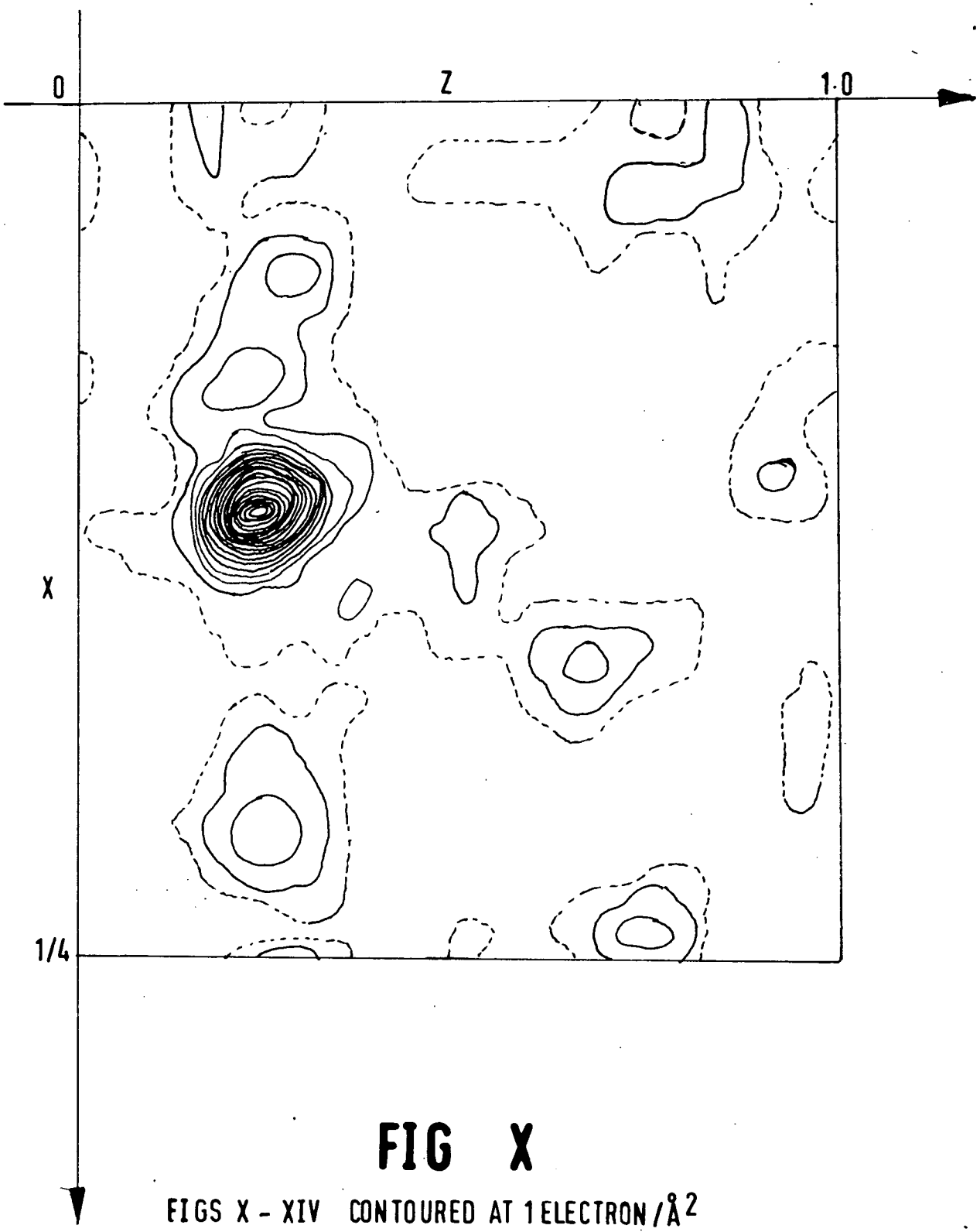


FIG X

FIGS X - XIV CONTOURED AT 1 ELECTRON/Å^2

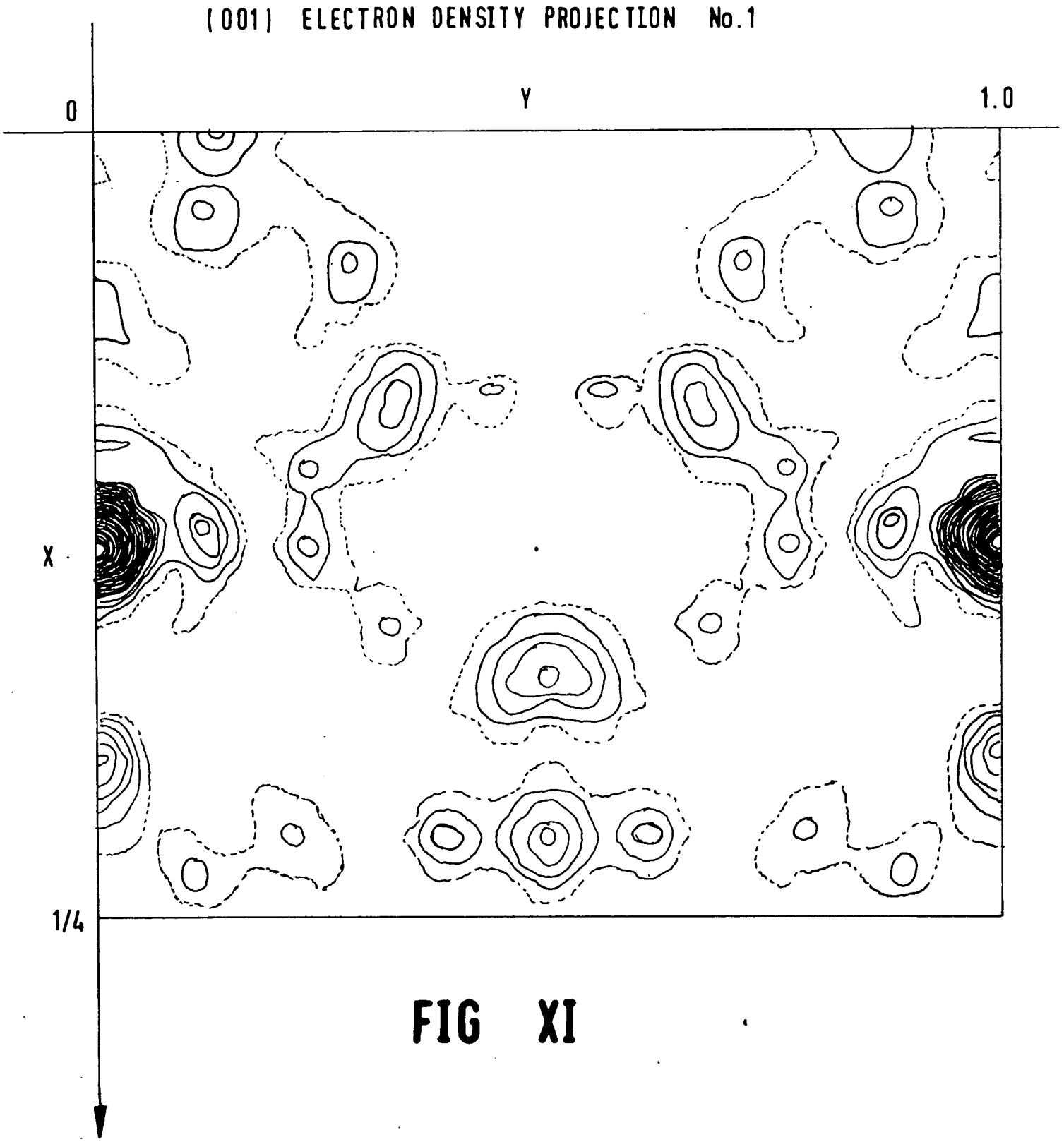


FIG XI

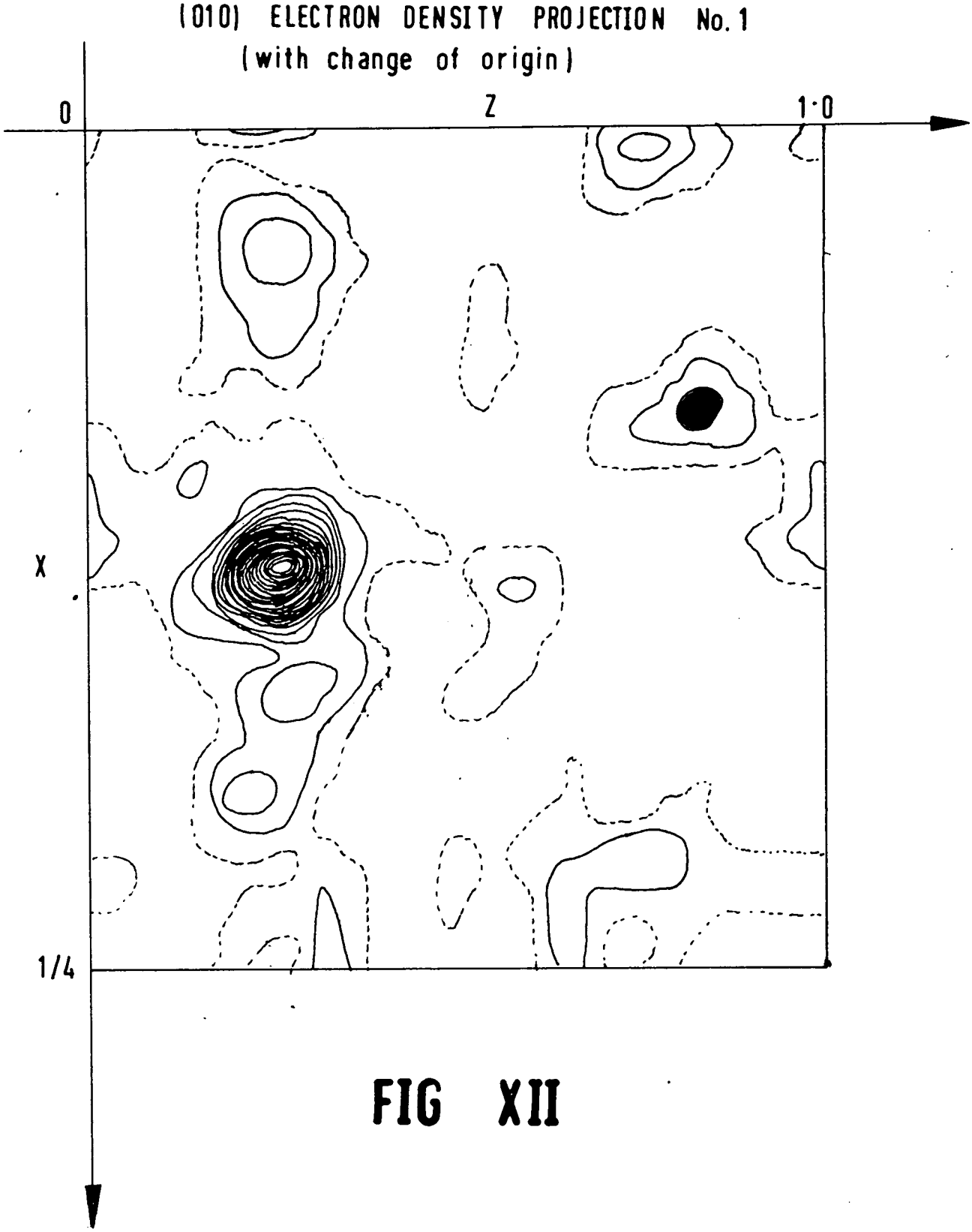

(010) ELECTRON DENSITY PROJECTION No. 1
(with change of origin)
0
Z
1·0
X
1/4

FIG XII

The two projections phased on cobalt alone were drawn on a scale of 2cm = 1Å. A model of the assumed structure of the complex on the same scale was built, fixing the imidazole nitrogen of each histidine residue on opposite sides of the central cobalt atom at distances corresponding to 2Å; the carboxyl and amino groups were not bonded to the cobalt atom but allowed freedom of movement. By superimposing shadows of the model in two beams of parallel light at 90° on to the contoured projections a solution of the structure was determined.

The Trial Structure

It was apparent, particularly from the (010) projection that the major concentration of atoms lay parallel to the a axis at roughly the same y and z values as those of the cobalt atom, this being substantiated in part by the Patterson superposition. The most plausible explanation was that the histidine imidazole rings lay along this line. Also it was noted from the (010) projection that if these peaks were assigned to the imidazole rings then the plane of the ring on the left of the cobalt atom, as seen by the observer (i.e. with the smaller x coordinate) was approximately in the plane of a and b, whereas the plane of the other imidazole ring was more nearly in the plane of a and c. This idea seemed to be substantiated by/

/by the appearance of the peaks on the (001) projection and parallel light projections of the model showed that this was allowable.

The real difficulty was to solve the ambiguous (001) projection. This turned out to be possible by considering the peak at (10/120, -5/30) (marked in red on Fig.XI). Assuming the imidazole rings to be positioned as indicated above, the only atom, other than a water of crystallisation oxygen which could be superimposed on this peak was one carboxyl oxygen of the left-hand histidine residue. Even assuming this to be true did not immediately resolve the ambiguity of the (001) projection, as one could twist the histidine residue through 180° about the imidazole nitrogen - cobalt bond and still achieve the same effect. When, however, the possibility of coordination of the amino and carboxyl groups to the cobalt atom was considered it was discovered that only one particular orientation of the ring would allow this coordination and that the alternative orientation at 180°, although satisfying the electron density syntheses could not do so.

Having fixed the position of one histidine residue, it was relatively simple to obtain a reasonable arrangement for the other. Assuming that both residues coordinated to the/

/the cobalt atom in the same manner, then the amino and carboxyl groups of the second histidine residue had to be coordinated on the opposite side of the cobalt atom from those groups of the first residue, and thus the ambiguous (001) projection was resolved.

In this manner a trial structure was obtained and approximate coordinates for all the atoms, other than the water oxygen were calculated. The projections were now recomputed on the Manchester Atlas, this time phasing on all atoms apart from the water oxygen. (Figs. XIII and XIV). The program used incorporates a device whereby structure factors can be omitted from the electron density synthesis if the value of a calculated structure factor is below a certain fraction of the observed structure amplitude. This fraction can be set at any desired value and here it was set at one third.

The (010) and (001) projections gave residual factors of 0·43 and 0·37, the residual factor R being:

$$R = \frac{\Sigma |F_o| - \Sigma |F_c|}{\Sigma |F_o|}$$

For a centrosymmetric projection a residual of less than 0·5 is a good indication that a trial structure is a reasonable approximation to the true structure, the corresponding residual for a non-centrosymmetric structure being/

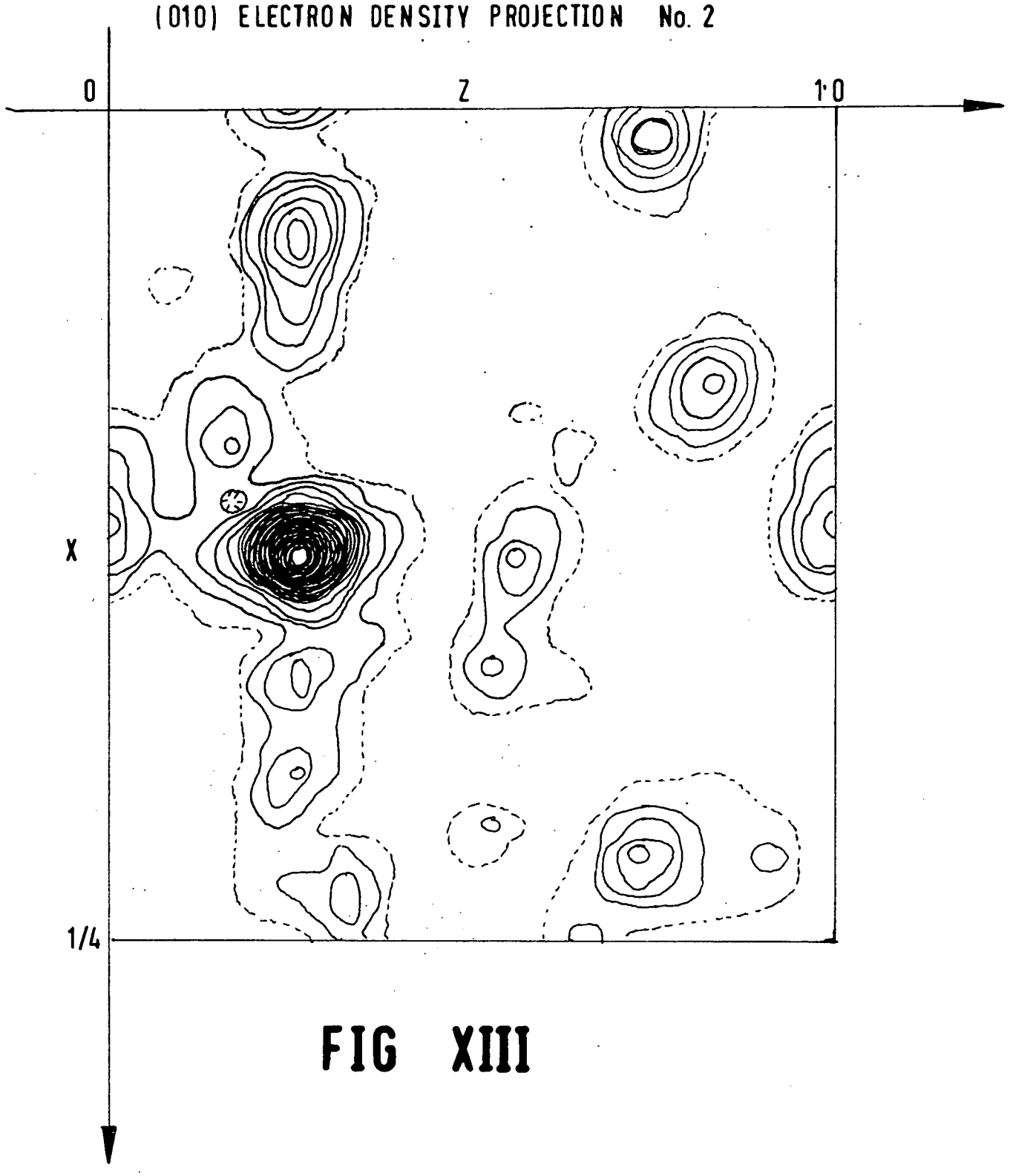


FIG XIII

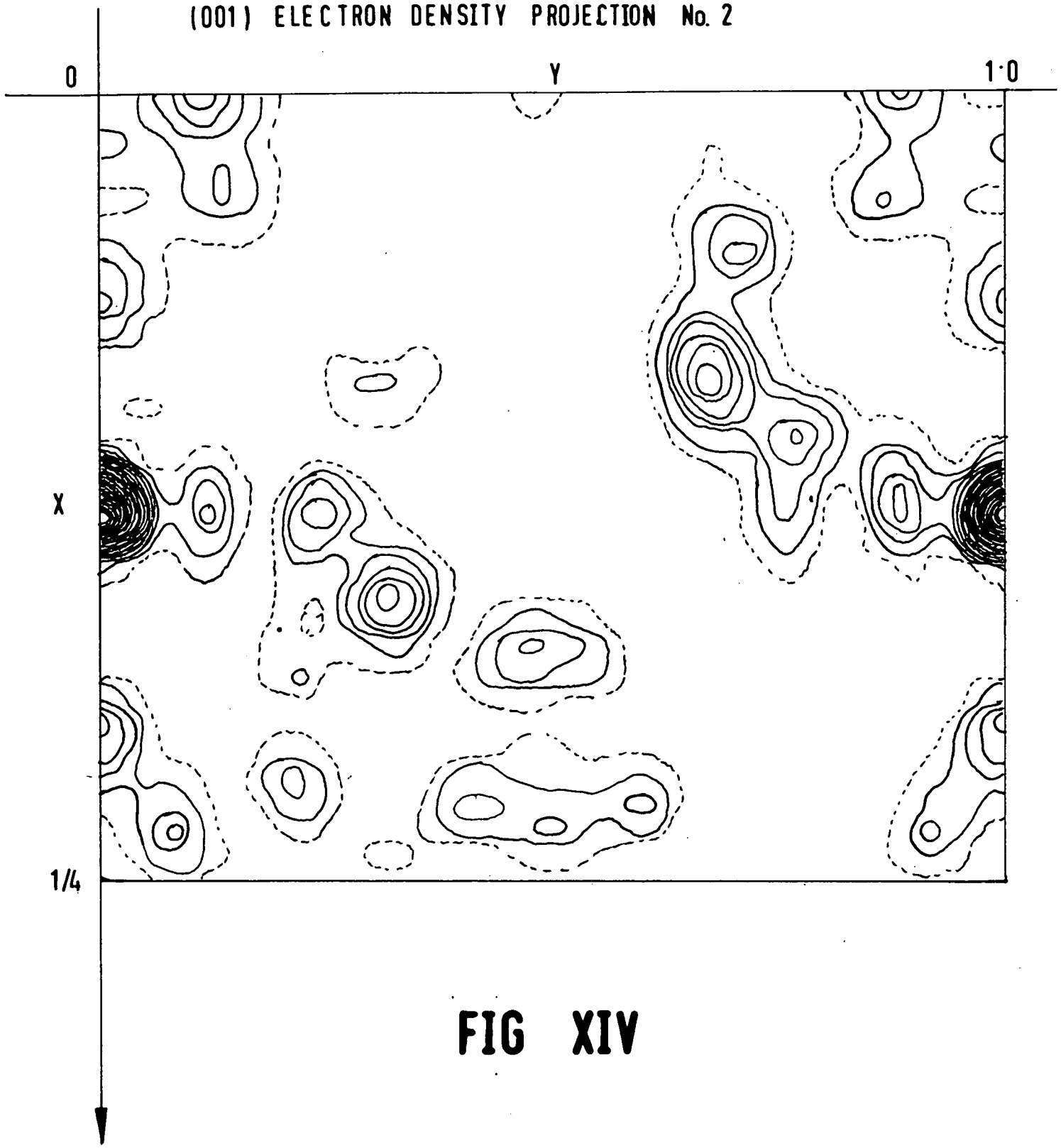


FIG XIV

/being 0·4 (46). The presence of a heavy atom can give a false indication of the correctness of a structure from this viewpoint, but it was nevertheless concluded that the right approach had been made and a difference electron density synthesis for each projection was calculated. These indicated that the structure was basically right but that minor shifts of the atoms were necessary. After making the indicated shifts, the projections and their corresponding difference syntheses were recalculated and the positions of the atoms corrected once more. In cases where the atoms overlapped, their positions were corrected by examination of the difference Fouriers; where the atoms were well resolved, Booth's extrapolation technique (47) was used to locate the centres of the Fourier peaks and these were then used as the new atomic positions.

In this way a series of Fourier syntheses, together with their corresponding difference syntheses, were calculated for each projection, the atomic positions being refined in each case, as well as the temperature and scale factors. A peak indicating the water of crystallisation was well resolved on the third set of electron density syntheses, and the water oxygen was inserted in the following structure factor calculations.

The final residual factors for the (010) and (001) projections were 0·21 and 0·17 respectively. An outline of the progress of the refinement of the projections is shown in the table of residuals given below.

Projection:	(010)	(001)
Synthesis No.	R%	R%
1	43·1	37·3
2	36·8	34·7
3	27·9	29·2
4	24·2	20·2
5	20·7	17·1

Conclusion

It was concluded from the good refinement of the structure, as indicated by the decreasing residuals, that it was in fact correct. However, because of the overlap of many of the peaks in the projections, it was decided that to obtain an accurate structure, three-dimensional refinement would be advisable, if not indeed essential.

SECTION VI

Refinement with Three-dimensional Intensity Data

Introduction

Some three-dimensional data had been collected at this stage using copper radiation. After consideration, however, it was decided not to proceed with the collection of any more copper data but to collect data using molybdenum radiation. There were two factors influencing this decision: the high linear absorption coefficient of the complex in copper Kα radiation and the anomalous dispersion effect obtained from cobalt in copper radiation.

The linear absorption coefficient of the complex in copper Kα radiation is $89{\cdot}0 cm^{-1}$, whereas the coefficient for molybdenum Kα radiation, calculated in the same manner, is only $12{\cdot}0 cm^{-1}$. Any absorption correction would have been made difficult owing to the irregular shape of the crystals, but absorption errors could be reduced considerably by using molybdenum radiation. If the crystals had been somewhat larger than they were it might have been possible to grind them into spheres or cylinders, but large crystals could not be obtained.

Cobalt has an absorption edge at 1·6081Å, very near to the wavelength of copper Kα at 1·5414Å. Friedel's Law(48) states that all crystals diffract X-rays as though there were a centre of symmetry present such that $|F(hkl)| = |F(\bar{h}\bar{k}\bar{l})|$. This law breaks down, however, when an atom in the crystal has an absorption edge at a slightly longer wavelength than the incident X-radiation, so that $|F(hkl)|$ no longer equals $|F(\bar{h}\bar{k}\bar{l})|$. This is the situation we have with cobalt in copper K radiation. It can be seen that the number of reflections that must be measured is automatically doubled when Friedel's Law breaks down, if the work is to be accurate, and although one can determine absolute configuration by means of the anomalous dispersion effect, the absolute configuration of L-histidine is already known. It was not considered, therefore, that anything could be gained by measuring copper radiation data. Further the high background on the copper films due to cobalt fluorescence in copper radiation made accurate intensity measurement difficult.

There are, however, disadvantages in using molybdenum radiation. The first is that the absorption of hard radiations by the crystals of silver halide in photographic film decreases considerably with shorter wavelength, necessitating much longer exposure times to obtain films comparable with their/

/their copper radiation counterparts. A second feature is that the atomic scattering factor curves fall off much more rapidly with sinθ for molybdenum radiation, so that, although one might expect to obtain a larger sphere of reflections, this, for a small crystal at any rate, is not obtained. In fact, because the copper limit occurs on molybdenum films in the region where the Lorentz-polarisation correction factor is about equal to one, the observed intensities near the copper limit are not enhanced as they are in copper radiation, with the result that the fall off in intensity is even steeper than might be expected.

It was, nevertheless, decided that these disadvantages were outweighed by the considerations in favour of using molybdenum radiation and the three-dimensional data was accordingly recorded with this radiation, the wavelength being taken as 0·7114Å.

Data Collection and Intensity Measurement

The three-dimensional data was collected by the Weissenberg method, photographs being taken about the b and c axes of the crystals used in the collection of the two-dimensional data.

For this work a Unicam non-integrating, equi-inclination Weissenberg camera was used in conjunction with a Philips PW1009 generator and a fine-focus molybdenum X-ray tube, working voltage 35Kv, tube current 20mA. The multiple film technique was used in the following manner. For each layer recorded, a pack of three Industrial G films and one Industrial B film was made up, the G films being nearest the crystal. Of this set the second G film served merely as an absorbing screen to cut down the intensity on the third G film and was discarded, after exposure. As Industrial B film has an exposure time approximately three times as long as Industrial G film, no form of absorbing screen was necessary between this and the third G film. This pack of films was given a 72 hour exposure and then another pack consisting of one Industrial G and one Industrial B film was given an exposure of 2 hours 40 minutes to complete the set of five films necessary to obtain all the measurable intensities on the same scale.

Seven layers from (hk0) to (hk6) along the c axis and the zero and first layers of the b axis were recorded in this manner. These photographs were measured by visual estimation against a calibrated intensity strip. Each set of films was calibrated internally by calculating the average film ratio of each adjacent pair of films as in section IV. The intensities were then corrected for the Lorentz-polarisation factor by computer. Finally, the corrected intensities were scaled to the c axis zero-layer intensities by applying the appropriate scale factors calculated from the ratios of reflections common to both the c axis and b axis films. No correction for absorption was made, absorption errors having been minimised by using molybdenum radiation.

Equi-inclination photographs show extension and contraction of reflections for all equi-inclination angles other than zero. All reflections were measured in the extended rather than the contracted form to avoid having a mixture of the two types of spots. The effect is most serious for high equi-inclination angles and low θ values. Since molybdenum radiation was being used , no very high equi-inclination angles were required, but a few low order reflections did show marked extension. It was possible, however to measure these on the b axis films where they were not subject to this effect, and no correction was therefore necessary.

In this way a total of 876 structure amplitudes were obtained for the refinement, and the number of unobserved reflections within the approximate sphere of reflection obtained was estimated to be 472.

Three-dimensional Electron Density Synthesis

The 876 structure amplitudes thus determined were used in the computation of a three-dimensional electron density synthesis phasing on the atomic positions found in the two-dimensional work. The formulae are:

Structure factors:

$$A = 4\Sigma f_{j(hkl)} \cos^2 2\pi\frac{h+k}{4} \cos 2\pi(hx + lz) \cos 2\pi ky$$

$$B = 4\Sigma f_{j(hkl)} \cos^2 2\pi\frac{h+k}{4} \cos 2\pi(hx + lz) \sin 2\pi ky$$

$$F_{(hkl)} = \sqrt{(A^2 + B^2)}$$

$$\tan \alpha_{hkl} = \frac{B}{A}$$

$$\rho(XYZ) = \frac{4}{V_c} \sum\sum\sum \Big\{ |F(hkl)| \cos 2\pi(hX + lZ) \cos[2\pi kY - \alpha(hkl)] + |F(\bar{h}kl)| \cos 2\pi(-hX + lZ) \cos[2\pi kY - \alpha(\bar{h}kl)] \Big\}$$

A residual of 22·0% was obtained and the majority of the atoms were well resolved, only C_{14} and N_{13} being rather poorly resolved from each other. The centres of the peaks were found by Booth's method(47) and it was decided to proceed with the refinement by the method of least squares.

Information regarding the formfactors used will be found in Appendix I, but two features will be pointed out here.

(1) In this and all subsequent calculations the formfactors used for the cobalt ion were corrected for anomalous dispersion.

(2) The two coordinating oxygens O_1 and $O_{1'}$ were treated as O^-.

Least Squares Refinement

Method

The least squares method is used for crystal structure refinement in order to obtain the best atomic parameters possible from the available data. The parameters which can be varied to achieve this are the positional and thermal parameters of the atoms and the scale factor between the observed and calculated structure factors. These parameters can be varied to give different calculated structure factors, which are absolute; it is the observed structure factors which are subject to error and these, once measured, should not be altered except as regards their overall scale factor (obvious mistakes excepted). Least squares refinement can only be employed when the differences between the experimental and calculated structure factors are small and evenly/

/evenly distributed in either direction. In other words a good approximation to the correct structure is necessary before least squares can be used.

To obtain the most probably correct set of parameters by least squares, the sum of the squares of the deviations between the observed and calculated structure factors is minimised, the observations being weighted according to their probable accuracy. Thus the term minimised is

$$R^1 = \sum_{hkl} w(|F_o| - |F_c|)^2$$

where $\sqrt{w}$ is the weight applied to each reflection. One cannot alter F_o as this is fixed and one must accordingly minimise R^1 by altering F_c. For R^1 to be a minimum we must have (49)

$$\frac{\delta R^1}{\delta p_i} = 0 \quad (i = 1,.....n)$$

$$= -\sum_{hkl} 2w\,(F_o - F_c)\,\frac{\delta F_c}{\delta p_i}$$

$$\text{i.e. } \sum_{hkl} w\Delta \frac{\delta |F_c|}{\delta p_i} = 0$$

where $\Delta = F_o - F_c$. Hughes (50) showed that with reasonably good trial parameters approximate equations can be set up in the form

$$\sum_{1}^{n}\left[\frac{\delta F_{hkl}}{\delta x_i}\right]\delta x_i = F_o - F_c = \Delta$$

In this expression $x_1 \ldots x_i \ldots x_n$ are the variable parameters which are to be refined, and there is one such equation for each F term. Writing p for x to include all variable parameters obtained in a three-dimensional structural analysis, this equation can be expanded to the form.

$$\left(\frac{\delta F_i}{\delta p_1}\right) p_1 + \left(\frac{\delta F_i}{\delta p_2}\right)\delta p_2 \cdots + \left(\frac{\delta F_i}{\delta p_i}\right)\delta p_i + \left(\frac{\delta F_i}{\delta p_n}\right) p_n - \Delta f_i = 0$$

there being a corresponding expansion for each term. The least squares solution of these equations is the solution of the normal equations (51). Normalisation with respect to any one unknown is carried out by multiplying each equation of the original set by the coefficient of the unknown and summing the new set. Thus for n variables we obtain a new set of equations:

$$\Sigma\left(\frac{\delta Fi}{\delta p_1}\right)^2\delta p_1 + \Sigma\left(\frac{\delta Fi}{\delta p_1}\right)\left(\frac{\delta Fi}{\delta p_2}\right)\delta p_2 + \cdots \Sigma\left(\frac{\delta Fi}{\delta p_1}\right)\left(\frac{\delta Fi}{\delta p_n}\right)\delta p_n = \Sigma\left(\frac{\delta Fi}{\delta p_1}\right)\Delta$$

$$\Sigma\left(\frac{\delta Fi}{\delta p_2}\right)\left(\frac{\delta Fi}{\delta p_1}\right)\delta p_1 + \Sigma\left(\frac{\delta Fi}{\delta p_2}\right)^2\delta p_2 + \cdots \Sigma\left(\frac{\delta Fi}{\delta p_2}\right)\left(\frac{\delta Fi}{\delta p_n}\right)\delta p_n = \Sigma\left(\frac{\delta Fi}{\delta p_2}\right)\Delta$$

$$\vdots \qquad \vdots \qquad \vdots \qquad \vdots$$

$$\Sigma\left(\frac{\delta Fi}{\delta p_n}\right)\left(\frac{\delta Fi}{\delta p_1}\right)\delta p_1 + \Sigma\left(\frac{\delta Fi}{\delta p_n}\right)\left(\frac{\delta Fi}{\delta p_2}\right)\delta p_2 + \cdots \Sigma\left(\frac{\delta Fi}{\delta p_n}\right)^2\delta p_n = \Sigma\left(\frac{\delta Fi}{\delta p_n}\right)\Delta$$

These equations, normally including the weight w, must be solved for the new values of the parameters, and, since the coefficients change with each change in the parameters, the refinement process must be carried out by cyclic approximation.

A block-diagonal least squares program SFLS DIAMAND/HARDING was available. Least squares refinement can be simplified by the diagonal approximation. This assumes that the off-diagonal terms, which are sums of products, these having an equal probability of being positive or negative, are generally small, whereas the diagonal terms are the sums of squares and thus are large positive terms. The diagonal terms therefore outweigh the off-diagonal terms and refinement can be made by means of the diagonal terms alone. The block diagonal program above uses not just the diagonal terms, which is a severe approximation, but carries out the refinement by computing small matrix blocks along the diagonal. These are the 3x3 matrix for each atomic position, the 6x6 matrix for each atomic vibration, and the 2x2 matrix for the scale factor and overall isotropic temperature factor. Only one weight routine was available for weighting the structure factors. This consisted of a set limit, all structure factors smaller than this limit being given unit weight such that $\sqrt{w} = 1$. Structure factors of higher value were weighted so that

$$\sqrt{w} = \text{limit}/F_o$$

For cobalt histidine structure factors the limit was set at 200. This is not a very good scheme, since very low F values which have a high percentage error are not weighted accordingly, but it was the only scheme available.

The Refinement

There are two values which show the progress of least squares refinement: the residual R and the value $R^1 = \Sigma w\Delta^2$ which is the value being minimised, and thus the most informative. The progress of the refinement is shown in the following table.

Round	R%	$\Sigma w\Delta^2 \times 10^{-4}$	Anisotropic Atoms	Hydrogens
	17·83	1238		
1	15·54	796	Cobalt only	-
2	16·86	910	"	-
3	16·46	1021	"	-
4	14·12	694	All atoms	-
5	13·95	655	Cobalt only	-
6	14·22	679	"	-
7	13·89	640	"	-
8	12·93	554	All atoms	-
9	12·98	544	"	-
10	12·86	522	"	-
11	12·86	525	"	-
12	12·64	511	"	-
13	12·51	505	"	-
14	12·58	502	"	-
15	12·56	497	"	Included
16	12·46		"	"

For the first 330 cycles of refinement all of the atoms excepting cobalt were put in with isotropic temperature factors. The program was altered so that whatever y shift was computed for the cobalt atom this was neglected and the cobalt atom reset to zero for the next cycle.

After the fourth cycle all of the atoms were allowed anisotropic vibration, but although there was a large drop in $\Sigma w\Delta^2$, anomalous effects such as negative temperature factors were obtained. A possible explanation is that some fairly large positional shifts were still required and, as the temperature factor and positional refinements were carried out independently (as outlined above), this could have led to discrepancies in the calculated anisotropic vibrations. The atoms other than cobalt were therefore allowed only isotropic vibrations for the next three cycles. The independent refinement of temperature factors and positional parameters is a disadvantage of this particular block diagonal least squares program. For the final cycles (8 - 16) all atoms were once again allowed anisotropic vibration. Hydrogen positions were calculated and included in the structure factor calculations in the last two cycles, but were not included in the refinement. It would probably have been wiser to include the hydrogen atoms in the structure factor calculations before allowing anisotropic vibrations to their bonded atoms, as the values of the vibrations were probably influenced by their absence. This is also a possible explanation for the very large anisotropic vibrations found for one or two of the light atoms.

On the whole convergence was very slow and it was decided after cycle sixteen that nothing further could be gained by proceeding with the refinement. The positions of the atoms no longer showed any shifts greater than 0·003Å, although in the fourteenth cycle shifts of up to 0·02Å were still being made. The least squares refinement was therefore concluded at this point giving the structure a final residual of 12·5%.

Final Three-dimensional Electron Density Syntheses

Two three-dimensional difference Fouriers were computed with the parameters obtained from the last cycle of least squares, one including the hydrogen atoms, the other omitting them. The residual of the latter was 12·8%. The only feature of any note on the difference maps was a positive region of electron density around the cobalt atom of up to two electrons per cubic angstrom. This was not isotropic but mainly in the y-z plane, possibly indicating that the anisotropic thermal parameters for cobalt were not as good as they might be, and it may be that the atomic scattering curve used for the cobalt ion could have been improved upon. The maps were otherwise rather featureless and it was not possible to pick out the hydrogen atoms with any degree of certainty, but this was hardly unexpected in the presence of cobalt.

Unobserved Reflections

The unobserved reflections, other than systematic absences, were each given an intensity value equal to half the minimum observable and these were corrected for the Lorentz and polarisation factors and put on the same scale as the observed intensities. They were then included in a final structure factor calculation giving an increase in the residual to 20·3%. On inspection it was seen that only a small proportion of the reflections had calculated structure factors greater than the minimum observable, although several were slightly greater than twice the minimum observable.

A table of the unobserved reflections with assigned values and calculated structure factors is given in Appendix No. III. The observed and calculated structure factors are given in Appendix No. II.

Scale Factor

The final scale factor K, where $\underline{KF}_o = Fc$ was 31·377.

SECTION VII

Results

Atomic Parameters and Temperature Factors

The following parameters were obtained for ~~cobalt(II)~~ cobalt(II) bis-(L-histidino)$_4$ monohydrate from the final least squares refinement cycle. The atomic positions are given in fractional coordinates.

Atom	x	y	z	$\sigma(x)\times10^5$	$\sigma(y)\times10^4$	$\sigma(z)\times10^4$
Co	0·13276	0·0000	0·2705	11	8	5
C1	0·16227	0·3258	0·3154	63	22	33
C2	0·17169	0·2501	0·5333	56	22	26
C3	0·22359	0·1937	0·5329	60	25	30
C4	0·23596	0·1024	0·3429	63	26	29
C5	0·22660	-0·0521	0·0764	72	28	27
C6	0·27710	0·1024	0·2371	64	27	35
C11	0·10577	-0·2428	-0·0205	55	20	25
C12	0·09366	-0·3152	0·1964	52	24	30
C13	0·04276	-0·2738	0·2626	61	23	25
C14	0·02937	-0·1104	0·2590	59	28	28
C15	0·03754	0·1643	0·2652	79	44	40
C16	-0·01265	-0·0350	0·2407	58	38	24
N1	0·13950	0·1176	0·5768	58	23	22
N2	0·20650	0·0186	0·2314	42	20	23
N3	0·27130	0·0059	0·0671	41	27	22
N11	0·12547	-0·2448	0·3524	52	19	27
N12	0·05940	0·0260	0·2687	51	21	20
N13	-0·00760	0·1299	0·2468	47	24	25
O2	0·17948	0·4623	0·2868	47	16	22
O12	0·09334	-0·3228	-0·1743	48	18	21
O1	0·13554	0·2508	0·2102	42	21	22
O11	0·12766	-0·1120	-0·0288	39	19	19
O3(H_2O)	0·07997	0·3451	-0·1610	43	18	22

The anisotropic thermal parameters for each atom are given below the temperature factor of each atom being given by $\exp^{-(h^2\beta_{11} + k^2\beta_{22} + l^2\beta_{33} + kl\beta_{23} + lh\beta_{31} + hk\beta_{12})}$

Atom	$\beta_{11}\times10^5$	$\beta_{22}\times10^5$	$\beta_{33}\times10^5$	$\beta_{23}\times10^5$	$\beta_{31}\times10^5$	$\beta_{12}\times10^5$
Co	49	983	2037	-370	-31	13
C1	75	295	3577	856	472	-31
C2	77	597	1640	825	65	62
C3	51	1159	2223	1980	-31	-259
C4	81	1324	1815	1714	-294	-414
C5	137	1954	903	542	16	0
C6	79	1511	3598	3596	154	-672
C11	68	282	1429	1854	182	-5
C12	22	1040	2993	2056	503	-308
C13	97	887	865	-659	253	-9
C14	65	1738	1650	1652	-161	-143
C15	82	3714	3664	3259	-445	-142
C16	76	3649	513	-544	150	-32
N1	123	1434	1979	-845	-175	-23
N2	49	580	3259	-2447	-268	525
N3	45	1321	2984	-2393	34	348
N11	83	456	3656	2225	-197	-91
N12	114	1362	1341	1902	-190	-252
N13	43	1449	2301	215	-71	38
O2	124	1007	3279	1994	-71	49
O12	111	1168	2309	-432	-224	234
O1	46	2014	3244	-2069	-326	46
O11	61	1628	1641	-605	58	198
O3(H_2O)	66	1158	3105	206	-118	110

Listed in the following table are the B_{ij} derived from the β_{ij} values as suggested by Cruickshank (52) and analagous to the isotropic temperature factor. The values given are the B_{ij} for each atom and also B the mean value of B_{11}, B_{22} and B_{33}. The B_{ij} values were calculated from $B_{12} = 4\beta_{12}/a^*b^*$, etc.

Atom	B_{11}	B_{22}	B_{33}	B_{23}	B_{31}	B_{12}	B
Co	1·70	2·72	3·28	-0·78	-0·23	0·13	2·57
C1	2·60	0·82	5·76	1·81	3·53	-0·30	3·06
C2	2·67	1·63	2·64	1·74	0·49	0·61	2·32
C3	1·77	3·21	3·58	4·18	-0·23	-2·54	2·85
C4	2·81	3·67	2·92	3·62	-2·20	-4·06	3·13
C5	4·75	5·42	1·45	1·15	0·12	0·00	3·87
C6	2·74	4·19	5·80	7·60	1·15	-6·59	4·24
C11	2·36	0·78	2·30	3·92	1·36	-0·05	1·81
C12	0·76	2·88	4·82	4·35	3·76	-3·02	2·82
C13	3·36	2·46	1·39	-1·39	1·89	-0·09	2·40
C14	2·25	4·82	2·65	3·49	-1·20	-1·40	3·24
C15	2·84	10·29	5·90	6·89	-3·33	-1·39	6·34
C16	2·63	10·11	0·83	-1·15	1·12	-0·31	4·52
N1	4·26	3·97	1·58	-1·79	-1·31	-0·23	3·27
N2	1·70	1·61	5·25	-5·17	-2·00	5·15	2·85
N3	1·56	3·66	4·81	-5·06	0·25	3·41	3·34
N11	2·88	1·26	5·89	4·70	-1·47	-0·89	3·34
N12	3·95	3·77	2·16	4·02	-1·42	-2·47	3·29
N13	1·49	4·02	3·71	0·45	-0·53	0·37	3·07
O2	4·30	2·79	5·28	0·41	-0·53	0·48	4·12
O12	3·85	3·24	3·72	-0·91	-1·67	2·29	3·60
O1	1·59	5·58	5·23	-4·37	-2·44	0·45	4·13
O11	2·11	4·51	2·64	-1·27	0·43	1·94	3·09
O3	2·29	3·21	5·00	0·44	-0·88	1·08	3·50

Finally the calculated hydrogen positions are given below, together with the assigned temperature factor, this being the isotropic temperature factor of the atom to which the hydrogen is bonded. These B values were determined after the fourteenth cycle of least squares refinement.

Atom	on Atom	x	y	z	B *
H1	C2	0·1662	0·331	0·670	2·46
H2	N1	0·1117	0·113	0·660	3·36
H3	N1	0·1525	0·030	0·677	3·36
H4	C3	0·2336	0·125	0·672	2·91
H5	C3	0·2452	0·290	0·586	2·91
H6	C5	0·2101	-0·139	-0·035	3·89
H7	N3	0·2937	-0·029	-0·042	3·31
H8	C6	0·3075	0·166	0·291	4·38
H9	O3	0·0986	0·317	-0·036	3·61
H10	O3	0·0850	0·468	-0·167	3·61
H11	C12	0·0975	-0·443	0·200	2·81
H12	N11	0·1546	-0·304	0·386	3·33
H13	N11	0·1067	-0·283	0·470	3·33
H14	C13	0·0366	-0·320	0·425	2·43
H15	C13	0·0173	-0·332	0·162	2·43
H16	C15	0·0556	0·285	0·279	3·06
H17	N13	-0·0328	0·210	0·234	2·94
H18	C16	-0·0456	-0·100	0·226	4·29

* Accuracy of the hydrogen temperature factors to three significant figures is not claimed. These are the calculated values for the light atoms.

Bond lengths and Angles

For speed and accuracy the bond lengths and angles were calculated by computer using a program of O. S. Mills. Standard deviations of the bond lengths were calculated by hand from the positional variances obtained from the least squares program and then checked by a program written by the author. Covariances were ignored and the formula used was: (53)

$$\sigma^2(l) = [\sigma^2(x_1)+\sigma^2(x_2)]\cos^2\alpha + [\sigma^2(y_1)+\sigma^2(y_2)]\cos^2\beta + [\sigma^2(z_1)+\sigma^2(z_2)]\cos^2\gamma$$

(l) = bond length

$\cos\alpha = \frac{x_2 - x_1}{l}$ etc.

Bond	Bond Length Å	e.s.d.	Difference between equivalent bonds.
Co - N1	2·186	0·015	
Co - N11	2·114	0·017	0·072
Co - N2	2·191	0·013	
Co - N12	2·171	0·016	0·020
Co - O1	2.124	0·019	
Co - O11	2·122	0·014	0·002
C1 - C2	1·545	0·028	
C11- C12	1·544	0·025	0·001
C1 - O2	1·257	0·023	
C11- O12	1·238	0·022	0·019
C1 - O1	1·206	0·024	
C11- O11	1·266	0·024	0·060
C2 - C3	1·598	0·024	
C12- C13	1·594	0·024	0·004
C2 - N1	1·480	0·025	
C12- N11	1·483	0·024	0·003
C3 - C4	1·471	0·027	
C13- C14	1·416	0·030	0·055
C4 - C6	1·385	0·027	
C14- C16	1·392	0·030	0·007
C4 - N2	1·319	0·024	
C14- N12	1·441	0·027	0·122
C5 - N2	1·290	0·024	
C15-N12	1·319	0·038	0·029
C5 - N3	1·403	0·026	
C15- N13	1·365	0·028	0·038
C6 - N3	1·356	0·028	
C16- N13	1·381	0·038	0·025

The bond angles obtained were as follows:

Bond	∠°
N2 - Co - N1	88·8
N2 - Co - N11	101·3
N2 - Co - O1	82·7
N2 - Co - O11	90·0
N12- Co - N1	92·9
N12- Co - N11	89·8
N12- Co - O1	86·5
N12- Co - O11	88·2
N1 - Co - N11	102·9
N11- Co - O11	77·9
O11- Co - O1	105·9
O1 - Co - N1	73·5

Bond	∠°	Bond	∠°
O1 - C1 - O2	130·6	O11 - C11 - O12	125·5
C2 - C1 - O1	113·7	C12 - C11 - O11	119·4
C2 - C1 - O2	115·2	C12 - C11 - O12	115·1
C1 - C2 - N1	110·9	C11 - C12 - O12	107·2
C1 - C2 - C3	106·9	C11 - C12 - C13	111·6
N1 - C2 - C3	113·2	N11 - C12 - C13	109·4
C2 - C3 - C4	112·9	C12 - C13 - C14	117·7
N2 - C4 - C3	123·4	N12 - C14 - C13	125·9
C5 - C4 - C6	127·9	C13 - C14 - C16	133·0
N2 - C4 - C6	108·4	N12 - C14 - C16	101·2
C4 - C6 - N3	106·0	C14 - C16 - N13	110·5
C6 - N3 - C5	106·8	C16 - N13 - C15	108·4
N3 - C5 - N2	107·8	N13 - C15 - N12	107·0
C5 - N2 - C4	110·4	C15 - N12 - C14	112·8

Hydrogen Bonds

A total of eight hydrogen bonds were found and are listed below.

		Å
N1 —— H·····O11	(x, y, z + 1)	3·17
N1 —— H·····O3	(x, y, z + 1)	3·07
N11—— H·····O9	(x, y - 1, z)	2·94
N11—— H·····O12	(x, y, z + 1)	3·22
N3 —— H·····O2	($^1/_2$ - x, $^1/_2$+y-1, -z)	2·70
N13—— H·····O3	(-x, y, -z)	2·84
O1····· H—— O3	(x, y, z)	2·97
O12·····H—— O3	(x, y - 1, z)	2·79

The upper limit for hydrogen bonds of the type O—— H ····· O has been suggested as 3·2A (54). The average magnitude of hydrogen bonds of the type N—— H····· O for amides and amines are 2·93A (e.s.d. 0·10A) and (3·04A (e.s.d 0.13A) respectively. Thus all the hydrogen bonds tabulated above are within the limits of hydrogen bonding and can be assumed to be true hydrogen bonds.

Molecular Geometry

A molecular geometry program was available by means of which best planes, projections along bonds and dihedral angles could be calculated. The following projections were calculated/

/calculated using this program; their results will be used in the discussion (Section VIII).

The best planes of (1) C3, C4, C5, C6, N2, N3.
(2) C13, C14, C15, C16, N12, N13.
(3) Co, O1, O11, N1, N11.
(4) O1, O11, N1, N11.
(5) N1, C1, C2, O1, O2.
(6) N11, C11, C12, O11, O12.

Eight dihedral angles were also calculated and these are listed below, the dihedral angle being the angle between the plane of the first three atoms and the plane of the last three.

	Dihedral angle ∠°
O1 C1 C2 C3	-113·9
O11 C11 C12 C13	-98·8
C1 C2 C3 C4	47·9
C11 C12 C13 C14	53·0
C2 C3 C4 N2	26·8
C12 C13 C14 N12	21·5
Co N1 C2 C3	81·3
Co N11 C12 C13	81·0

SECTION VIII

Discussion of Results

Description of the Structure

The complex is octahedral with coordination from the amino group, the carboxyl group and the imidazole nitrogen of each histidine residue, with the coordinating amino nitrogens and also the coordinating oxygens in adjacent positions (Fig.XV).

There are however some deviations from strict octahedral symmetry. The angles O11-Co-O1 and N1-Co-N11 are 106° and 103° whilst the N1-Co-O1 and N11-Co-O11 angles are only 74° and 78° as shown in Fig.XXII(a). This deviation arises from the formation of a five-membered ring by the coordination of the amino and carboxyl groups of a histidine molecule to the central atom, whereas the other coordination rings formed are six-membered. The atoms N2, Co and N12 do not lie on a straight line but give an angle about the cobalt atom of 168·1° (Fig.XVI). The explanation for this probably lies in the different environments of the two histidine residues and their different hydrogen bonding arrangements (see also page 70).

The two coordinating histidine residues are not identical in conformation, The main difference lies in a rotation about the C1-C2 and C11-C12 bonds as shown by the angle between the planes of O1, C1, C2 and C1, C2, C3 and the corresponding angle of the other histidine residue which are 113·9° and 98·8° respectively, differing by 15°. This can probably be explained in terms of the differing hydrogen bonding arrangements (see page 71).

Bond Lengths

The mean cobalt-nitrogen bond length obtained is 2·17Å, there being no significant difference between the cobalt - imidazole-nitrogen bond lengths and the cobalt - amino-nitrogen bond lengths, although the Co-N11 bond does seem somewhat shorter than the others. This value of 2·17 compares quite favourably with the Co-N bond lengths ranging from 1·92 to 2·30Å as shown in Table I.

The mean cobalt-oxygen bond length is 2·12A which is also in good agreement with other determinations which range from 2·01 to 2·18Å, neglecting carbon to water-oxygen bonds. (Table I)

In the structure single carbon-carbon bonds have a mean of 1·53Å, the carbon - carboxyl-oxygen bond lengths have a mean of 1·24Å, and the carbon - amino-nitrogen bonds have a mean of 1·48Å. These values can be compared with calculated bond lengths from covalent radii (55) as follows:

Bond	Calculated	Found
C-C	1·54	1·53
C-N	1·47	1·48
C-O(carboxyl)	1·29	1·24

All these values are quite comparable. It must be noted at this point that there is no significant difference between the bonds of the type C - O1⁻ and C - O2, and it is probably correct to assume that the negative charge is not concentrated on the coordinating oxygen but rather spread over both oxygens of the carboxyl group. It would therefore probably have been more accurate if the scattering factors for the carboxylic oxygens had been averaged to $O^{-1/2}$ for each oxygen, the method adopted by Harding and Cole (37) and Fraser (40).

The Imidazole Rings

The bond lengths obtained for the imidazole rings can be best compared with those obtained for imidazole itself whose/

/whose structure at -150°C was recently determined (56). Note that the nomenclature is different.

Imidazole Bonds	Å	cpdg. to	Mean of $Co(hist)_2$	Å
N1-C2	1·35	→	N3-C5	1·38
C2-N3	1·33	→	C5-N2	1·30
N3-C4	1·38	→	N2-C4	1·38
C4-C5	1·36	→	C4-C6	1·39
C5-N1	1·37	→	C6-N3	1·37

Imidazole Bond Angles	°		$Co(hist)_2$ Mean Angles	°
N1-C2-N3	111	→	N3-C5-N2	107
C2-N3-C4	105	→	C5-N2-C4	112
N3-C4-C5	110	→	N2-C4-C6	105
C4-C5-N1	106	→	C4-C6-N3	108
C5-N1-C2	107	→	C6-N3-C5	107

As can be seen there are no significant differences between the bond lengths although the bond angles involving N2 are somewhat different, possibly due to the coordination of N2 to cobalt.

The Histidine Residues

It is also worthwhile to compare the bond lengths found for the two histidine residues with those found in the structure determination of L-histidine hydrochloride (5).

Bond	Histidine HCl Å	Mean Co(hist)$_2$ Å
C1-C2	1·53	1·54
C2-C3	1·53	1·60
C3-C4	1·51	1·44
C4-C6	1·36	1·39
C6-N3	1·36	1·37
N3-C5	1·31	1·38
C5-N2	1·32	1·30
N2-C4	1·39	1·38
C1-O1	1·24	1·24
C1-O2	1·27	1·25
C2-N1	1·50	1·48

There are no really significant differences here but the position of C3 in the complex seems to be somewhat different giving a short C3-C4 bond and an increased C2-C3 bond. Reference to the table of bond lengths (page 57) will show that this is true for both histidine residues, although there is no reasonable explanation for this.

On comparison of the bond lengths of the two histidine residues of the complex itself (page 57), it is found that the agreement is good with one seeming exception. The bond length of C4-N2 is 1·319Å, the bond length of C14-N12 is/

/is 1·441Å, giving a difference of 0·122Å. However, on averaging the two a mean of 1·38Å is obtained, which compares very well with the value of this bond in imidazole and in histidine hydrochloride. Also the bonds differ from their mean by less than three times the standard deviation. It is concluded therefore that this difference is not significant but within the range of experimental error. It is also worth noting at this point that the e.s.d.'s of the bond lengths are probably underestimations because the inter-atomic interactions in the full matrix are ignored in the block-diagonal approximation.(49)

Anisotropic Vibrations

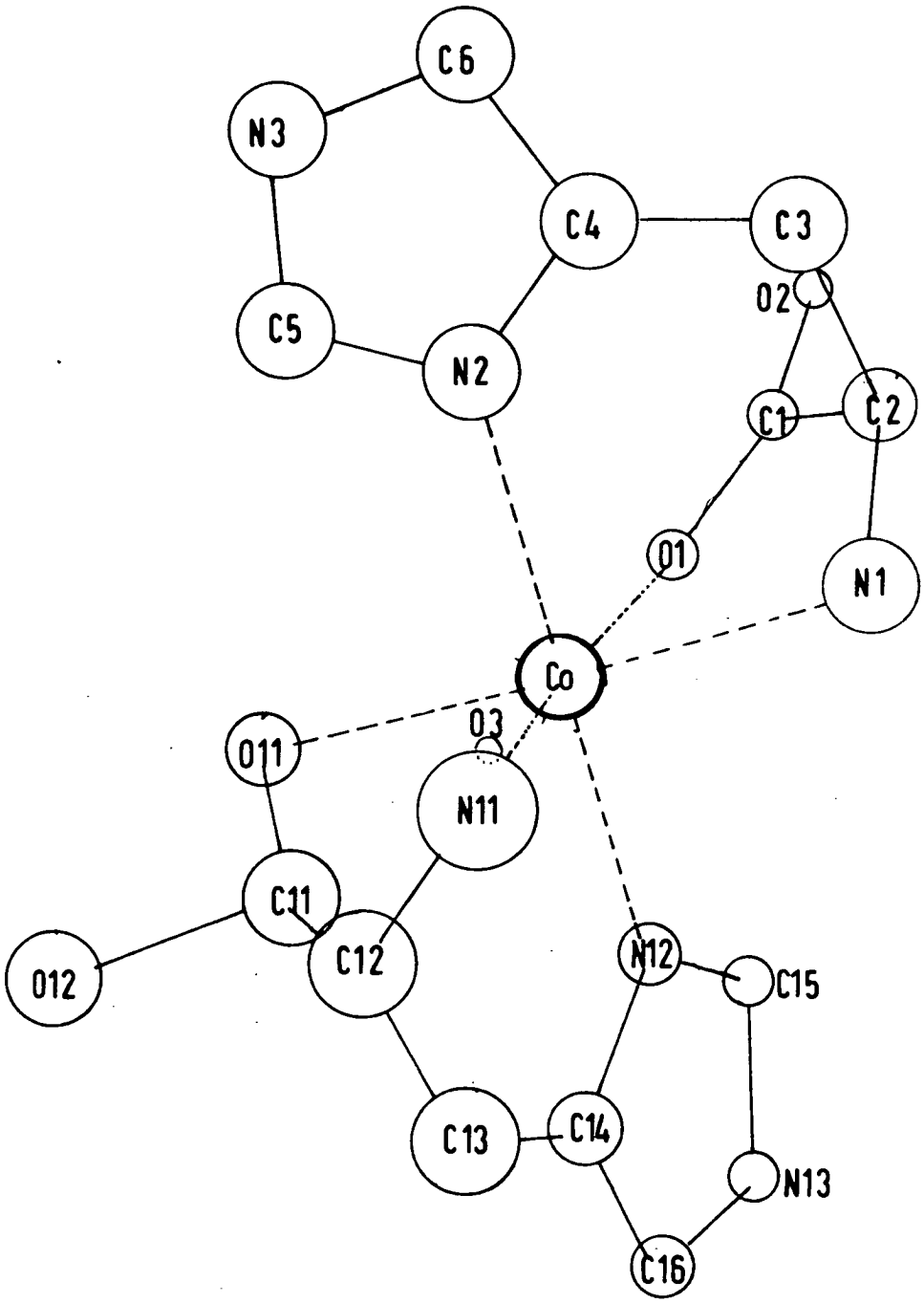
A study of the table of B_{11}, B_{22}, and B_{33} reveals that some of the atoms show very marked differences in vibration. In particular the atoms C15 and C16 show an extremely large vibration in the y direction which is difficult to explain. One would expect N13 to show a vibration of approximately the same magnitude if this were a rigid body vibration of the ring but this is not found. On examination of the positional variances it was found that $\sigma^2(y)$ for these two atoms was greater by a factor of two than those of the other atoms. Thus it is probable that the best y parameters of/

/of these atoms had not been obtained and the small shifts recorded in the final cycle gave a false indication of their accuracy. Some of the variation in anisotropic vibration can be presumed to have arisen through allowing the light atoms anisotropic vibration before including the hydrogen atoms in the structure factor calculations. Other sources from which differences in vibration can arise are errors in the observed data due to absorption, extinction and the fact that it was not completely isotropic.

Hydrogen Bonding and Molecular Packing

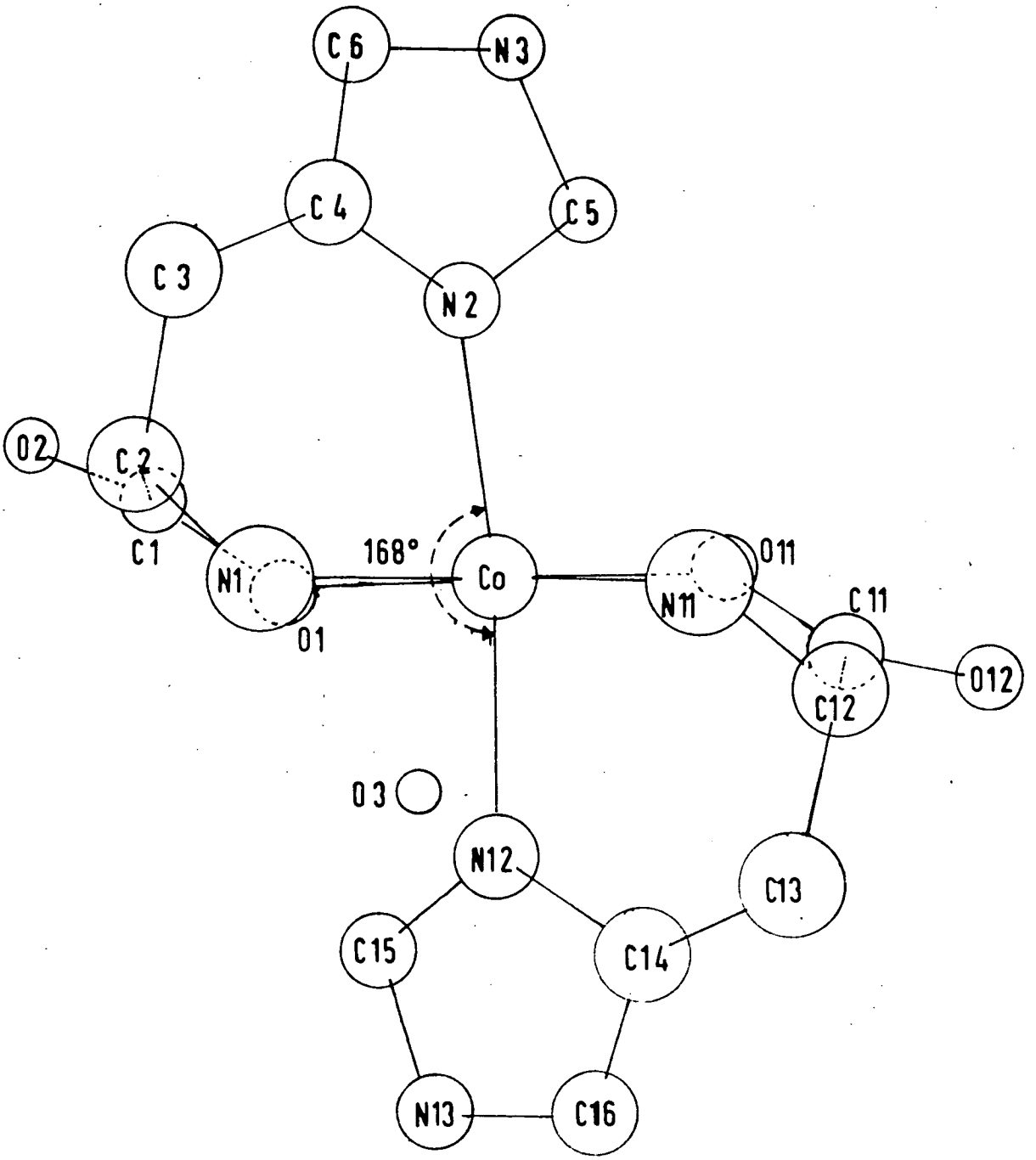
The structure is held together by a complex system of hydrogen bonds as listed on page 59, and by van der Waals' forces. Projections of the cell showing the hydrogen bonding system are given in Figs.XVII and XVIII although hydrogen bonds which cannot be shown clearly on one of the projections are shown only on the other. The closest and therefore most effective van der Waals' contacts are listed below.

C12 - O2 (x, y-1,z)	3·18Å
C14 - C16 (-x, y, -z)	3·27
C14 - C16 (-x, y, 1-z)	3·27
C15 - N13 (-x, y, -z)	3·38
C15 - N13 (-x, y, 1-z)	3·23
C16 - C16 (-x, y, -z)	3·14
C16 - C16 (-x, y, 1-z)	3·38
N13 - N13 (-x, y, -z)	3·17
N13 - N13 (-x, y, 1-z)	3·24



THE COMPLEX PROJECTED ON THE BEST PLANE OF C3,C4,C5,C6,N2 & N3

FIG XV



THE COMPLEX PROJECTED ALONG THE BEST PLANE OF N1,N11,O1 & O11

FIG XVI

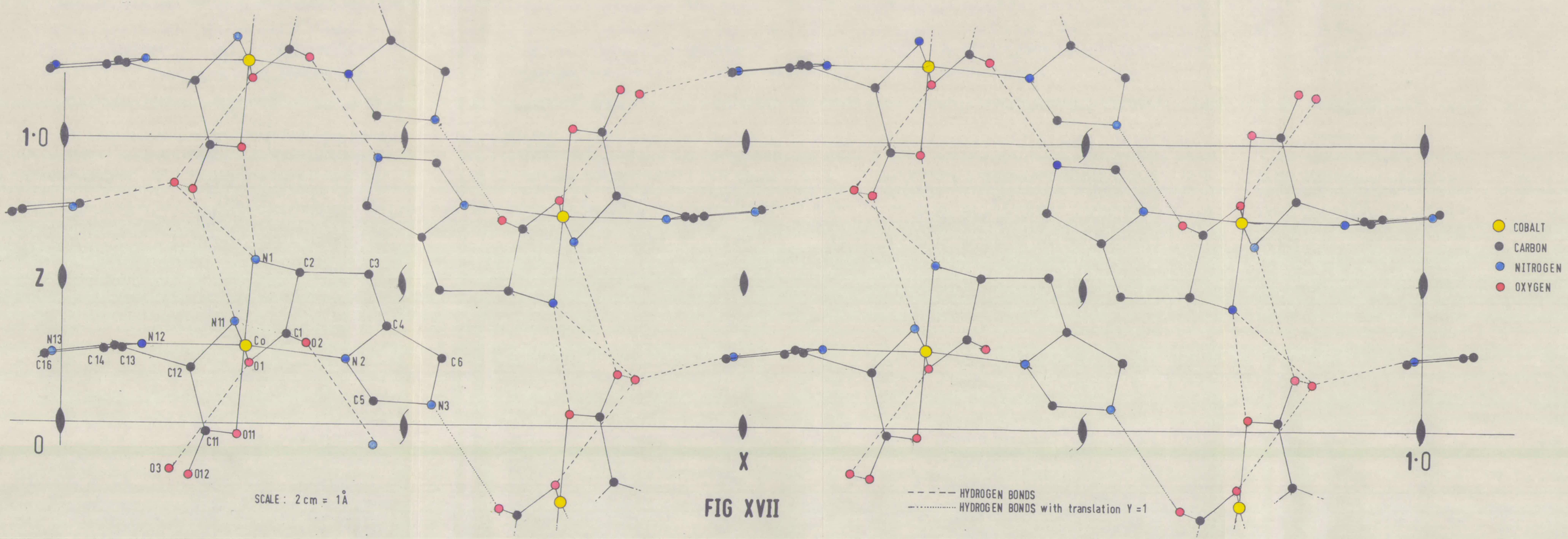

1·0
Z
0
X
1·0
N1
C2
C3
C4
C6
N2
N3
C5
C1
O2
Co
O1
N11
N12
N13
C16
C14
C13
C12
C11
O11
O3
O12
COBALT
CARBON
NITROGEN
OXYGEN
HYDROGEN BONDS
HYDROGEN BONDS with translation Y =1
SCALE : 2 cm = 1 Å

FIG XVII

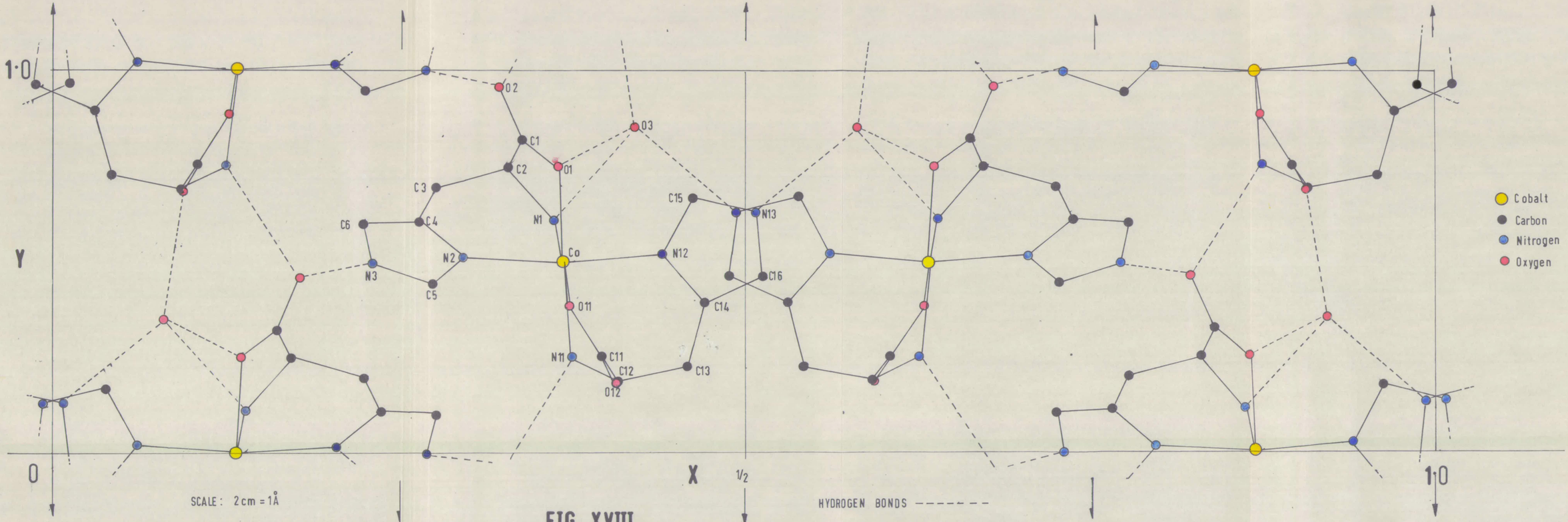

1·0
Y
0
X
½
1·0
O2
C1
O3
O1
C2
C3
C15
N13
C6
C4
N1
N2
Co
N12
N3
C5
C16
C14
O11
N11
C11
C12
C13
O12
Cobalt
Carbon
Nitrogen
Oxygen
SCALE : 2 cm = 1Å
HYDROGEN BONDS

FIG XVIII

As can be seen from the table the major part played by the van der Waals' contacts in holding the structure together is concerned with one of the histidine residues only, the contacts being principally between the imidazole rings giving the layer arrangement of the rings as seen in the (010) projection of the cell (Fig.XVII).

Comparison with Other Histidine Complexes

Table II shows a comparison of the structural work completed on the various histidine complexes which have been the subject of X-ray analysis (37,38,39,40). These are the Zn(II), Ni(II), Cd(II) and Co(II) complexes with L and DL-histidine, making eight in all. The zinc L-histidine complex is isomorphous with the cadmium L-complex, as is the cobalt L-histidine complex with the corresponding nickel complex. The zinc and cadmium DL-complexes are pseudo isomorphous, but the cobalt and nickel DL-complexes are not related in this manner.

The two zinc complexes are approximately tetrahedral with loose coordination by the carboxyl groups at 2·91Å in the DL-complex and 2·79Å in the L-complex (Fig.XIX). An interesting point as regards the DL-complex is that each/

TABLE II

Structures of Divalent Metal Histidine Complexes

Complex	Coordination
$Zn(DL\text{-hist})_2$	Distorted tetrahedral. Coordination by amino and imidazole nitrogens at 2·02A; weak association of one oxygen of each histidine at 2·91A.
$Zn(L\text{-hist})_2$	Similar to DL-complex with weak association of one oxygen of each histidine at 2·79A. Relation of one histidine to another differs from that in DL-complex. Nitrogens at 2·05A.
$Cd(DL\text{-hist})_2$	Space group determination shows some disorder making complete determination of crystal structure difficult, but some electron density syntheses have shown Cd environment similar to that in corresponding zinc complex.
$Cd(L\text{-hist})_2$	Nearer to more regular six-coordination than corresponding zinc complex. It has four nitrogens at 2·2A, two oxygens at 2·53A.
$Ni(DL\text{-hist})_2$	Very nearly regular octahedral. Each molecule contains two L-histidine residues or two D-residues but not one of each, as does zinc DL-complex. Two imidazole nitrogens are trans. Ni-N and Ni-O bond lengths close to 2·1A.
$Ni(L\text{-hist})_2$	Structure not refined; isomorphous with $Co(L\text{-hist})_2$ complex and thus structure very similar.
$Co(DL\text{-hist})_2$	Structure not yet determined.
$Co(L\text{-hist})_2$	Very nearly regular octahedral. Structure very similar to that of $Ni(DL\text{-hist})_2$ but arrangement in crystal lattice quite different. Co-N bonds between 2·1 and 2·2A; Co-O bonds close to 2·1A. Imidazole nitrogens trans.

/each zinc ion has either two L-histidine or two DL-histidine residues coordinated but not one of each. Compared with the L-complex however the DL-complex has one histidine residue twisted through 180°. Thus although the crystals contain the same molecule there are differences in stereochemistry and in coordination bond lengths caused by environmental conditions, a feature which also explains differences between the cobalt and nickel L-complexes.

The cadmium and zinc L-complexes are comparable but the cadmium complex is nearer to regular 6-coordination than the zinc, the coordinating oxygens having moved closer to the metal cation (2·53Å) and the nitrogens further away to 2·2A compared with 2·05A in the zinc complex. This difference is achieved mainly by rotation around the C3-C4 bond and it is rotation about this bond which accounts for the main differences in the conformation of complexed histidine groups in relation to the conformation of histidine in histidine hydrochloride. A complete structural study of the cadmium DL-complex has not been possible due to the disorder present in the crystal, but it has been shown that the cadmium ion has a similar environment to that of zinc.

Nickel $(DL\text{-hist})_2$ whose structural study is complete is more nearly octahedral than the cadmium complexes and the cobalt complex is more similar to this structure than those of the zinc and cadmium complexes. Although the position of the molecules in the nickel and cobalt complexes is crystallographically quite different, the actual molecules are very nearly the same as regards conformation. Nickel-$(DL\text{-hist})_2$ like zinc $(DL\text{-hist})_2$ has either two L-histidine or two D-histidine residues linked to each cation and not one of each type. Thus the structure of the cobalt complex is quite comparable to the nickel complex.

There are, however, some differences. The N2-Ni-N12 angle in the nickel complex is 177°, deviating by only 3° from the octahedral 180°. The cobalt complex on the other hand has a corresponding angle of 168°. This difference is probably due to the packing and hydrogen bonding systems of the molecules. As explained above the hydrogen bonding system and the van der Waals' contacts in the cobalt complex are different for the two histidine residues, whereas in the nickel complex the nickel atoms lie on special positions and the crystal symmetry is such that the histidine residues must have identical hydrogen bonds. If one considers merely the imidazole rings it is found that in the cobalt complex the hydrogen bonds act such that they tend to cause a deviation/

/deviation of the N2-Co-N12 bond from 180° (Fig.XX). In the nickel complex, however the hydrogen bonds must act equally in opposite directions and should give no deviation. The following table gives a comparison of the hydrogen bonds in the two complexes, those in the nickel complex occurring in symmetrical pairs, those of the cobalt complex being unsymmetrical.

Hydrogen Bonds

Co Complex	Ni Complex
N1 - O11	N1 - O2
N11 - O12	N11 - O12
N1 - O3	N1 - O3
N11 - O2	N11 - O3
N3 - O2	N3 - O2
N13 - O3	N13 - O12
O1 - O3	O1 - O3
O12 - O3	O11 - O3

Figure XXI(a) shows one histidine residue of the cobalt complex superimposed upon a histidine residue of the nickel complex both being projected on the best plane of C13, C14, C15, C16, N12 and N13; the projections of the two nickel histidine residues are of course identical. It is seen that the projections are almost identical, but Figure XXI(b) shows that the other histidine residue of the cobalt complex does not compare nearly as well when it is superimposed on

a/

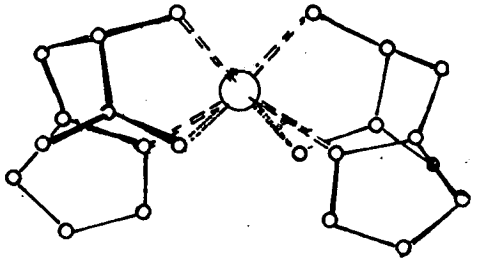
/a histidine residue of the nickel complex. The projection in this case is on the best plane of C3, C4, C5, C6, N2 and N3. There is some difference in conformation of the two side chains, which once again is probably due to hydrogen bonding and packing considerations.

Figure XXII shows the relationship between the coordination of the amino and carboxyl groups to the metal cations. In this they are quite similar for, in both cases, the amino nitrogen and carboxyl oxygen are not far enough apart to give the expected octahedral angle of 90°. In the cobalt complex there are further irregularites in this coordination, once more assignable to the uneven hydrogen bonding system pulling the coordinating atoms out of alignment. The deviations of the cations and coordinating atoms from their mean planes are also shown and although these deviations are larger for the cobalt complex than the nickel, they are not of great significance.

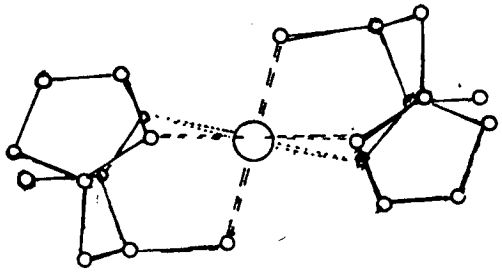
Finally, Figure XXIII shows the projections down the N1-C2 and N11-C12 bonds, which should be compared with Figs. 2 and 3 of Donohue and Caron (5) and Fig. 6(i) of Fraser (40). As in the nickel complex, the cobalt cation deviates from the staggered position, the two values in this case being approximately 15° and 25° for the two residues. Like the case/

/case of the nickel complex this can be explained by the strong coordination of the carboxyl oxygens and amino nitrogens giving fewer degrees of freedom to the histidine residues than in the zinc complexes for rotation about the N1-C2 bond.

In all other respects the structures are similar apart from small differences in bond lengths and angles. The dihedral angle between the plane of C2, C3 and C4 and the plane of the imidazole ring is 26·8° and for the C12, C13 and C14 plane and the corresponding ring the angle is 21·5°. These are very similar to Fraser's value of 23·0° for the nickel complex, showing once again the rotation about C3-C4 in comparison to the zinc complexes with a corresponding dihedral angle of about 40° and histidine hydrochloride with a dihedral angle of 121°.

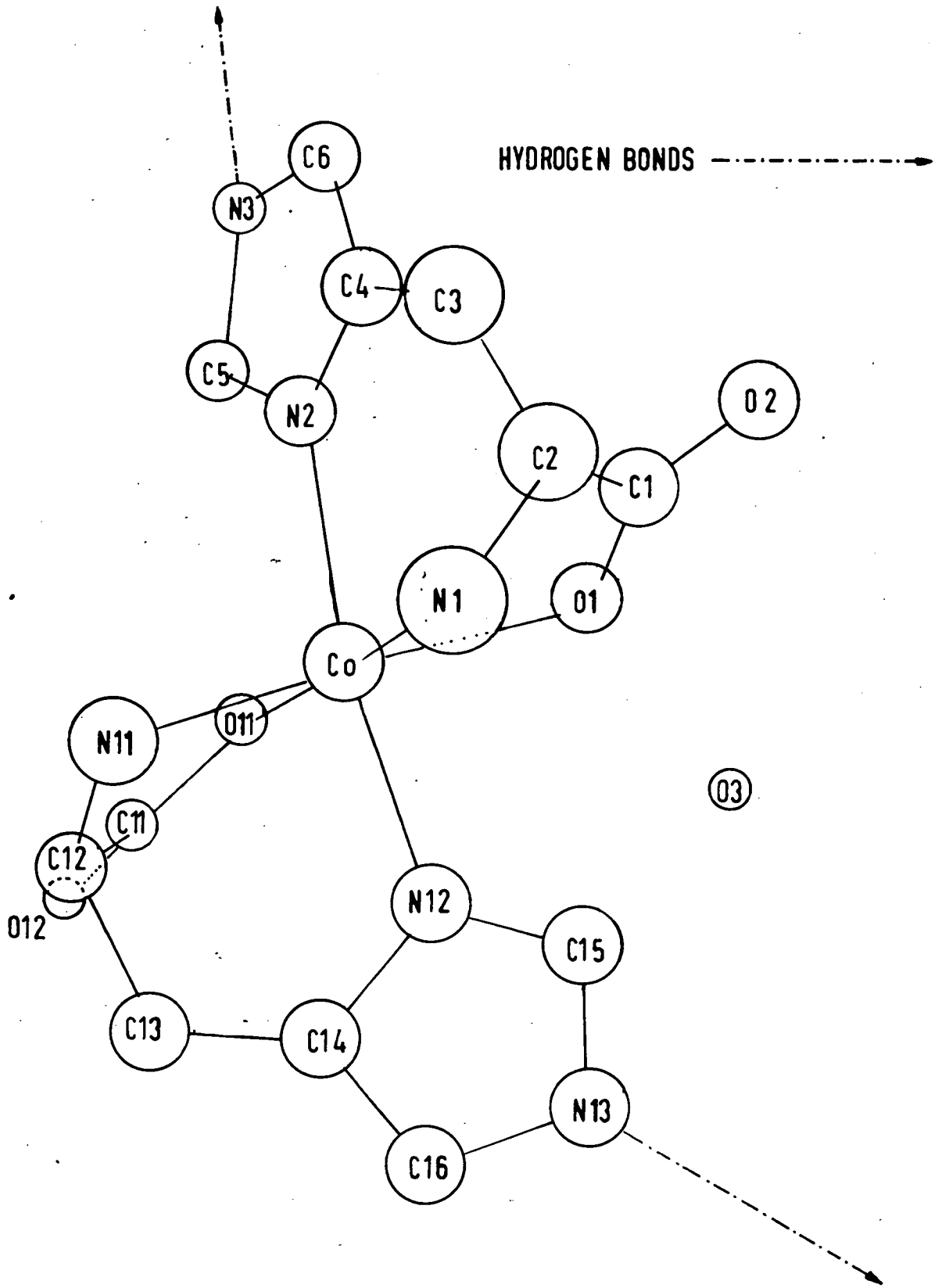


ZINC DL-(HIST)$_2$



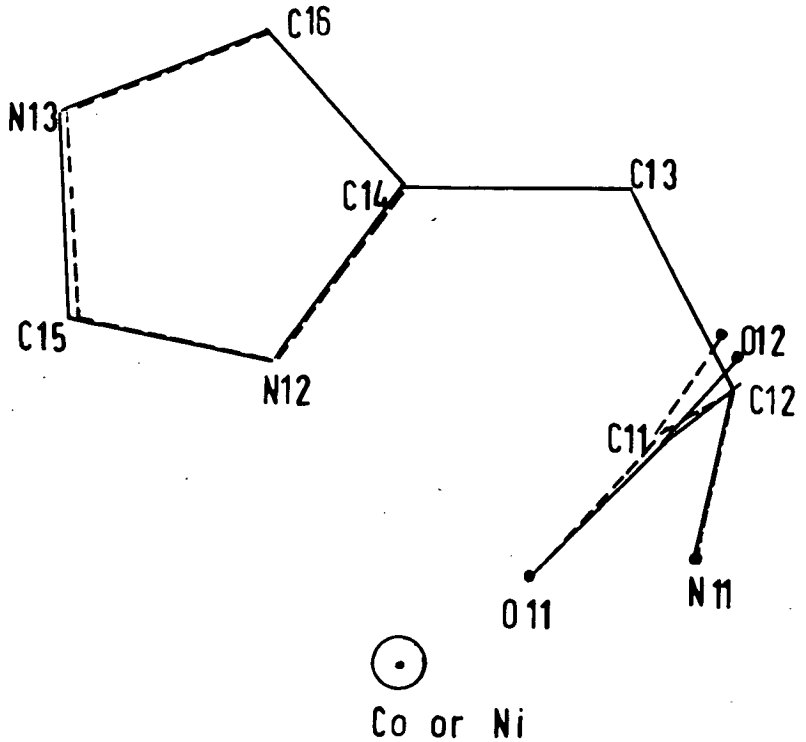
ZINC L-(HIST)$_2$

FIG XIX

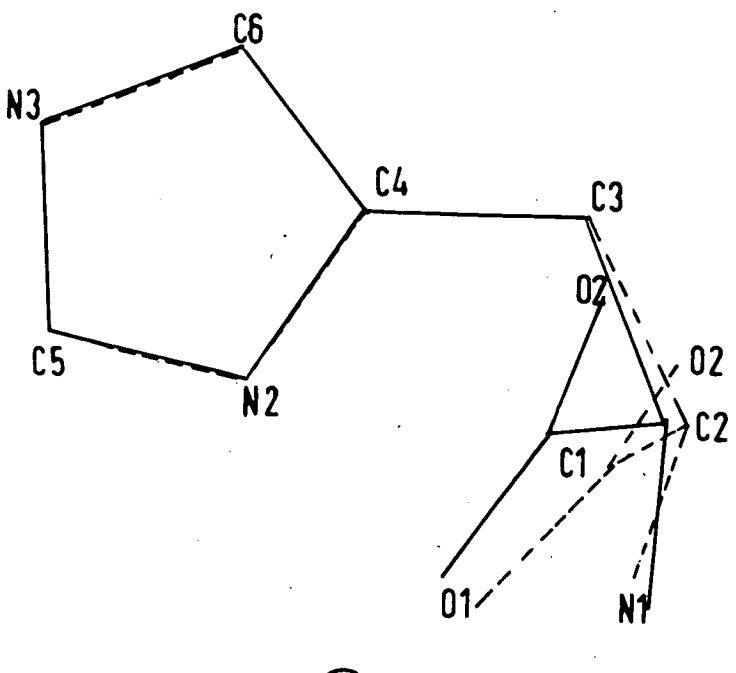


THE COMPLEX PROJECTED ON THE BEST PLANE OF C13, C14, C15, C16, N12 & N13
SHOWING IMIDAZOLE RING HYDROGEN BONDING

FIG XX



Projections on best plane of C14,C13,C15,C16,N12 & N13



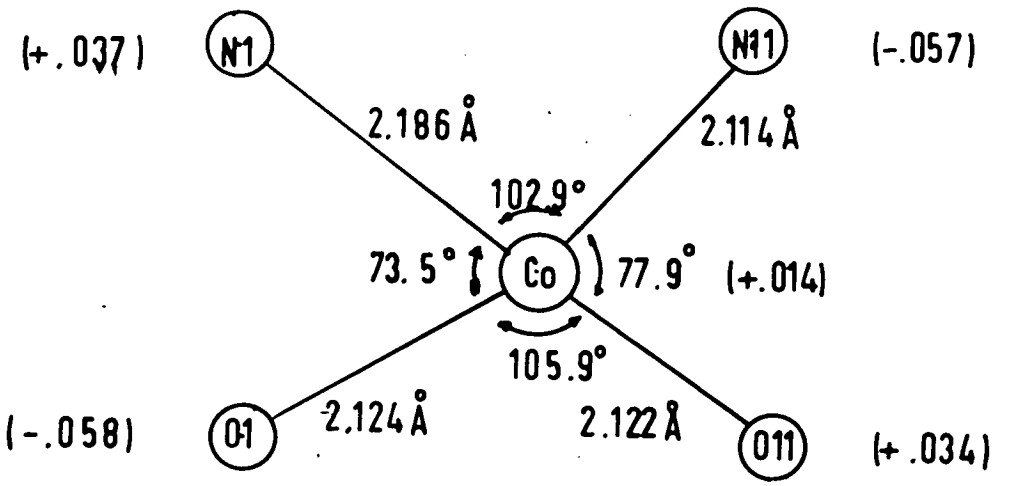
Cobalt histidine residues

Nickel histidine residues

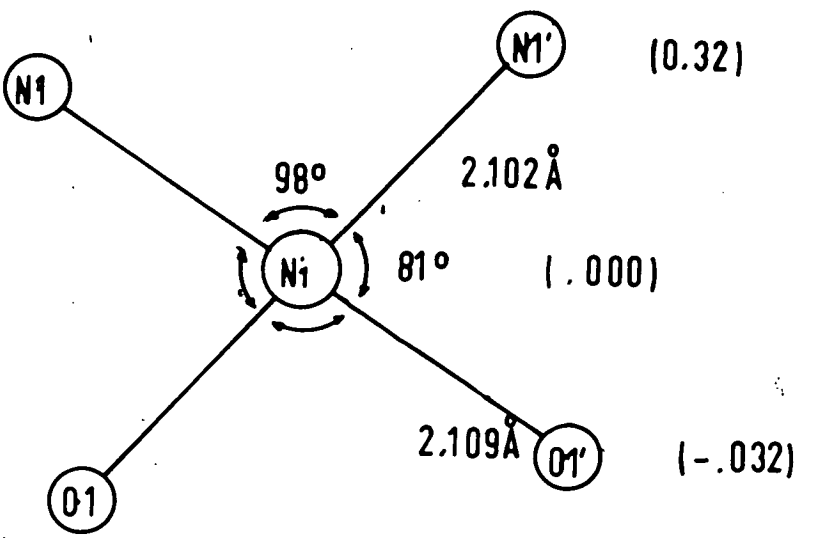
Projections on best plane of C3C4 C5 C6 N2 & N3

COMPARISON OF HISTIDINE RESIDUES OF NICKEL DL(HIST)$_2$ & COBALT L-(HIST)$_2$

FIG XXI

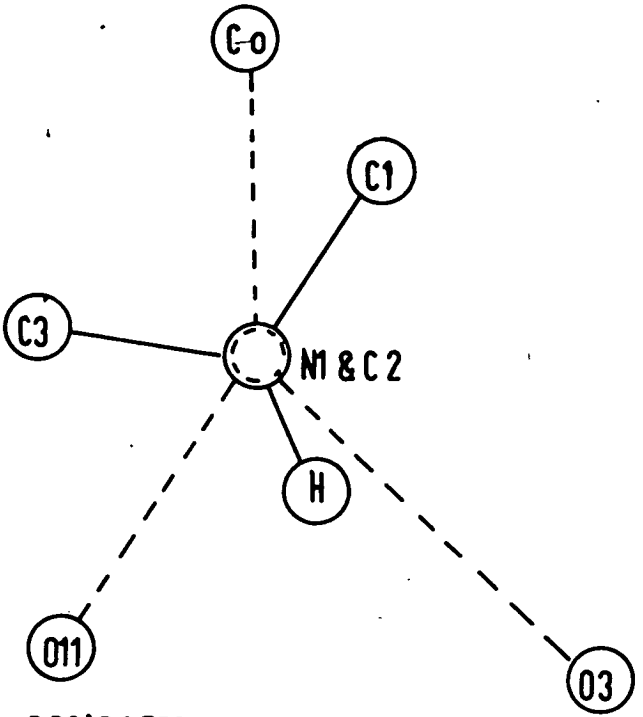


PROJECTIONS ON CATION, N1, N11, 01 & 011

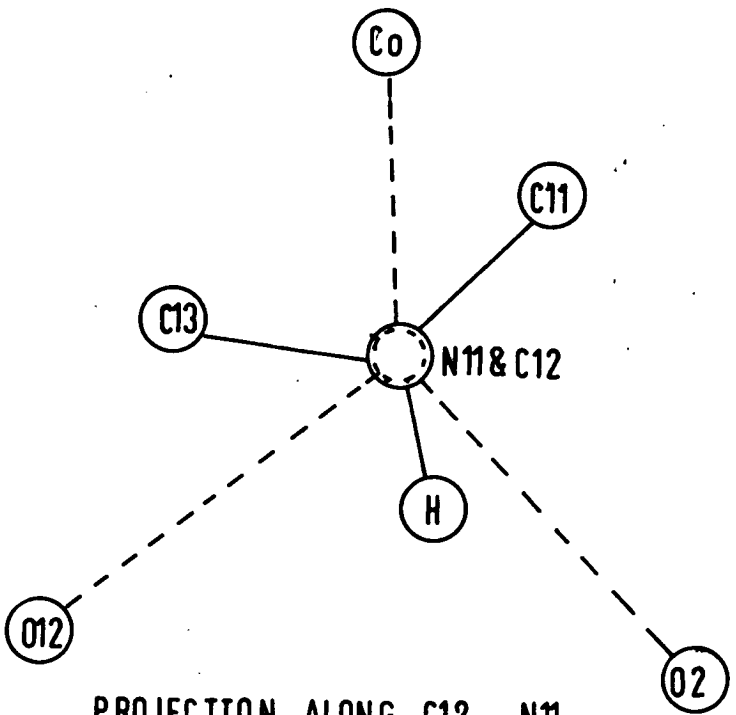


Values in brackets refer to deviations from best plane in Å

FIG XXII



PROJECTION ALONG C2 – N1



PROJECTION ALONG C12 — N11

FIG XXIII

SECTION IX

Conclusion

The structure of cobalt(II) bis-L-histidino monohydrate is octahedral confirming the N.M.R. evidence of McDonald and Phillips (36). It is comparable to nickel(II) bis-DL-histidino monohydrate but deviates from this structure in certain minor ways due to the different environmental conditions. Coordination of the histidine residues is attained in the same manner as in other histidine complexes by rotation about the C3-C4 bond giving the main difference in conformation from that of histidine in histidine hydrochloride. The bond lengths and angles obtained for the imidazole rings agree well with those determined for imidazole, although there does seem to be some difference in the bond angles around the imidazole nitrogen which is coordinated to cobalt in the complex. It has also been found that the bond lengths of the histidine residues are quite comparable to those in histidine hydrochloride.

Nickel(II) bis L-histidino monohydrate is isomorphous with the cobalt complex and has the same structure. (For a summary of the data determined for the nickel L-histidine complex see Table III). This leaves several interesting but/

/but unanswered questions. The principal of these are, why are the cobalt and nickel DL-complexes not isomorphous also as one might expect, and how will the structure of cobalt DL-histidine differ from that of nickel DL-histidine and compare with that of cobalt L-histidine? The structure of cobalt DL-histidine is now being studied by R. Candlin and the answers to these questions should be shortly forthcoming.

One question which remains unanswered and which this thesis has not dealt with is, how does the cobalt histidine complex absorb oxygen? This will not be answered until the oxygenated complex is crystallised in a suitable form and subjected to X-ray analysis. However, it is hoped that this thesis will be of help in answering this problem, by showing the form of the complex before oxygenation, thus allowing the mechanism of oxygenation to be determined once the structure of the oxygenated complex is known.

TABLE III

Bis-L-histidinato-nickel(II) monohydrate: Data

Isomorphous with bis-L-histidinato-cobalt(II) monohydrate

Space group: C2

No. of molecules per unit cell: 4

a	=	29·42 Å
b	=	8·32 Å
c	=	6·33 Å
β	=	90°
α	=	γ = 90°
Vc	=	1549 Å^3
Dc	=	1·65 g/cc
Dm	=	1·66 g/cc (flotation)

PART II

THE CRYSTAL STRUCTURE DETERMINATION OF L-CYSTEINE

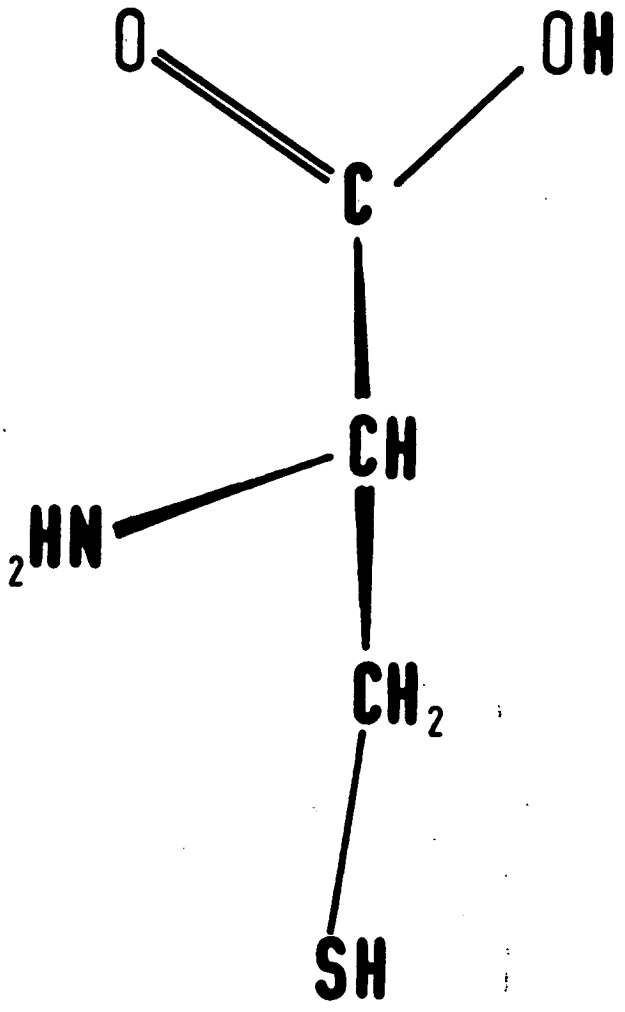
PART II

SECTION I

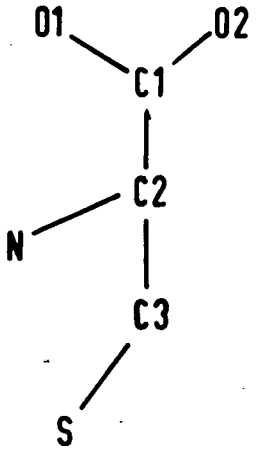
Introduction

L-cysteine ($SH.CH_2.CH(NH_2)COOH$, 2-amino-3-mercapto propanoic acid)(Fig.XXIV) is, like histidine, a naturally occurring α-amino acid which forms very strong coordination complexes with transition metals. The cobalt(II) complex of cysteine, like that of histidine, is rapidly oxidised by molecular oxygen but differs in that this oxidation is irreversible. Cysteine itself is slowly oxidised by molecular oxygen to cystine by the formation of a disulphide bridge. This part of the thesis deals with the structural analysis of pure L-cysteine.

Most of the natural amino acids have been subjected to X-ray examination, but since no work had apparently been carried out on pure cysteine it was decided that a structural analysis would be worthwhile. In the words of S.C. Nyburg (57), " Crystal structure analysis of the proteins themselves constitutes a formidable analytical task because of their very large molecular weights. Knowledge of the stereochemistry of the structural units from which they are made up is of great potential value."



L- CYSTEINE
[NON-ZWITTERION FORM]



NOMENCLATURE
(as used in this thesis)

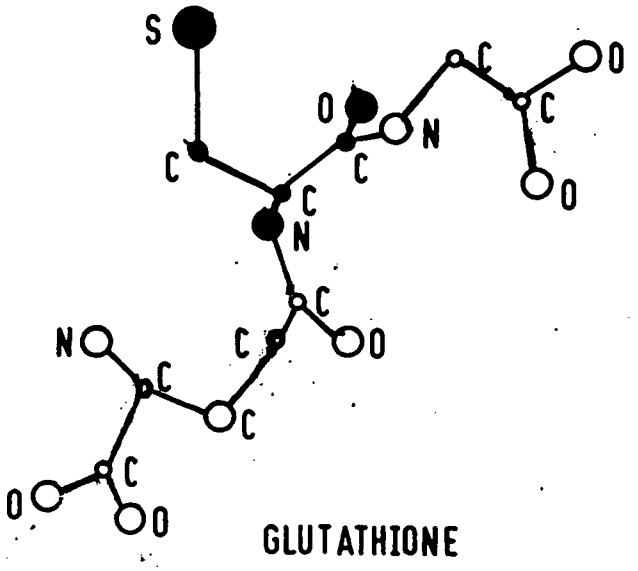
FIG XXIV

Although cysteine itself had not been structurally analysed when this work was commenced, the structures of several related compounds had been determined. These include the following:

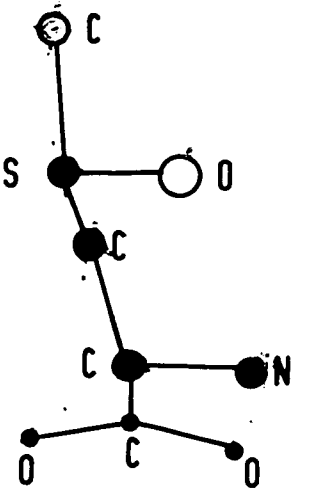
(a) Cysteinylglycine sodium iodide (58) (Fig.XXVI)
(b) Glutathione (γ-L-glutamyl-L-cysteinylglycine) (59) (Fig.XXV)
(c) (+)-S-methyl-L-cysteine sulphoxide (60) (Fig.XXV)
(d) L-cysteine ethyl ester hydrochloride -urea (1:1) (61)(Fig.XXV)
(e) L-cystine (Fig.XXVI) (62), L-cystine dihydrochloride (63), L-cystine dihydrobromide (64) and some related compounds containing cystine.

In addition, whilst the structural analysis of L-cysteine was in progress the structure determination of L-cysteine hydrochloride (65) was published.

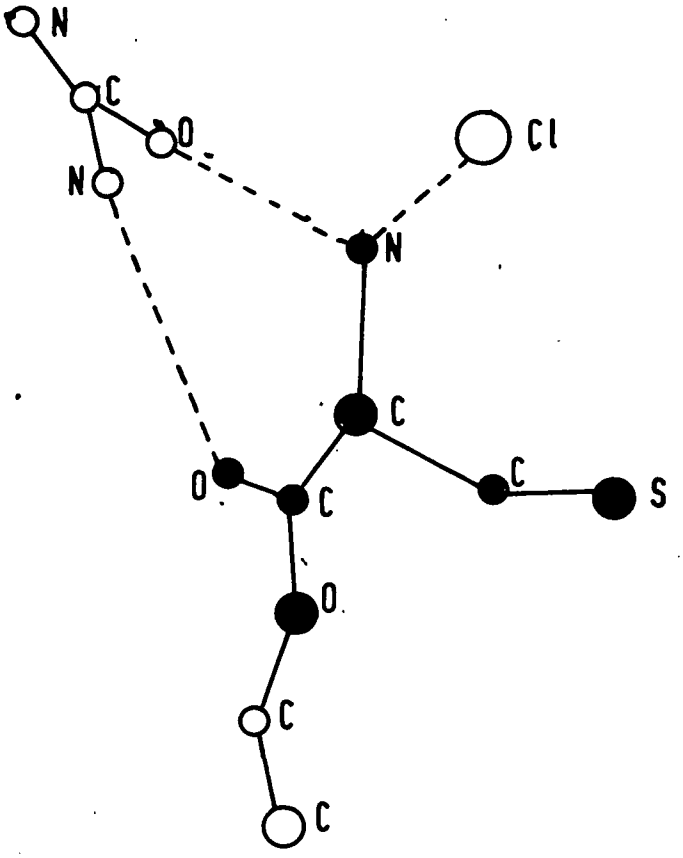
The best comparison that can be made between the cysteine residues in these molecules is a comparison of the relevant bond lengths and angles as given in Tables IV and V. There is not a great deal of difference in the bond lengths and angles overall. One deviation, however, is that the sulphur - Cl bond in cysteinylglycine sodium iodide does not follow the general pattern but is almost 0·2Å shorter than the mean of the other values determined for this bond. However, there is no obvious reason why this should be so, and, as this structure determination was of limited accuracy the difference will be presumed to be insignificant.



GLUTATHIONE



(+)-S-METHYL-L-CYSTEINE SULPHOXIDE



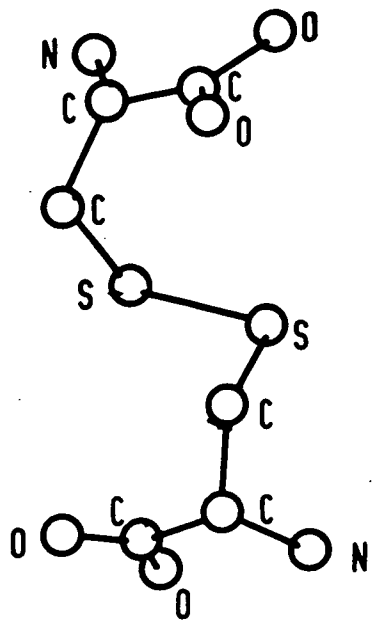
L-CYSTEINE ETHYL ESTER HYDROCHLORIDE

– UREA (1:1)

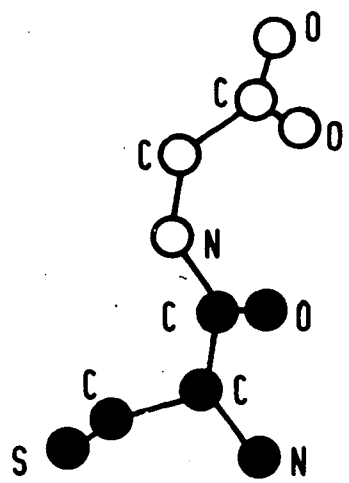
: CYSTEINE RESIDUES

: OTHER ATOMS

FIG XXV



L-CYSTINE



CYSTEINYLGLYCINE (SODIUM IODIDE)

FIG XXV

The conformation of the cysteine residue in all of these compounds is very similar. The peptide group ${}_{N}\!\!\diagup C - C\!\!\begin{smallmatrix}\diagup O\\ \diagdown O\end{smallmatrix}$ is coplanar or almost so in each compound as is generally found in α-amino acids. This, accordingly, leaves only rotation of the sulphur atom about C1–C2 to give differences in structural conformation. The normal position of the sulphur - C3 - C2 plane is one approximately bisecting the angle N - C2 - C1 as indicated by Oughton and Harrison (66).

TABLE IV

Cysteine-containing compounds

Bond Lengths Å

Compound	S-C3	C3-C2	C2-N	C2-C1	C1-O1	C1-O2	Ref.
S-methyl -L-cysteine-sulphoxide	1·83	1·49	1·52	1·57	1·24	1·22	(60)
Glutathione	1·78	1·55	1·46	1·51	1·24	----	(59)
Cysteinyl-glycine NaI	1·64	1·51	1·42	1·54	1·21	----	(58)
L-cysteine HCl	1·80	1·53	1·49	1·54	1·32	1·26	(65)
L-cystine	1·82	1·51	1·51	1·54	1·28	1·24	(62)
L-cystine 2HBr	1·86	1·51	1·49	1·51	1·27	1·22	(64)
L-cystine 2HCl	1·87	1·56	1·48	1·47	1·30	1·24	(63)

TABLE V

Cysteine-containing compounds

Bond Angles°

Compound	S-C3-C2	C3-C2-C1	C3-C2-N	N-C2-C1	C2-C1-O1	C2-C1-O2	O1-C1-O2
S-methyl-L-cysteine-sulphoxide	115	110	109	111	115	115	131
Glutathione	117	109	109	115	119	---	---
Cysteinyl-glycine NaI	125	111	108	110	109	---	---
L-cysteine HCl	116	112	112	105	111	126	123
L-cystine	116	114	112	109	118	115	127
L-cystine 2HBr	112	114	115	108	114	122	124
L-cystine 2HCl	113	112	111	111	118	123	119

SECTION II

Preliminary Work

Crystallisation and Analysis

Crystals of L-cysteine were obtained by dissolving crystals of L-cysteine hydrochloride supplied by B.D.H. in the minimum of hot water (c.80°C) and adding drops of 5M caustic soda solution until pH8 was reached. The solution was allowed to cool and crystallise. The crystals of the free base were recrystallised from hot water, washed with a 1:1 ethanol/water mixture and dried over calcium chloride in vacuo. A commercial analysis of the crystals for sulphur, carbon, nitrogen and hydrogen gave the following results.

	Calculated%	Observed%
Sulphur	26·46	23·65
Carbon	29·73	30·52
Nitrogen	11·56	10·46
Hydrogen	5·83	5·86

Space Group and Cell Dimensions

The crystals were transparent needles, showing extinction of polarised light parallel to the needle axis. A good single crystal was mounted along the needle axis and a preliminary oscillation/

/oscillation photograph recorded. For this and all following photographs a non-integrating Weissenberg equi-inclination camera was used in conjunction with a sealed vacuum X-ray tube and a Philips PW1009 generator. The radiation used was nickel filtered copper Kα, the working voltage of the tube being 35Kv, tube current 20mA.

The first oscillation photograph of the crystal about the needle axis, after the crystal had been accurately set showed a mirror plane perpendicular to b such that $I(hkl) = I(h\bar{k}l)$. A zero-layer Weissenberg photograph showed further that I(h0l) was not equivalent to $I(\bar{h}0l)$ and it was deduced that the cell was monoclinic. A first-layer Weissenberg photograph showed no evidence of systematic absences. Two axes were chosen such that $\beta = 109°$ and the following cell dimensions were calculated from the oscillation and zero-layer Weissenberg photographs by the chart method.

$a^* = 0{\cdot}142$
$b^* = 0{\cdot}296$
$c^* = 0{\cdot}171$
$\beta^* = 71°$

It was deduced that the space group must be P2 or $P2_1$. A zero-layer Weissenberg photograph of another crystal about the a axis showed that for the (0k0) reflections (k = 2n) was a condition/

/condition for reflection. It was therefore concluded that the space group of L-cysteine is $P2_1$. Accurate cell dimensions were measured by calibration against copper wire (see page 23) and the following values for the cell dimensions obtained.

$a = 11{\cdot}512 \pm 0{\cdot}01\text{Å}$

$b = 5{\cdot}240 \pm 0{\cdot}005\text{Å}$

$c = 9{\cdot}517 \pm 0{\cdot}01\text{Å}$

$\beta = 109^\circ\ 8'$

$\alpha = \gamma = 90^\circ$

$Vc = abc.\sin\beta = 542{\cdot}4\text{Å}^3$

The density of the crystals were measured by flotation in a mixture of carbon tetrachloride and iodomethane and the value obtained was 1·483g/cc. Space group $P2_1$ has two equivalent positions $(x,y,z;\ -x,y+{}^1/_2,-z)$. Assuming that there were two isostructural units per cell gave a calculated density of 0·7417g/cc. It was concluded therefore that there are two isostructural units per asymmetric unit giving a calculated density of 1·483g/cc. Thus L-cysteine has four molecules per unit cell and no water of crystallisation.

Linear Absorption Coefficient

The linear absorption coefficient of cysteine in copper Kα radiation is $61{\cdot}1\text{cm}^{-1}$.

SECTION III

Data Collection and Intensity Measurement

Three-dimensional data was collected using the Weissenberg equi-inclination technique. Data from the b axis was collected from (h0l) to (h4l) the maximum obtainable from the camera. Layers about the a axis were collected from (0kl) to (6kl). The crystal used for the a axis data was of approximately the same dimensions as the first crystal but it was cut in half across the needle in order to make absorption effects as isotropic as possible. The layers (7kl) to (9kl) were also photographed but it was discovered that the reflections on these photographs were beginning to blur indicating probably that oxidation to cystine was occurring. Intensities were therefore only measured to (6kl) where the reflections were still clear and sharp.

The multiple film technique with Industrial G film was used (page 26), and the intensities measured by visual estimation against a calibrated intensity strip. Each set of films was scaled internally by calculating average film ratios (page 27). The intensities were then corrected for the Lorentz and polarisation factors by computer program and then a spot-shape correction was applied using the chart method of Phillips (67). No absorption/

/absorption corrections were made. Finally each layer was scaled to the (h0l) reflections by applying the appropriate scale factors calculated from the ratios of reflections common to both the a axis and b axis films.

In this manner a total of 1239 intensities were measured, there being a total of 84 unobserved reflections within the layers recorded. These 1239 squared structure amplitudes were used in the determination of the structure.

A Wilson plot of the (h0l) reflections was calculated to determine the absolute scale factor and overall temperature factor (page 28). These were 0·487 and 3·1 respectively.

SECTION IV

The Three-dimensional Patterson Synthesis and Harker Section

The measured structure amplitudes were used in the computtation of a sharpened three-dimensional P(UVW) synthesis.

$$P(UVW) = \frac{4}{V_c}\sum_{0}^{\infty}\sum_{0}^{\infty}\sum_{0}^{\infty}\left\{|{}^{s}F(hkl)|^2\cos 2\pi(hU+lW) + |{}^{s}F(\bar{h}kl)|^2\cos 2\pi(hU-lW)\right\}\cos 2\pi kV$$

Sharpening of the synthesis was carried out by making

$$|{}^{s}F(hkl)| = \frac{|F(hkl)|}{f_{hkl}(\text{sulphur})e^{-3\sin^2\theta/\lambda^2}}$$

to sharpen the synthesis in favour of the sulphur atoms (68). This synthesis was computed in sections perpendicular to W.

Space group $P2_1$ has a Harker section (69) at $y = {}^1/_2$. The equivalent positions of space group $P2_1$ being (x,y,z) and $(-x, y+{}^1/_2, -z)$ means that every atom must have a vector peak at $(2x, {}^1/_2, 2z)$ and thus every atom gives a vector peak on the section $y = {}^1/_2$ which is the Harker section. The two largest peaks on the Harker section (Fig.XXVII) were at (24·8/30, $^1/_2$, 26/120) and (5·8/30, $^1/_2$, 28/120) with peak heights about $250e^2/\text{Å}^3$. It was assumed that these peaks were the Harker peaks of the two sulphur atoms in the assymetric unit. There are also the two/

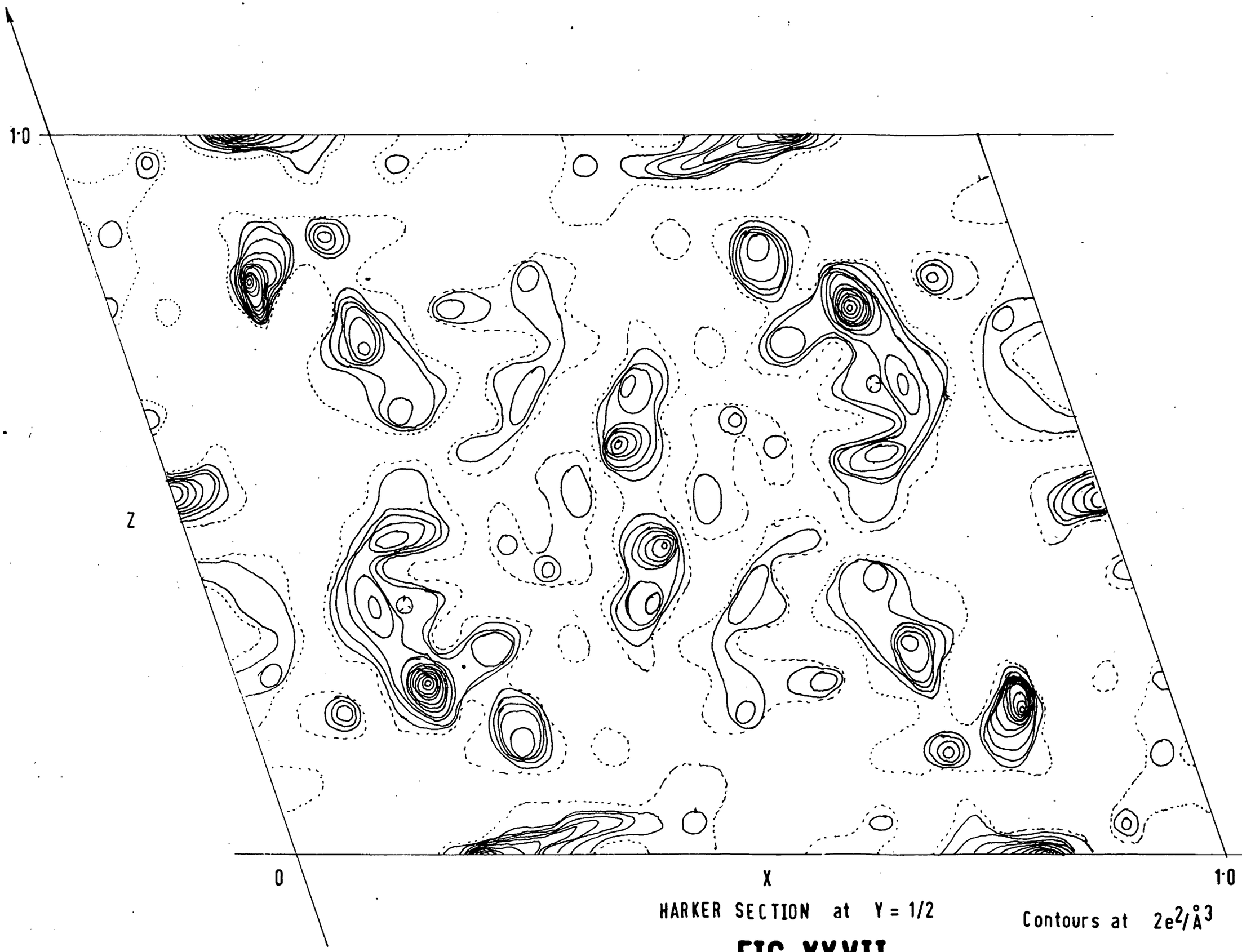
/two equivalent vector peaks at (-24·8/30, $^1/_2$, -26/120) and (-5·8/30, $^1/_2$, -28/120) and one can also include the equivalent peaks in the adjacent vector cells as possibilities since an atom at (29/30, y, 29/30) for instance will give a Harker peak at (58/30, $^1/_2$, 58/30). It was hoped to resolve this ambiguity however, by means of the cross vectors in the three-dimensional Patterson. Two atoms at (x_1, y_1, z_1) and (x_2, y_2, z_2) taken with their counterparts at $(-x_1, {}^1/_2+y_1, -z_1)$ and $(-x_2, {}^1/_2+y_2, -z_2)$ give the following series of vector peaks.

$(2x_1, {}^1/_2, 2z_1)$ $(2x_2, {}^1/_2, 2z_2)$	Harker vectors
$(x_1-x_2, y_1-y_2, z_1-z_2)$ $(x_2-x_1, y_2-y_1, z_2-z_1)$	Difference vectors
$(x_1+x_2, y_1-y_2-{}^1/_2, z_1+z_2)$ $(x_1+x_2, y_2-y_1-{}^1/_2, z_1+z_2)$	Sum vectors

Thus one must look for two pairs of cross vectors with a difference of $^1/_2y$. The solution obtained was the simple one with the (x,z) coordinates of the sulphur atoms being (12·4/30, 13/120), (2·9/30,14/120) as indicated by cross vectors at (9·5/30,1/120) and (15·3/30,27/120) of about 190$e^2/Å^3$. Taking the y-coordinate of the first sulphur atom to be zero, the y-coordinate of the second sulphur atom was deduced to be (2/15) to satisfy/

/satisfy the cross vectors. There is of course an alternative solution taking the y-coordinates as (0) and (-2/15) since the the vector set of space group $P2_1$ has symmetry P2/m. The ambiguous solutions correspond to the molecular configurations L-cysteine and D-cysteine, but it was not of course possible to tell which solution corresponded to which configuration at this stage.

It transpired later that this solution of the sulphur positions was incorrect, but a survey of the work completed using this solution is necessary to show how the true solution of the structure emerged as doubts grew about the original.



HARKER SECTION at Y = 1/2

Contours at $2e^2/Å^3$

FIG XXVII

SECTION V

The Three-dimensional Electron Density Synthesis and Other Methods

Structure factors were calculated from the sulphur atoms positioned at (12·4/30, 0, 13/120) and (2·9/30, 2/15, 14/120) and a three-dimensional electron density series phased accordingly was computed, the formulae for space group P2, being:

$$A = 2\cos 2\pi(hx+lz+k/4)\cos 2\pi(ky-k/4)$$

$$B = 2\cos 2\pi(hx+lz+k/4)\sin 2\pi(ky-k/4)$$

$$|F| = \sqrt{(A^2 + B^2)}$$

$$\rho(XYZ) = \frac{4}{V_c}\Bigg\{ \sum_{0}^{\infty}\sum_{0}^{\infty}\sum_{0}^{\infty}\Big\{ \sum_{l=2n}|F(hkl)|\cos 2\pi(hX+lZ)\cos[2\pi kY - \alpha(hkl)] + |F(\bar{h}kl)|\cos 2\pi(-hX+lZ)\cos[2\pi kY - \alpha(\bar{h}kl)]\Big\} - \sum_{0}^{\infty}\sum_{0}^{\infty}\sum_{0}^{\infty}\Big\{ \sum_{l=2n+1}|F(hkl)|\sin 2\pi(hX+lZ)\sin[2\pi kY - \alpha(hkl)] + |F(\bar{h}kl)|\sin 2\pi(-hX+lZ)\sin[2\pi kY - \alpha(\bar{h}kl)]\Big\}\Bigg\}$$

The peaks corresponding to the sulphur atoms were well resolved at 31 electrons/Å^3; there was one other peak at just under 4 e/Å^3 at (17/30, $^1/_2$, 54/120) and six others poorly resolved of about 2 e/Å^3. The residual was 55%.

Although it was not possible to fit the cysteine molecules to these peaks, a second set of structure factors and an electron density synthesis were computed with atoms in tentative positions indicated/

/indicated by the peaks. A total of ten atoms of the fourteen possible were included, these being the two sulphur atoms and atoms corresponding to two carboxyl groups and two nitrogen atoms. A corresponding difference synthesis was also computed; the residual was 45%. Although the atoms included in the structure factor calculations were well resolved in the electron density synthesis, not much sense could be made of the synthesis regarding the structure of cysteine and the difference fourier merely indicated that one carbon of a carboxyl group was definitely wrong, but was otherwise not particularly informative.

In order to try and interpret these syntheses a graphical three-dimensional Patterson superposition on the sulphur positions was made. This gave clear indication of some vector peak coincidences which corresponded with some of the postulated atomic positions from the sulphur-phased synthesis and also to some new peaks which appeared on the second synthesis and difference synthesis. It was possible from these positions to postulate a trial structure which, although fitting the indicated atomic positions, was rather unsatisfactory as regards the conformation of the molecules. To fit the trial structure meant breaking the planarity of the peptide groups severely, and adopting a conformation of the sulphur atom quite different from any found in the compounds referred to in the introduction. A further three-dimensional electron density series was nevertheless calculated, putting all of the atoms into the structure factor/

/factor calculation. This had a residual of 42% which might have indicated that the trial structure was reasonable but required fairly large atomic shifts. Examination of the large low-angle structure factors, however, showed several very severe discrepancies which could not be accounted for by mere shifts of the atoms. The residual of the centrosymmetric (h0l) reflections was 56% indicating very poor agreement and that something was radically wrong with the trial structure.

Since this work seemed to be making no progress, and it was not obvious how it could be corrected, it was laid aside and a study of the centrosymmetric (010) projection was made.

The (010) Projection

The solution of this projection was approached in two ways, by trial and error and by direct methods, neither of which proved to be entirely successful. These will be dealt with in turn.

(a) Trial and Error

The (010) electron density projection was calculated phasing the structure amplitudes on the sulphur positions. This projection (Fig.XXVIII) showed several features with (x,z) coordinates common to those obtained from the first three/

/three-dimensional electron density synthesis. The electron density formula is:

$$\rho(XZ) = \frac{2}{A_c} \sum_{\bullet\bullet}^{\infty\infty} \sum \left\{ F(hl)\cos 2\pi(hX+lZ) + F(\bar{h}l)\cos 2\pi(-hX+lZ) \right\}$$

A series of projections and their corresponding difference syntheses were calculated phasing on several trial structures until every reasonable structure had been tried but nothing which could be called successful was obtained. Although the residual for one attempt was 41%, severe discrepancies in the high value low-angle reflections still remained, and although an attempt was made to obtain an indication of what was wrong by examining structure factor graphs for these reflections no solution was found. One fact however did emerge which was eventually to provide the key to the solution of the structure. One atom in particular, designated as an oxygen atom at (27·5/30, 1/3) was well resolved on each projection and corresponded to a peak at (27·5/30, 9/15, 42/120) on the three-dimensional syntheses. It was therefore decided to compute structure factors and another three-dimensional synthesis phasing on the now refined positions of the sulphur atoms and this one 'oxygen' atom. Before describing this work, however, brief mention will be made of the direct method approach which was tried in parallel with the study of the (010) projection by trial and error.

(b) Direct Methods

Direct methods involve determining the phases of structure factors from mathematical relationships between their amplitudes. For space group $P2_1$ these methods are only applicable to the centrosymmetric (0l0) projection in which the phases are either positive or negative (0 or π radians) since the relationships used are effective only for these two phases.

To make use of sign relationships one normally uses unitary structure factors, instead of the simple structure factors. The unitary structure factor for each reflection is the ratio of the observed amplitude to the maximum possible amplitude. The observed structure amplitudes for the (0l0) projection were converted to unitary structure factors from

$$U_{hl} = F_{hl} / \sum_{j=1}^{N} f_j \exp -3\sin^2\theta/\lambda^2$$

where U_{hl} is the unitary structure factor for reflection (h0l).

Reflection (400) had the highest unitary structure factor this being 0·53. The method used in the determination of the phases is best described as Woolfson's Hit-or-Miss Method (70). Three of the largest independent reflections, namely (400),(00$\bar{4}$) and (302) had remained unchanged in phase throughout all of the various/

/various structure factor calculations and these were all π radians giving each reflection negative sign. By using the triple product sign relationship $s(h) \times s(h') \times s(h+h') \approx 1$ where $\approx$ means 'probably equals' and s(h) represents the sign of a structure factor of index (h), it was possible to determine probable signs of 58 reflections with unitary structure factors greater than 0·25. For example:

$$s(400) \times s(004) \times s(404) \approx 1$$

giving $s(404) \approx (+)$. No attempt was made to assess the probability of the relationships holding. Figure XXIX shows the electron density projection obtained from the structure factors phased in this way. It bears many similarities to the projection phased on the sulphur positions, but did not help in the solution of the structure. This, however, was not entirely unexpected as an electron density synthesis derived from only 58 out of a total of 238 non-zero (h0l) reflections could not be relied on to give a clear picture of the electron density distribution, even although they were amongst the strongest reflections and even assuming that all of the phases were correct.

In fact 74% of the 58 signs so determined were correct. An attempt was made to extend the triple product relationship to reflections with unitary structure factors between 0·15 and 0·25 but the relationship failed and the work was not extended.

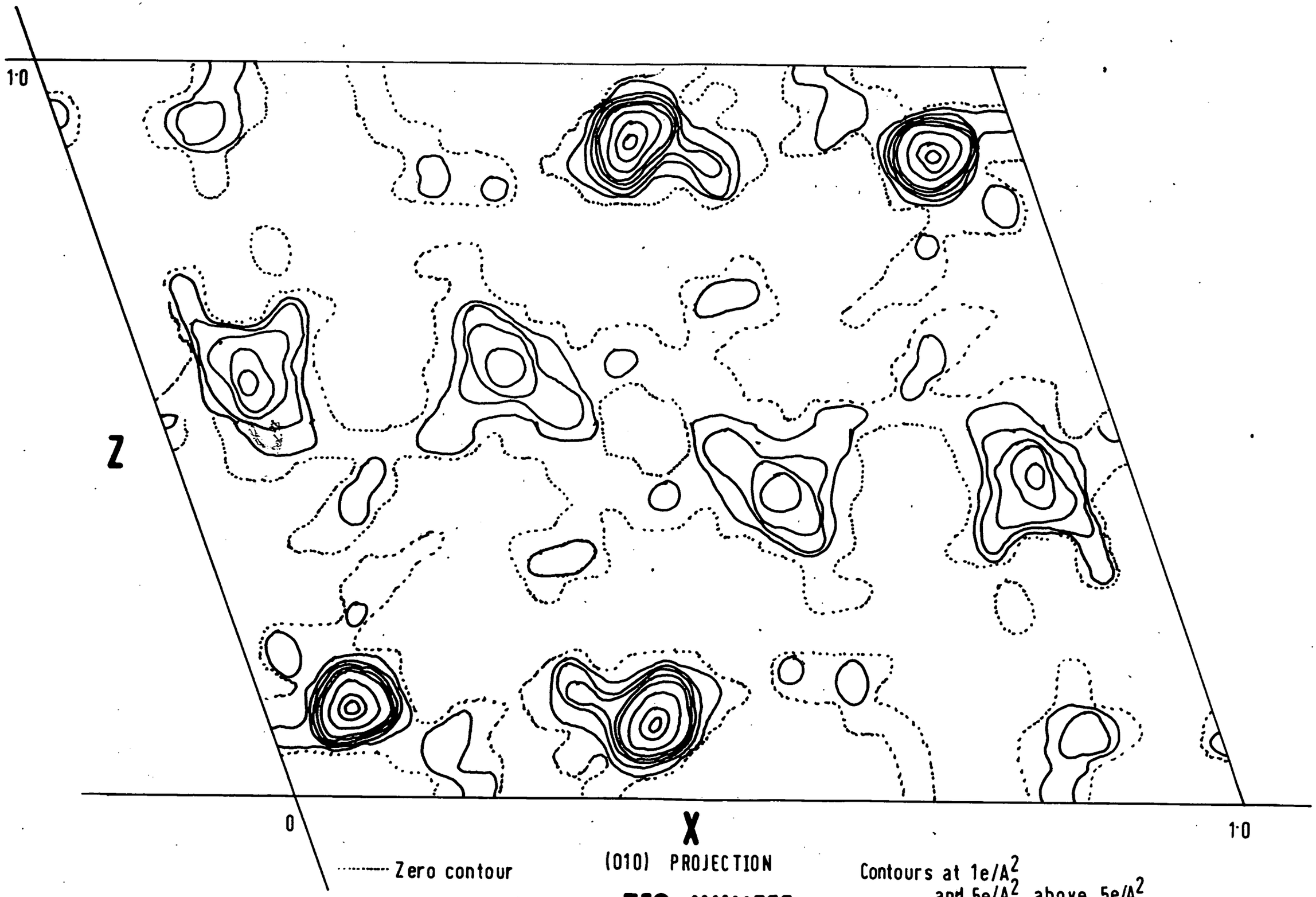
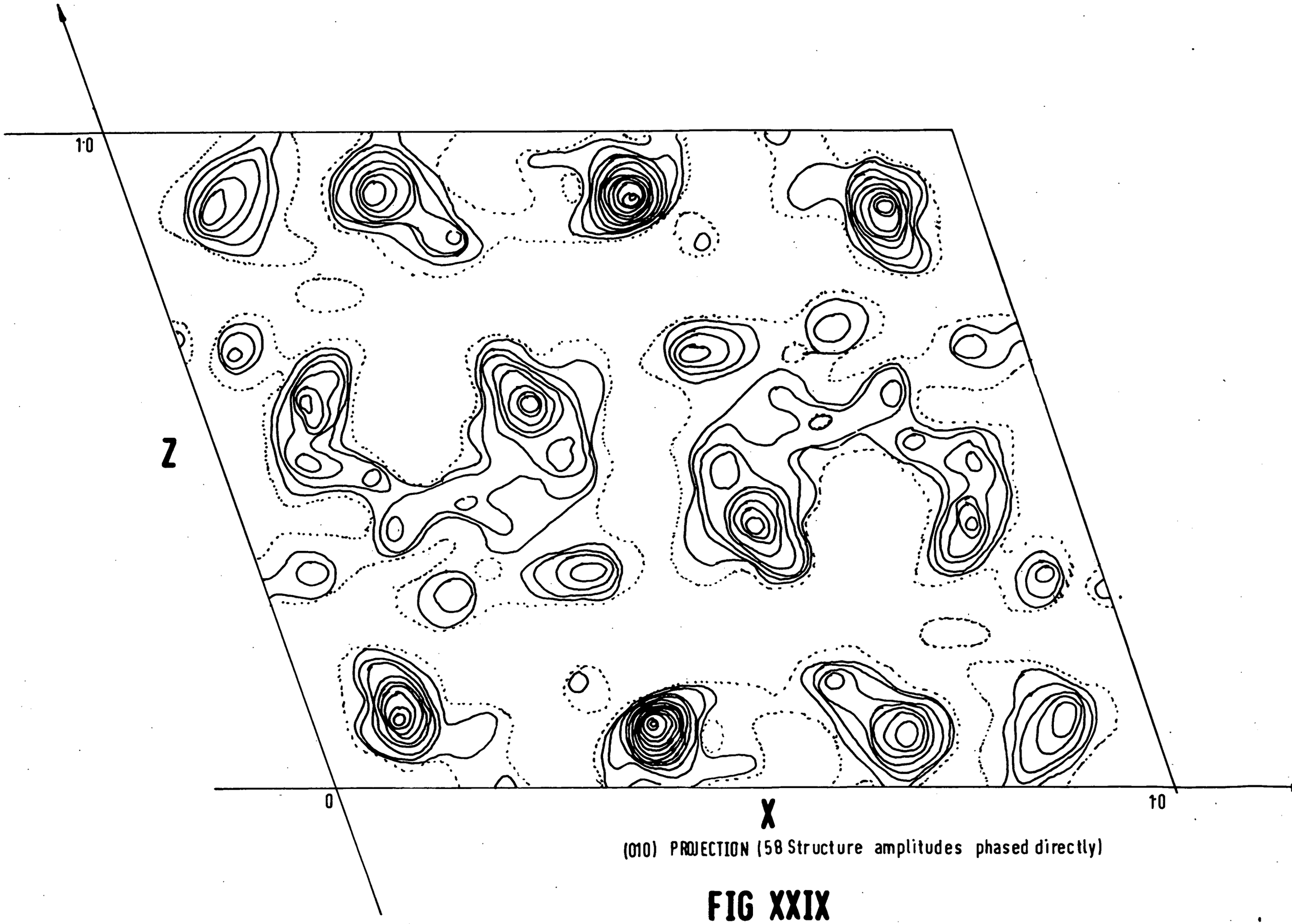

1·0
Z
0
X
1·0
Zero contour
(010) PROJECTION
Contours at 1e/A2
and 5e/A2 above 5e/A2

FIG XXVIII



(010) PROJECTION (58 Structure amplitudes phased directly)

FIG XXIX

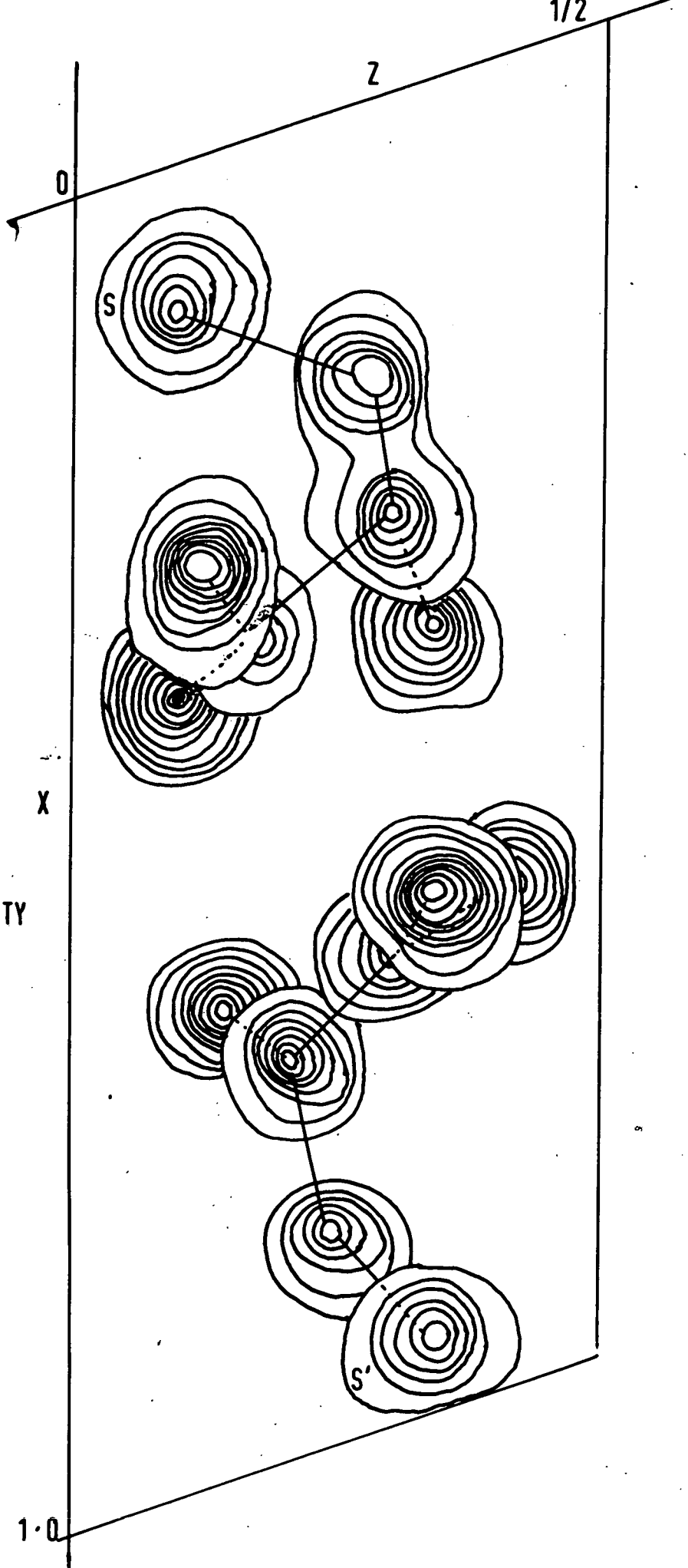
SECTION VI

The Solution of The Structure

It was decided to compute structure factors and a three-dimensional electron density synthesis phasing on the two sulphur atoms and the atom at (27·5/30, 9/15, 42/120) (page 90). The residual obtained was 45% and the synthesis showed numerous peaks between three and four electrons per cubic Angstrom. A further synthesis was computed phasing on a total of nine atoms placed tentatively in the positions of the highest peaks in the first synthesis. This had a residual of 40% and a difference synthesis was also computed. It now became apparent that if the sulphur atom at (12·4/30, 0, 13/120) was not in fact a sulphur but the oxygen of a carboxyl group in accordance with the peaks which were resolved on this synthesis, then one cysteine molecule of which the other sulphur was part would fit the electron distribution very well. The atom at (27·5/30, 9/15, 42/120) which had been thought to be an oxygen atom had a peak height of 14electrons/$Å^3$ and a positive difference peak of 2 electrons/$Å^3$ compared with the other suspected oxygen atoms which had peak heights of 10 electrons/$Å^3$. It was found that if this atom was regarded as the other sulphur atom then the other cysteine molecule could be fitted quite well.

The only tentative atom used in the electron density synthesis phasing which did not fit with this structure had a negative difference peak of 2 electrons/$\mathring{A}^3$ and this atom was presumed to be quite wrong. The only reason for doubting this new interpretation was that both of the original sulphur atoms had the same peak height and appeared equally good on the Fourier map and the difference map. It was, however, decided to try this new interpretation and structure factors and an electron density synthesis were computed. There was an immediate drop in the residual to 34% and the large discrepancies in the low angle reflections disappeared. It was apparent that the solution of the structure had been obtained. A corresponding difference synthesis indicated shifts of some of the atoms but had no large features suggesting that the trial structure was wrong in any other way. It was therefore decided to proceed with refinement. The composite electron density synthesis is shown in Figure XXX.

Contours at 1 e./Å^3 starting at 1. except sulphurs contoured at 5e./Å^3 starting at zero



COMPOSITE ELECTRON DENSITY

FIG XXX

SECTION VII

Refinement of the Structure

Refinement of the structure of L-cysteine was carried out by the block-diagonal least squares method (Part I, SectionVI). No attempt was made to fix the y-origin and it was allowed simply to float; the final y-coordinates are therefore purely relative with arbitrary origin. The progress of the refinement is shown in the following table.

After cycle No.	R%	$\Sigma wA^2 \times 10^{-4}$	Scale factor	Anisotropic Atoms	H atoms
Input:	33·3	160	4·87	---	---
1	27·8	117	5·79	---	---
2	25·0	89	5·43		
3	24·1	80	5·07	---	---
4	23·7	79	5·02	---	---
5	14·6	28	4·81	All	6
6	15·0	27	4·66	"	"
7	15·8	30	4·64	"	"
8	14·8	26	4·64	"	"
9	15·4	--	4·60	"	"

The refinement was halted at this point as convergence seemed to be practically complete.

In parallel with this refinement, cycles 5 to 9 were calculated with only the sulphur atoms anisotropic after some adjustments were made to rationalise the temperature factors obtained from cycle 4. This showed the following progress.

After cycle No.	R%	$\Sigma w\Delta^2 \times 10^{-4}$	Scale factor	Anisotropic Atoms	Hydrogen Atoms
Input	27·0	98	5·02	---	---
5	17·1	44	5·27	2S	6
6	16·6	34	4·77	2S	6
7	18·5	42	4·70	2S	6
8	17·1	36	4·72	2S	6
9	16·9	--	4·68		

The results from both of these refinement procedures are given in the following section.

Hydrogen atoms

Only six of the fourteen hydrogens of the two molecules were included in the structure factor calculations, there being too much uncertainty in the positions of the nitrogen and sulphur hydrogens to allow the postulation of probable positions. The positions of the six hydrogen atoms included are given below, the temperature factor of each was taken arbitrarily as 3.

Hydrogen	on Atom	x	y	z
H1	C2	0·383	0·833	0·370
H2	C3	0·163	0·787	0·270
H3	C3	0·173	0·080	0·345
H1^{1}	C2^{1}	0·677	0·773	0·125
H2^{1}	C3^{1}	0·867	0·987	0·325
H3^{1}	C3^{1}	0·877	0·987	0·185

A list of the final observed and calculated structure factors from the positions obtained from the final cycle of least squares refinement in which all atoms were anisotropic is given in Appendix IV.

SECTION IX

Results

Positional and Thermal Parameters from First Refinement

The final atomic positions in fractional coordinates obtained from the refinement in which all of the atoms were allowed anisotropic vibration are listed below together with their estimated standard deviations.

Atom	x	$\sigma(x)\times10^4$	y	$\sigma(y)\times10^4$	z	$\sigma(z)\times10^4$
S	0·0974	4	0·1805	12	0·1242	5
S^1	0·9199	2	0·6150	5	0·3232	3
C3	0·1946	6	0·0156	14	0·2871	7
C3^1	0·8353	5	0·9130	15	0·2760	6
C2	0·3179	5	0·9928	11	0·2863	5
C2^1	0·6980	4	0·8809	11	0·2154	4
C1	0·3450	4	0·8784	9	0·1548	4
C1^1	0·6448	4	0·7912	11	0·3292	5
N	0·3996	4	0·2439	10	0·3319	5
N^1	0·6396	4	0·1352	9	0·1554	4
O1	0·3976	3	0·0085	8	0·0871	3
O1^1	0·6240	3	0·9522	8	0·4133	3
O2	0·3047	4	0·6560	9	0·1234	4
O2^1	0·6264	4	0·5502	8	0·3454	4

The anisotropic β_{ij} values are as follows:

Atom	β_{11}	β_{22}	β_{33}	β_{23}	β_{31}	β_{12}
S	0·00490	0·04895	0·01755	-0·00774	0·00569	0·00115
S¹	0·00703	0·04472	0·02781	0·02592	0·01436	0·01228
C3	0·01657	0·04377	0·01715	-0·03902	0·02964	-0·03272
C3¹	0·00269	0·03189	0·00923	-0·00549	0·00312	-0·00612
C2	0·01144	0·01355	0·00644	-0·00616	0·01613	-0·00684
C2¹	0·00902	0·04092	0·00339	-0·00504	0·00953	-0·00642
C1	0·01147	0·00944	0·00316	-0·00866	0·00661	-0·00499
C1¹	0·00364	0·01641	0·00370	0·00503	0·00002	-0·00318
N	0·00787	0·01858	0·00290	-0·01543	0·00548	-0·00566
N¹	0·00907	0·02109	0·00275	0·01388	0·00468	-0·00227
O1	0·00910	0·03322	0·00530	0·00085	0·01091	0·00310
O1¹	0·01136	0·01837	0·00550	0·01094	0·01224	0·01093
O2	0·01185	0·01774	0·00541	-0·01034	0·01082	-0·00335
O2¹	0·01255	0·00892	0·00821	-0·00224	0·01542	-0·00708

Converted to B_{ij} values these are

Atom	B_{11}	B_{22}	B_{33}	B_{23}	B_{31}	B_{12}
S	2·32	5·37	5·68	-1·46	2·22	0·26
S¹	3·32	4·91	8·99	4·89	5·61	2·80
C3	7·83	4·81	5·55	-7·36	11·59	-7·46
C3¹	1·27	3·50	2·98	-1·03	1·22	-1·39
C2	5·41	1·49	2·08	-1·16	6·31	-1·56
C2¹	4·26	4·49	1·10	-0·95	3·73	-1·46
C1	5·42	1·04	1·02	-1·63	2·58	-1·14
C1¹	1·72	1·80	1·20	0·95	0·01	-0·72
N	3·72	2·04	0·94	-2·91	2·14	-1·29
N¹	4·29	2·32	0·89	2·62	1·83	-0·52
O1	4·30	3·65	1·71	0·16	4·27	0·71
O1¹	5·37	2·02	1·78	2·06	4·79	2·49
O2	5·60	1·95	1·75	-1·95	4·23	-0·76
O2¹	5·93	0·98	2·66	-0·42	6·03	-1·61

Bond Lengths and Angles

The bond lengths and angles calculated from these preceding parameters are given below; the e.s.d.'s, being calculated from the position variances obtained from the final least squares cycle, were 0.01Å° for all bonds and 0·5° for the bond angles.

			Å°				Å°
S	-	C3	1·807	S^1	-	$C3^1$	1·817
C3	-	C2	1·427	$C3^1$	-	$C2^1$	1·504
C2	-	C1	1·510	$C2^1$	-	$C1^1$	1·485
C2	-	N	1·593	$C2^1$	-	N^1	1·517
C1	-	O1	1·226	$C1^1$	-	$O1^1$	1·239
C1	-	O2	1·254	$C1^1$	-	$O2^1$	1·299

					∠°						∠°
S	-	C3	-	C2	111·9	S^1	-	$C3^1$	-	$C2^1$	114·1
C3	-	C2	-	C1	120·9	$C3^1$	-	$C2^1$	-	$C1^1$	112·9
C3	-	C2	-	N	115·4	$C3^1$	-	$C2^1$	-	N^1	109·1
N	-	C2	-	C1	106·8	N^1	-	$C2^1$	-	$C1^1$	108·3
C2	-	C1	-	O1	119·1	$C2^1$	-	$C1^1$	-	$O1^1$	118·0
C2	-	C1	-	O2	114·0	$C2^1$	-	$C1^1$	-	$O2^1$	121·1
O1	-	C1	-	O2	126·8	$O1^1$	-	$C1^1$	-	$O2^1$	120·8

On inspection of the bond lengths and also the temperature factors of the atoms it was discovered that these were not altogether satisfactory. In particular atom C3 bonded to S has rather high vibrational parameters and the bond length C3 - C2 is much shorter than expected. Both of the sulphur atoms have fairly large temperature factors and the positional accuracy of the first/

/first sulphur is only half that of the second. It was decided that, on account of these discrepancies the positional parameters were not the best obtainable and that it would be worth looking at the results of the second refinement in which only the sulphur atoms were allowed anisotropic vibration. These results turned out to be extremely satisfactory giving good bond lengths and rational temperature factors. The parameters from this refinement are listed below and should be taken as the final ones.

Positional and Thermal Parameters from Second Refinement

Atom	x	$\sigma(x)\times10^{-4}$	y	$\sigma(y)\times10^{-4}$	z	$\sigma(z)\times10^{-4}$	B
S	0·0946	4	0·1768	15	0·1173	6	-
S¹	0·9202	2	0·6135	6	0·3234	3	-
C3	0·1953	6	0·0102	15	0·2874	7	2·57
C3¹	0·8343	5	0·9060	13	0·2749	6	2·78
C2	0·3262	5	0·9942	13	0·2926	6	1·29
C2¹	0·6963	5	0·8774	13	0·2131	6	1·59
C1	0·3447	5	0·8832	11	0·1558	5	1·71
C1¹	0·6491	5	0·7903	13	0·3290	6	2·03
N	0·3950	4	0·2470	10	0·3291	5	1·53
N¹	0·6439	4	0·1322	11	0·1552	5	1·55
O1	0·3986	4	0·0096	10	0·0857	4	1·62
O2	0·3033	4	0·6552	11	0·1207	5	2·81
O1¹	0·6245	4	0·9530	9	0·4132	4	2·10
O2¹	0·6286	4	0·5459	10	0·3413	5	3·28

The vibrational parameters for the sulphur atoms are given below.

	β_{11}	β_{22}	β_{33}	β_{23}	β_{31}	β_{12}
S	0·00669	0·05125	0·01731	-0·00896	0·00775	-0·00060
S¹	0·00839	0·05143	0·02686	0·02688	0·01801	0·01356

	B_{11}	B_{22}	B_{33}	B_{23}	B_{31}	B_{12}
S	3·16	5·63	5·60	-1·68	3·03	-0·14
S¹	3·97	5·65	8·68	5·07	7·04	3·09

Bond Lengths and Angles from Second Refinement

			Å				Å
S	-	C3	1·795	S¹	-	C3¹	1·800
C3	-	C2	1·493	C3¹	-	C2¹	1·510
C2	-	C1	1·504	C2¹	-	C1¹	1·515
C2	-	N	1·524	C2¹	-	N¹	1·494
C1	-	O1	1·241	C1¹	-	O1¹	1·226
C1	-	O2	1·289	C1¹	-	O2¹	1·298

The estimated standard deviations of these bond lengths were calculated from the position variances and were all found to be slightly less than or equal to 0·01Å, including the sulphur-carbon bonds. This value is probably an underestimation.

The bond angles are:

	∠°		∠°
S - C3 - C2	115·1	S^1 - $C3^1$ - $C2^1$	115·8
C3 - C2 - C1	114·9	$C3^1$ - $C2^1$ - $C1^1$	112·7
C3 - C2 - N	113·7	$C3^1$ - $C2^1$ - N^1	107·5
N - C2 - C1	108·8	N^1 - $C2^1$ - $C1^1$	108·8
C2 - C1 - O1	119·8	$C2^1$ - $C1^1$ - $O1^1$	117·3
C2 - C1 - O2	116·0	$C2^1$ - $C1^1$ - $O2^1$	116·6
O1 - C1 - O2	124·2	$O1^1$ - $C1^1$ - $O2^1$	125·6

SECTION X

Discussion of Results

The final bond lengths and angles obtained agree well with the results from the similar structures as listed in Tables IV and V, and there is good agreement for these parameters between the two independent molecules in crystalline cysteine itself.

The major difference between the molecules of L-cysteine and the cysteine residues in other compounds lies in the conformation of the sulphur atom which can vary by rotation about the C3 - C2 bond. This is shown by the angle between the planes of S - C3 - C2 and C2- C3 - N. This angle was calculated for the majority of the compounds listed in Section I and was found to vary between 55° and 75°. The angles obtained for the two molecules of cysteine were 72° and 190°, respectively, and thus it would seem that the position of the sulphur atom in relation to the rest of the molecule and favoured in similar compounds only occurs in one of the two independent molecules of L-cysteine. The preference the sulphur atom has for this position must therefore be overruled by packing considerations and it would seem that it is only because the favoured position has also been the best position from packing considerations in other compounds that alternative conformations have not been found previously.

The projections of the molecules on the best planes through (C2, C1,O1,O2) and (C2^1,C1^1,O1^1,O2^1) are shown in Figure XXXIII. The (C2,C1,O1,O2) groups are quite coplanar with the amino nitrogens lying a little way out of the planes at distances of 0·35 and 0·85Å respectively.

There is no really significant difference between the carbon - oxygen bond lengths, which are of the same values as found for other α-amino acids, showing that the amino acid is in the zwitterion form, this being assumed before refinement was commenced. Better agreement would possibly have been obtained if the amino nitrogen had been given a form factor equivalent to N^+, but this was not done.

Hydrogen Bonding and Molecular Packing

The crystal structure is held together by a system of hydrogen bonding between the amino (NH_3^+) groups and carboxylic groups, and by van der Waals forces between the sulphur atoms. Projections of the cell showing the hydrogen bonding system are given in Figures XXXI and XXXII. Each amino group has four oxygen atoms at distances between 2·7 and 3·1Å in association. These give rise to two normal hydrogen/

/hydrogen bonds from one amino group together with a bifurcated hydrogen bond to the two carboxyl oxygens of one cysteine molecule. The amino group of the other cysteine molecule appears to make three normal hydrogen bonds which give a good tetrahedral arrangement, and it is possible that the fourth oxygen at the slightly longer distance is held by electrostatic attraction. The bifurcated hydrogen bond has been observed in several compounds including α-glycine (71,72) and β-glycine (73) in which the amino nitrogens form two normal hydrogen bonds and one bifurcated hydrogen bond. The hydrogen bonds found in L-cysteine are listed below.

N	-	O2 (x, y, z)	2·87	
N	-	$O1^{1}$(1-x, y-$^{1}/_{2}$, 1-z)	2·76	
N	-	$O1^{1}$(x, 1-y, z)	2·93	
N	-	$O2^{1}$(x, y, z)	3·08	or electrostatic
N^{1}	-	$O2^{1}$(x, y, z)	2·84	
N^{1}	-	O1 (x, y, z)	2·76	
N^{1}	-	O1 (1-x, y+$^{1}/_{2}$, -z)	2·94	Bifurcated
N^{1}	-	O2 (1-x, y-$^{1}/_{2}$, -z)	2·89	Bifurcated

It can also be seen from this table that every oxygen atom makes two close contacts with the amino nitrogens.

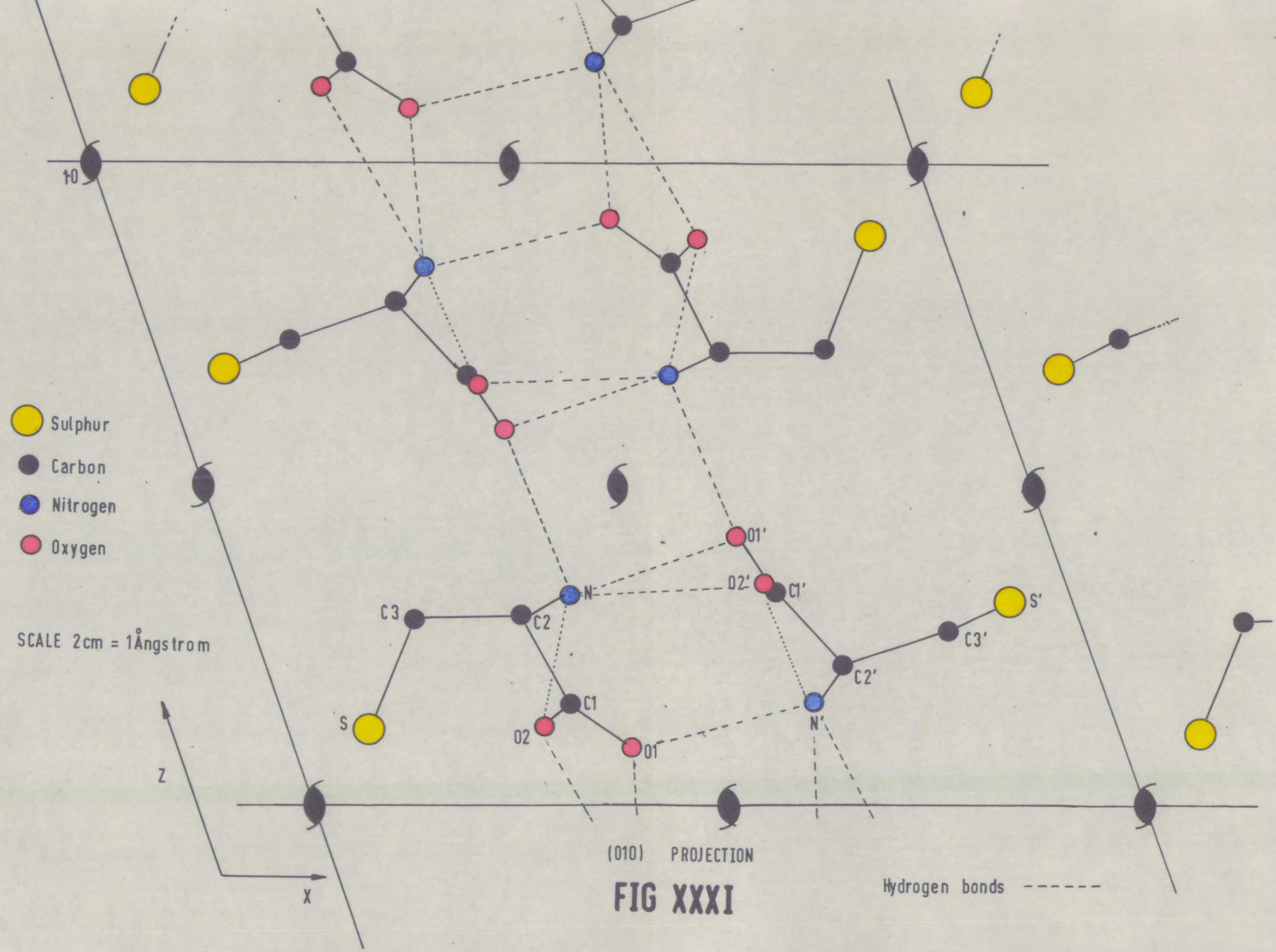
The three-dimensional structure, however, could not be held together by this hydrogen bonding system alone. The crystal structure is also held together by the sulphur atoms making van der Waals contacts with each other. The nearest approach distances of the sulphur atoms are listed below.

			Å
S	-	S $(-x, y-^1/_2, -z)$	3·76
S	-	S $(-x, y+^1/_2, -z)$	3·76
S	-	S^1 $(x-1, y, z)$	3·89
S^1	-	S^1 $(-x, y-^1/_2, -z)$	4·18
S^1	-	S^1 $(-x, y+^1/_2, -z)$	4·18
S	-	S^1 $(x-1, y-1, z)$	4·31
S	-	S^1 $(1-x, y-^1/_2, -z)$	4·27

The van der Waals radius of sulphur as reported by Pauling (55) is 1·85Å, giving an expected van der Waals contact distance of 3·7Å, although in hexagonal L-cystine (62) a sulphur - sulphur contact distance of 3·47Å has been found. However, as the sulphur atom in cysteine is in the form of an SH group it is reasonable to assume that the contact distances should be longer than those for sulphur alone.

The adjacent positions of the sulphur atoms explain the ease in oxidation of cysteine to cystine in the solid, crystalline state by the formation of a disulphide bridge. If the sulphur atoms were not adjacent but remote from each other it is hard/

/hard to see how cystine could be formed without the crystal lattice being first broken down by some external influence. In fact the reverse would seem to be true:- the formation of cystine through combination of adjacent sulphur atoms leads to the breakdown of the crystal lattice, this being observed in the latter stages of data collection. The large temperature factors of the sulphur atoms could also indicate that there is some slight disorder in their positions possibly due to movement nearer to each other as a preliminary to oxidation.



(010) PROJECTION

Hydrogen bonds ------

FIG XXXI

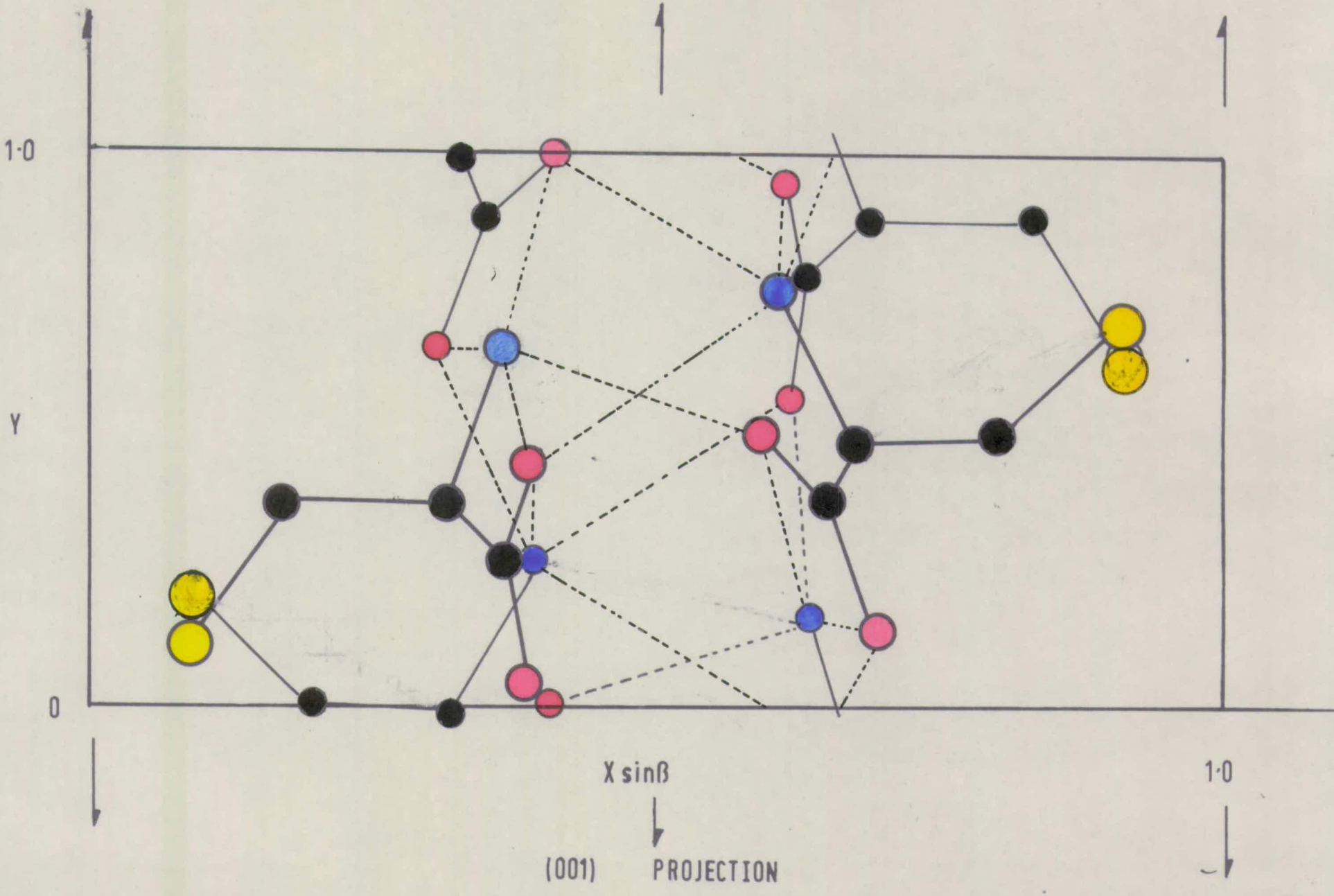
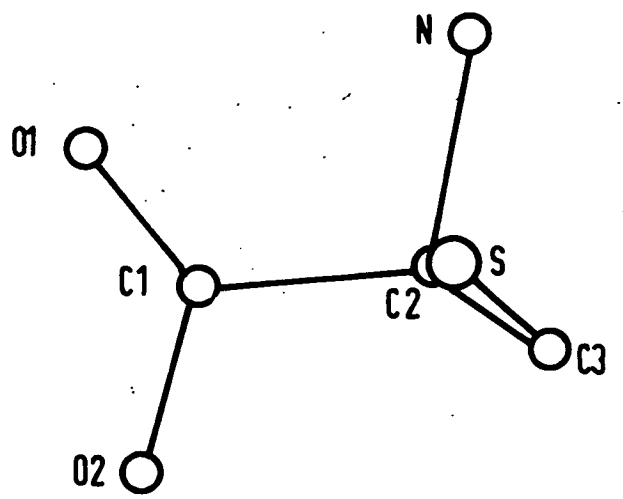
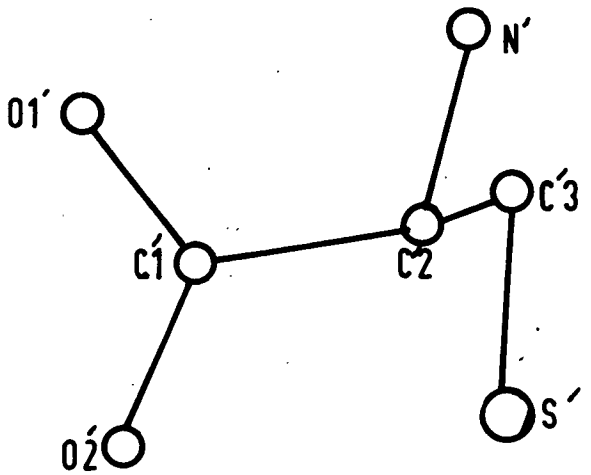


FIG XXXII





Projections on best planes of O1,O2,C1,C2 and O1',O2',C1',C2'

FIG XXXIII

SECTION XI

Conclusion

The L-cysteine molecule adopts two different conformations in the crystalline state, arising from rotation of the sulphur atom about the C3 - C2 bond. The structure is held together by a system of hydrogen bonding and contacts between the mercapto groups, which are adjacent, this presumably giving ease of oxidation to cystine in the crystalline state.

The solution of the structure would have been much simpler if the correct sulphur positions had been obtained in the first instance. The ambiguity arose through the sulphur S^1 and the oxygen O1 having overlapping Harker vectors. Cross-vectors for the final sulphur positions should appear on the 3D-Patterson synthesis at (0·4/30,2/30,53/120), (0·4/30,28/30,53/120) and (25/30,17/30,25/120), (25/30,13/30,25/120). On re-examining the Patterson these were found with values of $200e^2Å^{-3}$ and $190e^2Å^{-3}$ respectively; the latter peak, however, overlapped the edge of the Harker vector of S^1 making the correct solution less obvious than the original.

There are still a few features concerning the structure which require some explanation. The anisotropic temperature factors of the sulphur atoms are high and the position of the sulphur atom (S) did not refine well, as indicated by the positional estimated standard deviations. It is possible that there is some type of disorder in the position of this atom. This would certainly seem feasible since the van der Waals contacts between the sulphur atoms are fairly long and could almost certainly allow fractional movement. Refinement of the structure by the full matrix least squares method would be interesting, since this should give a clearer picture of any irregularities in the structure of L-cysteine.

A C K N O W L E D G E M E N T S

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APPENDIX I

Formfactors used in the Determination of the Structures of

(a) Bis-L-histidinato-cobalt(II) monohydrate

Cobalt $^{2+}$: Int. Tab. Cryst. Vol. III corrected for anomalous dispersion

Carbon, Nitrogen, Oxygen, Oxygen^{-}, Hydrogen : Int. Tab. Cryst. Vol. III.

(b) L-cysteine

Sulphur : A. Vaciago, Rend. Acc. Lineei 28, 851 (1960) corrected for anomalous dispersion.

Carbon : J. Berghius et al , Acta Cryst., 8, 478 (1955)

Nitrogen : A.J. Freeman, Acta Cryst., 12, 261 (1959)

Oxygen $^{1/2-}$: Average of O and O^{-}, ibid.

Hydrogen : R. McWeeny, Acta Cryst., 4, 513 (1951).

APPENDIX II

Observed and Calculated Structure Factors for Bis-L-histidinato-cobalt(II) monohydrate

k	h	l	31·38Fo	10Fc	l	31·38Fo	10Fc	l	31·38Fo	10Fc
0	0	1	89	73	2	1354	1098	3	177	168
		4	648	551	6	198	170			
0	2	-6	126	147	-5	222	193	-4	366	354
		-3	978	806	-2	907	787	-1	1450	1006
		0	279	128	1	933	721	2	154	128
		3	634	574	4	183	130	7	172	171
0	4	-7	140	139	-6	63	92	-5	54	65
		-4	133	134	-3	89	68	-2	94	58
		-1	193	175	0	932	560	1	198	138
		2	317	303	3	160	161	4	44	70
		5	154	150						
0	6	-5	99	95	-4	191	198	-3	426	399
		-2	210	197	-1	1008	655	1	264	238
		2	349	313	4	201	193	6	186	166
0	8	-6	147	150	-5	113	72	-4	279	262
		-2	77	51	-1	863	614	0	1134	756
		1	615	460	2	208	205	3	465	450
		4	83	103						
0	10	-5	140	130	-3	514	431	-2	94	100
		-1	964	716	0	257	168	1	169	147
		2	126	138	4	54	80	5	163	119
		6	163	156						
0	12	-6	144	136	-4	380	349	-2	217	206
		-1	299	261	0	585	437	1	315	257
		2	235	227	3	470	456	4	169	172
		5	109	91	6	169	132			
0	14	-5	175	162	-4	109	92	-3	299	252
		-2	196	201	-1	755	542	0	422	323
		1	809	623	2	210	177	3	412	399
		4	63	55	5	117	151	6	201	171
0	16	-4	154	133	-3	117	116	-2	117	125
		-1	63	23	0	117	92	1	137	136
		2	215	200	3	354	320	4	104	98
		5	166	140						
0	18	-2	157	158	-1	63	68	0	198	188
		1	126	107	2	509	432	3	104	84
		4	160	135	6	89	85			
0	20	-4	126	120	-3	206	203	-2	94	89
		-1	332	295	0	409	343	1	355	339
		3	366	352	5	257	213			
0	22	-5	104	112	-3	166	135	-2	117	136
		-1	154	165	0	559	485	1	203	196
		2	282	278	3	175	170	4	201	179

0	24	-4	122	100	-3	144	116	-2	89	123
		-1	77	106	0	147	133	1	129	154
		2	169	168	3	154	144			
0	26	-4	163	116	-2	89	116	0	217	235
		2	257	286	4	166	158			
0	28	-3	188	188	-1	344	361	1	309	301
		3	282	267						
0	30	-4	109	113	0	264	259	2	188	181
		4	122	119						
0	32	3	99	117						
0	34	4	77	79						
0	36	-1	147	132	3	126	111			
0	38	3	77	72						
1	1	-6	177	193	-4	424	436	-3	284	274
		-2	239	242	-1	488	469	0	856	673
		1	642	735	2	556	578	3	415	443
		4	208	228	5	160	174	6	89	144
		7	99	106						
1	3	-5	77	95	-4	44	51	-3	375	424
		-2	231	252	-1	296	230	0	366	218
		1	501	517	2	77	74	3	247	258
		4	54	66	6	154	143			
1	5	-4	89	90	-3	140	156	-2	150	138
		-1	322	293	0	549	453	1	99	121
		2	315	330	3	440	524	4	77	101
		5	126	146	6	126	94	7	140	113
1	7	-4	126	136	-3	157	159	-2	404	404
		-1	309	239	0	840	693	1	482	439
		2	358	322	3	99	111	4	268	294
		5	109	100	6	109	127			
1	9	-4	268	291	-3	177	180	-2	306	322
		-1	237	220	0	586	479	1	438	379
		2	198	202	3	351	381	4	104	122
		5	140	136	6	104	95			
1	11	-5	94	121	-4	169	143	-3	303	294
		-2	312	314	-1	177	165	0	930	760
		1	70	91	2	435	429	3	94	76
		4	175	187	5	122	114	6	113	93
1	13	-5	166	182	-4	186	172	-3	492	452
		-2	183	159	-1	763	714	0	427	372
		1	508	412	2	160	132	3	404	414
		4	104	94	5	183	169	6	144	91
1	15	-4	247	268	-3	191	209	-2	315	316
		-1	172	166	0	733	609	1	198	188
		2	478	454	3	126	123	4	222	217
		5	109	109	6	191	131			
1	17	-3	220	234	-1	251	251	1	166	151
		2	183	161	3	193	190	4	129	109
1	19	-4	109	116	-2	140	174	0	237	243
		1	140	153	2	117	117	3	275	279
		4	129	127	5	117	92			
1	21	-4	94	73	-3	206	238	-1	331	357
		1	226	214	2	70	44	3	113	113
		4	94	70						

1	23	-4	166	206	-3	133	130	-2	94	100
		0	137	160	3	54	44			
1	25	-3	122	137	-1	147	138	0	63	59
		2	129	135	3	117	135			
1	27	-4	133	137	-3	77	71	-2	172	200
		0	208	227	2	129	137	3	243	240
		4	89	99						
1	29	-3	133	144	-1	99	190	0	172	173
		1	183	187	2	180	182	3	109	108
		4	150	129						
1	31	0	104	113	3	99	89			
1	33	3	70	58	4	70	66			
1	35	3	104	104						
2	0	0	341	308	1	345	402	2	397	503
		3	217	279	4	175	216	5	137	178
		6	144	187						
2	2	-6	109	101	-5	186	191	-4	109	115
		-3	349	330	-2	147	171	-1	126	87
		0	347	358	1	612	678	2	203	263
		3	157	205	4	89	111	5	137	159
		6	144	154						
2	4	-6	137	135	-4	188	193	-3	99	112
		-2	389	376	-1	423	336	0	339	340
		1	468	493	2	109	117	3	217	270
		4	144	169	5	177	235			
2	6	-5	109	94	-4	94	104	-3	245	224
		-2	239	213	-1	150	129	0	251	241
		1	140	169	2	268	304	3	188	228
		4	180	209	5	140	175	6	54	75
2	8	-6	150	130	-4	222	184	-2	210	215
		-1	291	288	0	220	243	1	147	135
		2	188	218	3	84	94	4	83	93
		5	140	137						
2	10	-5	117	86	-4	133	115	-3	245	225
		-2	157	168	0	147	141	1	222	205
		3	104	95	4	140	146	5	54	67
2	12	-4	186	156	-3	193	203	-2	315	280
		-1	133	150	0	281	299	1	203	206
		2	89	115	3	117	131	4	122	117
		5	140	131						
2	14	-5	177	158	-3	382	325	-2	133	131
		-1	379	286	0	428	370	1	312	274
		2	154	155	3	166	164	4	266	291
		5	122	123	6	191	132			
2	16	-4	241	258	-3	150	119	-2	277	262
		-1	113	128	0	398	332	1	264	230
		2	268	268	3	210	202	4	83	91
		5	147	119						
2	18	-5	94	133	-4	109	120	-3	293	263
		-1	325	299	0	157	170	2	177	173
		3	122	110	4	222	204	6	177	118
2	20	-4	163	161	-2	193	200	-1	226	255
		0	266	224	1	133	145	3	210	197
2	22	-4	70	86	-3	224	208	-2	83	144
		0	203	194	1	109	95	3	54	92

2	24	-4	129	128	0	77	83	1	117	131
		3	104	82						
2	26	-4	154	133	-3	150	140	-2	206	225
		0	160	155	2	201	181	3	63	69
		4	126	115						
2	28	-4	117	113	-3	154	127	-1	208	232
		1	126	149	3	169	158			
2	30	0	117	107						
2	32	3	99	72						
3	1	-5	140	117	-4	172	183	-3	160	174
		-2	533	473	-1	421	390	0	471	468
		1	354	361	2	286	359	3	99	113
		4	77	50	5	147	224			
3	3	-5	160	165	-4	133	164	-3	306	296
		-2	193	184	-1	175	165	0	298	313
		1	222	235	2	532	691	3	183	196
		4	196	240	6	89	111			
3	5	-4	144	130	-3	312	289	-2	396	376
		-1	450	410	0	485	496	1	268	299
		2	163	174	3	147	158	4	44	78
		5	89	111						
3	7	-5	245	231	-4	89	92	-3	281	244
		-2	183	173	-1	332	260	0	77	100
		1	166	181	2	122	134	3	89	93
		4	166	203	5	54	62	6	70	67
3	9	-6	175	159	-4	140	128	-3	191	169
		-2	198	214	-1	470	422	1	322	358
		2	193	210	4	122	116	5	83	103
3	11	-5	160	150	-4	231	224	-3	224	223
		-2	428	398	-1	382	299	0	177	167
		1	261	241	2	270	295	3	157	165
		4	166	205	5	137	142	6	113	105
3	13	-6	175	97	-5	77	137	-4	117	142
		-3	281	221	-1	499	393	0	70	83
		1	605	538	2	104	101	3	231	247
		4	70	46	5	169	182			
3	15	-4	213	188	-3	129	130	-2	126	299
		-1	272	228	0	304	296	2	224	209
		3	83	66	4	169	172	5	147	138
		6	144	104						
3	17	-4	94	110	-3	249	216	-2	104	103
		-1	144	160	0	193	197	1	279	276
		2	154	170	3	243	244	4	104	109
		5	140	120						
3	19	-4	70	72	-2	198	207	-1	175	181
		0	126	140	2	117	111	3	77	51
		4	169	167	5	70	50			
3	21	0	109	126	4	144	120			
3	23	-3	77	79	-2	126	173	0	160	172
		2	208	195						

3	25	-3	198	200	1	213	238	2	172	148
		3	175	153	4	109	108			
3	27	-2	169	204	0	264	256	2	160	181
		3	94	77	4	147	146			
3	29	-3	113	95	3	113	92	4	122	106
3	31	3	117	106						
4	0	0	511	491	2	245	302	3	104	124
		4	188	248						
4	2	-5	186	154	-3	210	208	-2	169	177
		-1	642	537	0	169	203	1	654	675
		2	160	180	3	288	350	4	99	96
4	4	-4	188	229	-2	462	457	0	413	408
		1	208	219	2	341	409	3	191	219
		4	133	174	5	83	121			
4	6	-4	94	104	-1	491	399	0	83	120
		1	261	248	2	239	273	3	129	137
		4	63	62						
4	8	-4	117	108	-3	54	78	-2	289	259
		-1	126	123	0	147	160	2	150	159
		5	89	98						
4	10	-5	169	178	-3	99	125	-1	263	252
		0	163	165	1	237	262	2	286	339
		3	140	177	4	99	134			
4	12	-3	160	166	-2	430	397	-1	196	206
		0	318	350	1	237	233	2	309	355
		3	154	183	4	99	96	5	177	176
4	14	-3	163	138	-2	231	241	-1	328	332
		0	154	160	1	351	336	2	335	349
		3	104	94	4	177	178			
4	16	-4	117	86	-3	129	105	-2	301	287
		-1	169	186	0	264	234	1	388	389
		2	253	276	3	94	95	5	147	152
4	18	-3	150	149	-2	220	240	-1	201	221
		0	44	92	1	133	142	2	257	285
		3	77	87	4	150	148			
4	20	0	89	92	1	282	286	2	70	82
		3	129	135	5	177	146			
4	22	-3	77	82	-2	157	153	0	175	199
		1	117	125	2	104	132	4	140	151
4	24	-3	188	170	1	354	360	3	154	165
4	26	-2	193	218	0	208	217	2	160	182
		4	150	151						
4	28	-3	140	102	3	77	99			
4	30	4	70	101						
4	32	3	117	122						
5	1	-4	160	146	-3	140	147	-2	104	105
		-1	133	135	0	63	75	1	235	251
		2	169	156	3	140	133	5	157	164
5	3	-4	172	171	-3	154	151	-2	270	243
		-1	277	261	0	198	200	1	133	158
		2	309	329	3	70	83	4	180	204

5	5	-3	83	56	-2	220	205	-1	237	212
		1	272	284	2	54	97	3	247	261
		5	177	187						
5	7	-3	150	136	-2	198	184	-1	175	188
		0	99	125	1	183	189	2	172	204
		3	54	35	4	104	108			
5	9	-4	180	177	-2	188	173	0	133	146
		1	275	276	2	70	74	3	70	101
		5	99	118						
5	11	-4	133	113	-2	304	284	-1	237	176
		0	222	252	2	304	323	4	183	189
5	13	-4	117	90	-3	172	140	-2	113	113
		-1	325	311	0	63[illegible]	101	[illegible]	282	274
		3	166	211	4	147	149	5	70	114
5	15	-4	122	127	-3	117	91	-2	315	302
		0	309	312	2	284	303	3	54	87
		4	140	147						
5	17	-3	233	239	-2	83	95	-1	263	247
		1	293	302	3	113	103	4	104	71
5	19	-3	144	117	-2	183	203	2	129	123
		3	63	59	4	137	122			
5	21	-3	77	65	4	129	131			
5	23	-2	94	112	0	113	111	2	140	133
5	25	-3	163	147	-2	104	134	0	126	117
		2	117	124	3	70	101			
5	27	-2	104	139	0	109	99	3	70	76
6	0	0	237	248	2	270	279	4	109	112
6	2	-5	233	184	-4	133	78	-3	231	200
		-2	163	136	-1	169	178	0	83	78
		1	156	180	2	70	86	3	140	126
		4	133	127						
6	4	-2	312	296	0	160	143	1	226	230
		2	228	269	3	104	90	4	122	125
6	6	-4	70	80	-3	253	223	-2	140	121
		-1	196	180	0	122	122	1	77	109
		2	117	95	3	144	134	4	157	159
6	8	-4	186	139	-3	63	85	-2	113	112
		0	94	84						
6	10	-4	70	89	-3	341	291	-1	160	138
		4	54	78						
6	12	-4	180	140	-2	126	128	0	89	108
		1	140	145	2	104	43	3	172	159
		4	83	133						
6	14	-3	175	131	-1	188	163	0	144	164
		1	117	139	4	150	146			
6	16	-4	188	184	-2	157	140	3	122	125
6	18	0	129	135						
6	20	3	109	98						
6	22	0	89	100	4	99	67			
6	24	0	77	82	3	99	79			

7	1	-4	196	160	-3	160	114	-2	175	187
		-1	160	157	0	144	163	1	166	197
		2	160	162	3	183	197	4	104	102
7	3	-3	188	171	-2	157	131	-1	160	152
		0	113	119	1	77	140	2	129	146
		3	113	108	4	104	79			
7	5	-3	175	154	0	44	51	2	177	184
		3	144	137						
7	7	-3	77	56	-1	188	160	0	186	188
		3	117	111	4	122	113			
7	9	-4	109	92	-3	104	100	-2	183	140
		2	150	156	3	117	101			
7	11	-2	163	114	0	144	154	2	186	183
		4	109	105						
7	13	-3	201	129	0	129	118	1	126	142
		3	140	120						
7	15	-3	133	102	0	206	205	2	109	124
		4	133	117						
7	17	-3	113	104	0	44	53	3	94	78
7	19	3	117	98						
7	21	-3	83	67	0	133	113			
7	23	0	126	111						
7	25	-3	94	88						
8	0	0	251	241	3	63	94	4	129	125
8	2	-4	77	72	-3	188	152	-1	201	143
		0	44	37	1	126	137	3	154	136
8	4	0	163	157	3	109	86	4	63	71
8	6	0	70	72	3	169	149	4	94	95
8	8	-4	117	97	0	196	190	2	140	153
		3	109	128						
8	10	-1	183	162	2	144	142	3	147	139
8	12	-2	206	171	0	201	188	2	117	119
		3	133	121						
8	14	0	70	80	2	169	158	3	70	70
8	16	0	154	158	2	89	91			
8	18	0	77	82	2	129	136			
8	22	0	83	79						
9	1	-3	117	98	0	140	146	3	117	99
9	3	-3	117	102	0	160	147			
9	5	-3	117	99	3	99	77			
9	7	-3	117	73	0	94	121			
9	15	0	113	106						

APPENDIX III

Cobalt-histidine: Unobserved Reflections

k	h	l	$Kx^{1}/_{2}$ min.Fo	10Fc	l	$Kx^{1}/_{2}$ min.Fo	10Fc	l	$Kx^{1}/_{2}$ min.Fo	10Fc
0	0	5	39	69						
0	2	-7	49	65	5	39	60	6	44	59
0	4	6	44	0	7	49	99			
0	6	-6	45	33	0	19	8	5	40	38
0	8	-3	32	2	5	41	36	6	45	21
0	10	-6	46	3	-4	37	33	3	33	51
0	12	-5	42	2	-3	34	24			
0	14	-6	48	9						
0	16	-5	44	23						
0	18	-6	50	15	-5	46	77	-4	42	46
		-3	39	12	5	46	33			
0	20	-5	47	45	4	44	18			
0	22	-4	45	64	5	49	45			
0	24	4	47	12						
0	26	-3	46	3	-1	43	94	1	43	6
		3	46	16						
0	28	-4	50	1	-2	46	35	0	44	91
		2	46	10	4	50	50			
0	30	-3	50	95	-2	48	104	-1	47	42
		1	47	27						
0	32	-4	54	42	-3	52	92	-2	50	64
		-1	49	105	1	49	99	2	50	11
		4	54	16						
0	34	-4	56	126	-3	54	27	-2	52	80
		-1	51	31	0	51	142	1	51	28
		2	52	92	3	54	68			
0	36	-2	54	67	0	53	6	1	53	93
		2	54	14						
0	38	-3	57	91	-2	56	55	-1	55	24
		0	55	72	2	56	103			
1	1	-7	49	9	-5	39	81			
1	3	-6	44	92	5	40	23			
1	5	-7	50	25	-6	45	103	-5	40	31
1	7	-6	45	87	-5	40	59			
1	9	-6	46	124	-5	41	65			
1	11	-6	46	25						
1	13	-6	47	58						
1	15	-6	48	102	-5	44	89			
1	17	-4	41	29	-2	35	101	0	33	54
		5	45	81						
1	21	-5	48	121	-2	39	66	0	37	50
1		5	48	70						
1	23	-1	40	18	1	40	75	2	41	98
		4	46	73						
1	25	-4	48	23	-2	43	41	4	48	32
1	27	44	44	124	-1	44	122			
1	29	-4	51	24	-2	47	85			
1	31	-4	53	104	-3	51	28	-2	49	72
		-1	48	62	1	48	107	2	49	58
		4	53	21						

1	33	-4	55	40	-3	53	87	-2	51	57
		-1	50	64	0	50	100	1	50	20
		2	51	100						
1	35	-3	55	60	-2	53	33	-1	52	133
		0	52	90	1	52	63	2	53	56
2	4	-5	40	51	6	45	101			
2	6	-6	45	91						
2	8	-5	41	106	-3	32	55	6	46	39
2	10	-1	26	72	2	29	63			
2	12	-5	43	76						
2	14	-6	48	58						
2	16	-5	45	61						
2	18	-6	50	83	-2	36	63	1	35	66
		5	46	24						
2	20	-5	47	38	-3	41	47	2	38	63
		4	44	57	5	47	60			
2	22	-1	39	75	2	40	40	4	45	25
2	24	-3	44	79	-2	42	115	-1	41	141
		2	42	83	4	47	22			
2	26	-1	43	59	1	43	88			
2	28	-2	46	24	0	45	56	2	46	67
		4	50	47						
2	30	-4	52	49	-3	50	91	-2	48	75
		-1	47	27	1	47	51	2	48	90
		3	50	18	4	52	49			
2	32	-3	52	73	-2	50	31	-1	49	59
		0	49	25	1	49	67	2	50	63
3	3	-6	45	7	5	40	27			
3	7	-6	46	65						
3	9	-5	42	75	3	33	74	6	46	66
3	11	-6	47	106						
3	13	-2	32	65	6	48	61			
3	15	-6	49	75	-5	44	96	1	32	45
3	17	-5	46	90						
3	19	-5	47	63	-3	40	81	1	36	37
3	21	-4	45	51	-3	42	21	-2	40	31
		-1	38	75	1	38	88	2	40	66
		3	42	52						
3	23	-1	40	88	1	40	67	3	44	35
3	25	-4	48	28	-2	43	48	-1	42	191
		0	42	84						
3	27	-4	50	79	-3	47	68	-1	44	78
		1	44	112						
3	29	-4	52	12	-2	47	43	-1	46	180
		0	46	81	1	46	142	2	47	31
3	31	-4	53	77	-3	51	27	-2	49	140
		-1	48	102	0	48	101	1	48	123
		2	49	97	4	53	43			
4	0	1	17	51	5	40	130			
4	2	-4	35	55	5	40	110			
4	4	-5	41	67	-3	31	29	-1	20	97
4	6	-3	31	91	-2	27	18			
4	8	-5	42	21	1	24	34	3	32	33
		4	37	51						
4	10	-4	38	52	-2	30	115	5	42	44
4	12	-5	43	94	-4	39	66			
4	14	-4	40	71						

4	16	-5	45	49	4	42	81			
4	18	-5	47	89	-4	43	37	5	47	24
4	20	-5	48	32	-4	44	31	-3	41	81
		-2	39	38	-1	37	102	4	44	15
4	22	-4	46	2	-1	39	138	3	43	57
4	24	-2	43	170	-1	42	110	0	41	66
		2	43	65						
4	26	-4	49	95	-3	47	62	-1	44	181
		1	44	54						
4	28	-2	47	85	-1	46	87	0	45	33
		1	46	158	2	47	46			
4	30	-4	53	61	-3	50	13	-2	49	90
		-1	48	32	0	47	138	1	48	27
		2	49	120	3	50	32			
4	32	-3	52	115	-2	51	41	-1	50	63
		0	49	62	1	50	159	2	51	41
5	1	-5	41	50	4	36	55			
5	5	-5	41	50	-4	36	70	4	36	32
5	7	-4	37	56						
5	9	-5	42	87	-3	34	81	-1	26	56
		4	38	25						
5	11	-3	35	15	1	28	90	3	35	61
5	13	-5	44	109	2	33	65			
5	15	-1	32	138	1	32	21			
5	17	-4	43	42	0	34	18	2	36	83
5	19	-4	44	60	-1	37	27	0	36	79
		1	37	99						
5	21	-4	46	82	-2	40	75	-1	39	89
		0	38	61	1	39	95	2	40	34
		3	43	3						
5	23	-1	41	66	1	41	112			
5	25	-1	43	176	1	43	128			
5	27	-1	45	140	1	45	84	2	46	71
6	0	1	17	72	3	31	70			
6	2	5	41	135						
6	4	-4	37	70	-3	32	68	-1	20	102
6	8	-1	25	77	1	25	147	2	29	88
		3	33	42	4	38	76			
6	10	-2	30	39	0	26	14	1	27	132
		2	30	55	3	35	59			
6	12	-3	36	86	-1	30	42			
6	14	-4	41	39	-2	34	97	2	34	22
6	16	-3	39	77	-1	34	125	0	33	87
		1	34	104	2	36	86	4	43	28
6	18	-2	38	117	-1	36	79	1	36	29
		2	38	98						
6	20	-3	42	20	-2	40	80	-1	38	34
		0	38	27	1	38	51	2	40	75
6	22	-3	44	48	-2	42	29	-1	40	49
		1	40	51	2	42	89	3	44	29
6	24	-3	46	63	-2	44	100	-1	42	145
		1	42	74	2	44	32			

7	3	-4	37	48						
7	5	-2	27	72	-1	22	85	1	22	103
7	7	-4	38	44	-2	28	76	1	24	94
		2	28	102						
7	9	-1	26	76	0	25	30	1	26	114
		4	39	48						
7	11	-4	40	113	-3	36	82	-1	29	53
		1	29	18	3	36	91			
7	13	-2	34	80	-1	31	191	2	34	11
7	15	-2	36	117	-1	34	13	1	34	34
		3	39	37						
7	17	-2	38	38	-1	36	121	1	36	121
		2	38	14						
7	19	-3	42	37	-2	40	48	-1	38	32
		0	37	71	1	38	92	2	40	73
7	21	-2	42	106	-1	40	177	1	40	114
		2	42	54	3	44	82			
7	23	-3	46	86	-2	43	137	-1	42	80
		0	42	111	1	42	30	2	43	81
		3	46	48						
7	25	-2	45	30	-1	44	108	0	44	38
		1	44	78	2	45	44	3	48	77
8	0	1	18	47	2	26	101			
8	2	-2	26	31	2	26	58	4	38	43
8	4	-4	38	94	-3	33	40	-2	27	46
		-1	21	30	1	21	61	2	27	22
8	6	1	23	86	2	28	67			
8	8	-3	35	64	-2	30	51	-1	26	59
		1	26	74	4	39	7			
8	10	-3	36	44	-2	32	42	0	27	76
		1	28	125						
8	12	-3	37	35	-1	31	69	1	31	54
8	14	-3	39	128	2	35	110	1	33	191
		1	33	147						
8	16	-2	37	95	-1	35	105	1	35	148
8	18	-2	39	48	-1	37	89	1	37	39
9	1	-2	26	110	-1	19	118	1	19	110
		2	26	73						
9	3	-2	27	72	-1	20	47	1	20	31
		2	27	100	3	33	81			
9	5	-2	28	54	-1	22	147	0	19	95
		1	22	76	2	28	52			
9	7	-2	30	72	-1	25	48	1	25	32
		2	30	95						

APPENDIX IV

Observed And Calculated Structure Factors

For L-cysteine

h	k	l	4·68Fo	10Fc	l	4·68Fo	10Fc	l	4·68Fo	10Fc
0	0	1	87	69	2	512	655	3	196	174
		4	490	510	5	351	342	6	118	125
		7	99	111	8	41	2	9	96	74
		10	22	16	11	49	55			
1	0	-11	83	57	-8	100	80	-7	62	41
		-6	219	215	-5	119	126	-4	277	252
		-3	173	177	-2	336	423	-1	71	41
		0	338	179	1	96	75	2	452	563
		3	178	156	4	54	10	5	70	60
		6	163	150	7	37	131	8	52	40
		10	49	37	11	23	5			
2	0	-12	41	24	-10	64	66	-9	74	62
		-7	125	114	-6	68	101	-5	85	88
		-4	338	427	-3	274	304	-2	253	258
		-1	91	65	0	196	163	1	55	53
		2	51	70	3	65	57	4	163	163
		5	34	50	6	19	17	10	73	57
3	0	-12	50	36	-11	51	53	-10	47	36
		-9	68	54	-8	151	123	-7	123	99
		-6	129	105	15	109	90	-4	278	361
		-3	89	98	-2	422	519	-1	131	144
		0	493	529	1	157	139	2	515	494
		3	166	162	4	179	157	5	42	2
		6	116	107	7	92	62	8	32	29
		9	49	28	10	22	30			
4	0	-12	88	88	-11	83	75	-10	33	23
		-9	44	45	-8	157	174	-7	173	194
		-6	224	279	-5	159	153	-4	219	236
		-3	22	18	-2	458	552	-1	101	114
		0	745	854	1	147	100	2	169	156
		3	209	150	4	315	326	5	143	106
		6	63	31	7	61	44	8	40	63
		9	70	62	10	17	47			

5	0	-12	119	149	-9	61	52	-8	108	124
		-7	84	105	-6	47	17	-5	67	68
		-4	18	9	-3	298	292	-2	92	67
		-1	168	127	0	171	154	1	252	236
		2	86	107	3	104	114	4	129	81
		5	75	67	6	36	13	7	17	51
		8	60	51	9	49	17			
6	0	912	39	65	-11	37	31	-10	112	88
		-9	171	178	-8	41	49	-7	119	135
		-6	98	72	-5	267	297	-4	220	233
		-3	252	276	-2	116	59	-1	134	173
		0	99	133	1	32	92	2	85	49
		3	90	58	4	106	103	6	20	54
		7	36	20						
7	0	-12	72	69	-11	52	41	-10	65	39
		-8	183	188	-6	160	172	-5	63	50
		-4	169	160	-3	224	201	-2	141	138
		-1	84	40	0	309	248	1	108	99
		2	257	194	3	20	3	4	140	125
		5	50	19	6	100	98	7	88	44
		8	46	19						
8	0	-11	94	78	-10	41	43	-8	129	129
		-7	60	54	-6	70	73	-5	33	47
		-4	32	23	-3	34	46	-2	67	81
		-1	32	22	1	28	15	2	205	207
		4	115	121	5	48	33	6	49	20
		7	58	55						
9	0	-10	34	15	-9	51	42	-8	40	5
		-7	132	150	-8	40	5	-7	132	150
		-6	155	165	-5	49	49	-4	37	41
		-3	249	260	-2	127	103	0	25	16
		1	141	140	2	105	95	5	50	51
		6	50	26						
10	0	-11	29	36	-10	111	95	-9	66	67
		-8	62	62	-7	45	58	-6	99	94
		-5	173	185	-4	33	9	-3	48	55
		-2	133	132	-1	194	207	0	145	131
		2	119	80	4	80	85	5	43	17
11	0	-10	46	53	-9	29	2	-8	28	4
		-7	76	65	-5	113	107	-4	21	25
		-3	46	25	-2	65	46	-1	103	111
		0	159	134	1	69	72	2	18	26
		3	35	9	4	118	98			
12	0	-10	12	18	-8	50	16	-6	13	18
		-5	91	110	-4	68	51	-1	64	28
		0	89	85	2	77	86			

13	0	-9	37	39	-8	45	44	-7	50	35
		-6	48	34	-5	92	98	-4	12	2
		-3	126	135	-2	40	25	-1	64	32
		0	63	54	1	81	63	2	41	62
14	0	-7	44	62	-6	41	38	-4	34	25
		-3	76	101	-2	64	58	-1	62	68
		0	8	10						
0	1	1	414	463	2	386	415	3	169	126
		4	133	135	5	135	124	6	109	93
		7	92	82	8	106	99	9	100	57
		10	49	36						
1	1	-11	53	56	-9	126	100	-8	44	40
		-7	35	26	-6	112	90	-5	201	155
		-4	129	112	-3	379	333	-2	402	470
		-1	250	262	0	311	366	1	242	203
		2	153	140	3	363	393	4	101	97
		5	207	197	6	80	57	7	161	144
		8	72	37	9	112	92	10	59	42
		11	50	58						
2	1	-11	62	84	-10	57	66	-9	88	81
		-8	139	127	-7	257	263	-6	58	69
		-5	150	139	-4	152	157	-3	484	505
		-2	318	332	-1	247	236	0	308	344
		1	457	530	2	230	201	3	211	220
		4	193	190	5	146	122	6	67	52
		7	73	97	8	76	63	9	71	60
		10	39	24						
3	1	-12	42	42	-11	57	43	-10	79	66
		-9	213	181	-8	62	43	-7	153	117
		-6	72	60	-5	377	374	-4	280	258
		-3	291	274	-2	216	173	-1	223	216
		0	500	598	1	221	214	2	119	123
		3	133	102	4	270	285	5	158	130
		6	76	85	7	48	41	8	73	59
		9	74	64	10	29	40			
4	1	-12	25	31	-11	43	7	-10	69	53
		-9	120	122	-8	84	85	-7	113	96
		-6	57	63	-5	68	73	-4	144	122
		-3	402	390	-2	294	267	-1	58	21
		0	126	133	1	217	208	2	349	357
		3	83	53	4	57	77	5	66	60
		6	121	94	7	108	91	8	62	42
5	1	-12	17	21	-11	9	21	-10	72	75
		-9	80	77	-8	68	81	-7	105	100
		-6	30	45	-5	72	48			
		-4	168	188	-3	147	106	-2	141	129
		-1	146	137	0	113	128	1	170	123
		2	135	146	3	166	156	4	61	58
		5	50	14	6	81	62	7	93	75
		9	41	39						

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		-9	116	109	-8	97	90	-7	225	239
		-6	107	118	-5	147	131			
		-4	103	92	-3	391	371	-2	207	219
		-1	151	125	0	124	58	1	253	261
		2	236	209	3	105	110	4	53	46
		5	68	30	6	93	72	7	32	15
		8	51	31						
7	1	-11	105	86	-10	0	29	-9	111	100
		-8	99	80	-7	144	156	-6	118	121
		-5	178	180	-4	101	84	-3	140	120
		-2	272	270	-1	147	174	0	233	228
		1	101	37	2	185	167	3	55	55
		4	200	194	5	93	74	6	61	23
		7	64	34	8	50	52			
8	1	-11	80	76	-10	78	70	-9	94	79
		-8	41	44	-7	52	62	-6	75	64
		-5	167	180	-4	110	99	-3	182	168
		-2	110	97	-1	149	139	0	183	161
		1	47	35	2	102	87	3	57	52
		4	90	69	5	59	28	6	26	26
		7	45	30						
9	1	-11	42	36	-10	62	65	-8	35	48
		-7	166	158	-6	52	25	-5	182	201
		-4	77	68	-3	47	66	-2	38	31
		-1	98	88	0	169	169	1	48	24
		2	94	85	3	97	62	4	53	37
		5	24	11	6	32	32			
10	1	-11	54	59	-10	46	53	-9	60	42
		-8	76	50	-7	145	127	-6	106	84
		-5	139	114	-4	20	36	-3	154	150
		-2	42	33	-1	44	46	0	142	89
		1	101	94	2	80	72	4	75	47
		5	35	20						
11	1	-10	23	25	-9	70	66	-8	81	59
		-7	87	86	-6	66	34	-5	72	51
		-4	118	91	-3	134	130	-2	162	127
		-1	47	50	0	32	6	1	47	39
		2	115	119	3	32	20	4	43	51
12	1	-9	46	33	-8	68	67	-7	92	85
		-6	59	27	-5	61	44	-3	94	92
		-2	99	70	-1	49	58	0	28	36
		1	37	28	2	67	46	3	17	23
13	1	-8	37	27	-7	80	70	-6	56	31
		-5	103	105	-4	58	33	-3	65	43
		-2	40	27	-1	93	86	0	82	74
		1	24	14						

14	1	-7	41	40	-6	22	34	-5	67	80
		-4	23	22	-3	20	22	-2	37	24
		-1	63	75						
0	2	0	315	357	1	207	181	2	32	51
		3	130	118	4	272	264	5	197	179
		6	150	163	7	50	43	8	94	71
		9	107	88	10	54	54	11	25	42
1	2	-11	38	30	-10	123	109	-9	42	26
		-8	55	55	-7	112	102	-6	117	95
		-5	121	93	-4	205	217	-3	113	98
		-2	111	107	-1	151	155	0	268	288
		1	113	108	2	223	228	3	188	185
		4	61	64	5	129	121	6	113	107
		7	118	121	8	64	55	9	68	44
		10	34	32						
2	2	-11	52	61	-10	67	77	-9	123	97
		-8	70	53	-7	49	26	-6	75	101
		-5	95	96	-4	144	133	-3	119	105
		-2	232	270	-1	31	11	0	173	186
		1	156	142	2	415	484	3	48	68
4		4	97	94	5	83	69	6	123	137
		7	64	57	8	98	95	9	43	43
		10	53	52						
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		-8	73	68	-7	74	64	-5	121	127
		-4	136	155	-3	144	138	-2	258	262
		-1	230	236	0	28	33	1	237	224
		2	145	134	3	89	84	4	37	43
		5	122	91	6	95	97	7	103	84
		10	40	45						
4	2	-11	73	72	-10	96	115	-9	62	44
		-8	85	90	-5	93	103	-6	176	173
		-5	120	95	-4	36	25	-3	159	179
		-2	321	363	-1	147	145	0	143	137
		1	65	59	2	175	167	3	99	75
		4	119	136	5	44	45	6	50	64
		7	108	64	8	41	25	9	39	29
5	2	-11	27	10	-10	77	71	-9	36	48
		-8	64	48	-7	82	95	-6	99	70
		-5	88	90	-4	201	210	-3	78	59
		-2	173	186	-1	136	151	0	264	258
		1	139	99	2	227	159	3	118	113
		4	183	180	5	153	132	6	117	80
		7	75	72	8	68	53	9	31	31

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		-8	46	42	-7	42	36	-6	37	39
		-5	207	226	-4	101	76	-3	92	104
		-2	118	105	-1	156	139	0	253	249
		1	127	127	2	240	241	3	35	38
		4	63	52	5	44	8	6	66	63
		7	64	31	8	25	35			
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		-8	61	59	-7	63	55	-6	68	76
		-5	113	114	-4	103	89	-3	179	175
		-2	103	88	-1	186	178	0	141	127
		1	88	65	2	171	140	3	48	53
		4	60	43	5	35	30	6	63	48
		7	41	28						
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		-8	46	32	-7	128	131	-6	68	51
		-5	86	78	-4	114	122	-3	149	160
		-2	194	210	-1	140	123	0	45	40
		1	53	30	2	66	77	3	116	61
		4	38	9	5	47	16	6	30	9
		7	28	32						
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		-8	75	67	-7	109	112	-6	73	50
		-5	66	40	-4	116	114	-3	112	114
		-2	174	174	-1	40	21	0	45	61
		1	80	82	2	172	130	3	33	24
		4	55	55	5	42	56	6	41	48
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		-7	133	114	-6	72	58	-5	186	165
		-4	88	77	-3	77	53	-2	109	83
		-1	88	77	0	129	100	1	76	54
		2	46	6	3	32	35	4	58	53
		5	29	22						
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		-6	46	27	-5	153	127	-4	88	60
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		0	109	82	1	39	21	2	62	39
		3	41	58	4	23	30			
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		-6	33	29	-5	49	52	-4	51	30
		-3	62	61	-2	97	69	0	51	45
		2	24							
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		-4	51	48	-3	60	67	-2	41	41
		-1	32	49	0	24	34			
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		-2	29	32						

0	3	1	266	280	2	216	195	3	38	20
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		7	57	60	8	30	12	9	91	77
		10	47	37						
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		-7	115	113	-6	155	165	15	39	56
		-4	145	143	-3	172	164	-2	85	96
		-1	97	87	0	178	138	1	114	126
		2	257	276	3	140	133	4	46	35
		5	139	149	6	85	83	7	95	89
		8	24	20	9	46	37	10	40	53
2	3	-10	50	49	-9	74	47	-8	43	36
		-7	84	92	-6	165	180	-5	204	219
		-4	188	208	-3	99	78	-2	234	268
		-1	124	108	0	355	350	1	168	163
		2	86	86	3	57	54	4	126	131
		5	153	148	6	140	114	7	77	62
		8	45	46	9	51	49			
3	3	-10	57	45	-9	36	21	-8	69	63
		-7	28	26	-6	79	73	-5	44	46
		-4	75	83	-3	132	136	-2	70	57
		-1	38	39	0	165	188	1	123	125
		2	76	75	3	112	100	4	110	103
		5	75	76	6	72	74	7	105	101
		8	38	29	9	48	44			
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		-8	48	49	-7	83	87	-6	80	57
		-5	181	193	-4	67	65	-3	59	54
		-2	151	131	-1	210	226	0	58	59
		1	83	110	2	137	124	3	84	88
		4	71	69	5	62	81	6	36	42
		7	41	50	8	18	29			
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		-7	89	92	-6	130	134	-5	52	54
		-4	88	92	-3	150	150	-2	124	123
		-1	46	7	0	115	110	1	80	81
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		5	92	74	6	79	74	7	36	20
		8	18	10						
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		-7	67	51	-6	121	135	-5	124	110
		-4	122	145	-3	26	8	-2	149	163
		-1	116	115	0	35	33	1	50	64
		2	54	71	3	40	23	4	62	66
		5	52	36	6	70	43	7	41	55
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		-7	102	100	-6	39	44	-5	51	31
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		-1	29	8	0	99	95	1	52	72
		2	81	84	3	58	10	4	60	56
		5	86	67	6	34	25			

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		-7	51	44	-6	117	101	-5	126	142
		-4	26	24	-3	109	86	-2	39	21
		-1	159	173	0	104	97	1	55	64
		2	52	27	3	84	81	4	91	77
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		-7	64	76	-6	94	77	-5	87	57
		-4	90	74	-3	126	96	-2	165	129
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		-5	35	27	-4	66	63	-3	61	73
		-2	10	28	-1	90	93	0	79	79
		1	24	21	2	43	58	3	24	39
		4	9	26						
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		-5	85	83	-3	62	70	-2	57	51
		-1	61	47	0	32	27	1	34	55
		2	49	60						
12	3	-7	40	66	-6	41	42	-5	25	42
		-4	54	60	-3	84	82	-2	36	26
		-1	43	59	0	32	35	1	36	61
13	3	-5	38	55	-4	44	61	-2	39	66
0	4	0	94	95	1	101	100	2	86	73
		3	166	180	4	89	101	5	72	53
		6	92	90	7	88	75	8	90	71
		9	31	35						
1	4	-9	97	110	-8	59	54	-7	66	53
		-6	25	17	-5	174	185	-4	111	108
		-3	212	224	-2	60	31	-1	187	180
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		-6	41	34	-5	75	62	-4	75	98
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3	4	0	13	16	1	135	148	2	50	36
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		7	31	40						
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		-6	55	57	-5	159	163	-4	75	64
		-3	83	73	-2	105	96	-1	95	90
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		0	26	34						
12	4	-3	5	32						

0	5	1	72	81	2	67	66	3	44	22
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		0	93	113	1	76	59	2	98	102
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		7	40	34						
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		-5	38	18	-4	132	112	-3	68	63
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		-5	69	43	-4	126	102	-3	67	56
		-2	146	147	-1	83	68	0	21	33
		1	74	77	2	48	44	3	82	84
		6	42	32						
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		-4	80	65	-3	87	89	-2	44	37
		-1	77	66	0	51	51	1	79	76
		2	24	28	3	68	68	4	52	38
		5	25	32						
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		-3	44	37	-1	53	53	0	101	100
		2	56	52	4	73	66	5	28	19
6	5	-7	22	39	-6	49	56	-5	52	43
		-2	114	96	-1	81	72	0	82	64
		1	52	61	2	42	39	3	15	19
		4	35	49						
0	6	1	70	59	2	24	15	3	46	57
		4	19	22	5	7	16			
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		1	72	68	3	62	52	4	61	65
2	6	-4	26	42	-3	49	46	-1	32	24
		0	34	26	2	41	39	3	24	21
		4	42	55						
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		0	27	36	1	32	33	2	23	30
		3	31	42						

4	6	-5	22	33	-4	24	33	-3	48	42
		-2	14	6	0	21	17	1	65	68
		2	29	37	3	21	45			
5	6	-4	31	35	-3	26	25	-2	41	53
		-1	25	25	0	34	42			
6	6	-3	22	37	-2	26	30	-1	37	53

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The Structures of Bishistidino-nickel(II), -cobalt(II), and -cadmium(II)

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THE above complexes were prepared from the metal carbonates and either L- or DL-histidine ($C_3H_3N_2{\cdot}CH_2{\cdot}CH(NH_2){\cdot}COOH$), and crystallised from aqueous solutions.

The racemic nickel compound, $Ni(C_6H_8N_3O_2)_2$, H_2O, is orthorhombic, *Aba*2, with $a = 15{\cdot}18$, $b = 13{\cdot}05$, $c = 7{\cdot}72$ Å, $Z = 4$. Its structure was solved with three-dimensional *X*-ray intensity data (Cu—K_α radiation), and after least-squares refinement of the parameters, the agreement factor, *R*, is 0·075. There are equal numbers of molecules of bis-L-histidinonickel and bis-D-histidinonickel. The nickel atoms lie on two-fold axes and are octahedrally co-ordinated by two nitrogens and an oxygen of each histidine group. The bond angles around the nickel atom are all within 10° of the ideal values of 90° and 180°.

Bis-L-histidinocobalt(II), $Co(C_6H_8N_3O_2)_2,H_2O$, is monoclinic, C_2, with $a = 29{\cdot}44$, $b = 8{\cdot}32$, $c = 6{\cdot}35$ Å, $\beta = 90°$, $Z = 4$. Its structure was solved from electron density projections and the parameters are being refined using three-dimensional *X*-ray intensity data (Mo—K_α radiation). At present $R = 0{\cdot}14$. The configuration of the molecule (which does not lie on a crystallographic two-fold axis) is similar to that in the nickel complex above. The average distances are Co–N(1) 2·14, Co–N(2) 2·17, Co–O(1) 2·13 Å. The conformations of the two histidine groups are slightly different (by up to 10° in dihedral angles) from each other and from that in the nickel complex.

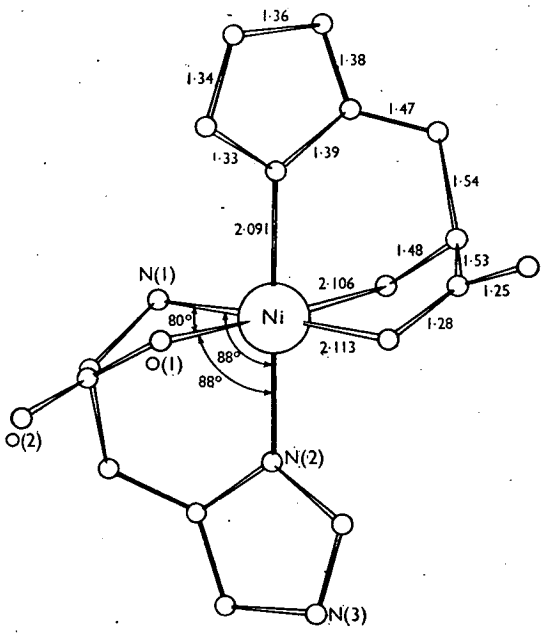
The cell dimensions and (010) electron density projection of bis-L-histidinonickel show that its crystal structure is very like that of bis-L-histidinocobalt. Racemic bis-histidinocobalt crystallises in space group $P2_1/c$, different from both the above structures; it is being studied further.

Bis-L-histidinocadmium is tetragonal, $P4_12_12$, with $a = 7{\cdot}39$, $c = 30{\cdot}5$ Å, and a structure similar to that of the corresponding zinc compound.[1] The (100) electron density projection gave approximate values for all the atomic positional parameters. The metal atoms are on two-fold axes. In the zinc compound the co-ordination group is essentially tetrahedral [to N(1) and N(2) of each histidine] and the carboxyl oxygen atom, O(1) is loosely associated. In the cadmium compound the histidine groups have tilted to allow these six atoms to be at more nearly equal distances from the metal atom.

Cd–N(1) 2·26, Cd–N(2) 2·25, Cd–O(1) 2·49 Å, (e.s.d. ~ 0·05 Å)

Zn–N(1) 2·05, Zn–N(2) 2·04, Zn–O(1) 2·79 Å, (e.s.d. < 0·02 Å)

In no case is the metal atom quite in the plane of the imidazole group co-ordinated to it; the displacements from the imidazole plane range from 0·13 Å in the nickel compound to 1·1 Å in the cadmium compound.



(*Received, June 22nd,* 1965; *Com.* 386.)

[1] R. H. Kretsinger, F. A. Cotton, and R. F. Bryan, *Acta Cryst.*, 1963, **16**, 651.