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The Crystal Structure of L-Ergothioneine Dihydrate, $C_9H_{15}N_3O_2S.2H_2O$

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Crystals of L-ergothioneine dihydrate are monoclinic, space group $P2_1$, with $a=7{\cdot}171(2)$, $b=6{\cdot}129(2)$, $c=15{\cdot}051(5)$ Å, $\beta=90{\cdot}83(2)°$, $Z=2$. The structure was deduced from Patterson and minimum functions, and refined by block-diagonal least-squares calculations. The final R value is 0·057 for 2461 non-zero reflexions. The tautomer obtained assumes a thione form, with no evidence for the coexistence of a thiol form. The two nitrogen atoms of the imidazole ring are protonated. The quaternary nitrogen atom and the imidazole ring are *trans* to one another across the C^{α}–C^{β} bond, the torsion angle being 168·6°. The ring is planar together with S and C^{β}. The molecules are linked by hydrogen bonds N–H···O and N–H···S, and O–H···O *via* water molecules.

Introduction

L-Ergothioneine is the only naturally occurring compound discovered to date that contains the imidazole-2-thione moiety (Stowell, 1961). It was first isolated from ergot, the fungus infection of rye grain, and later from many microbial, plant and animal sources. Interest in this compound was greatly stimulated by its discovery in blood (Hunter & Eagles, 1925; Benedict, Newton & Behre, 1926; Eagles & Johnson, 1927). The time sequence of biosynthetic reactions in fungi has been established (Askari & Melville, 1962), while its origin and function in the animal body remain to be established. Wide variation in the amounts of L-ergothioneine in different regions of the central nervous system may indicate that this compound has a role to play in certain areas of the system (Briggs, 1972). It is notable in this connexion that L-ergothioneine can have excitatory effects on the neurons in the brain stem (Avanzino, Bradley, Comis & Wolstencroft, 1966).

There are three possible tautomers, one imidazolethione and two imidazolethiol structures, as shown in Fig. 1. The purpose of this study is to obtain information on the molecular dimensions and conformation, and to compare them with those of related biologically active compounds.

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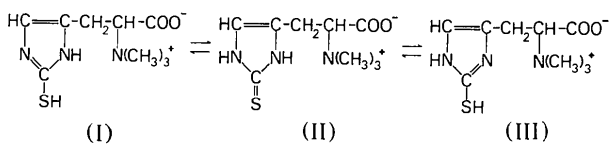

Fig. 1. Three possible tautomeric structures for L-ergothioneine.

Experimental

Crystals of ergothioneine dihydrate from aqueous solution are colourless, transparent regular prisms elongated along the b axis, with well developed faces. Since they gradually deteriorate on exposure to air, each crystal used for X-ray work was given a thin coating of collodion. The systematic absences in the X-ray spectra ($0k0$ with k odd) and the known optical activity of the compound ($[\alpha]_D+115°$, in water) uniquely defined the space group as $P2_1$. Unit-cell dimensions were obtained from 14 diffractometer settings, each collected at $\pm 2\theta$. The density was measured in a mixture of bromobenzene and chlorobenzene. The crystal data are given in Table 1.

Table 1. *Crystal data*

$C_9H_{15}N_3O_2S.2H_2O$; F.W. 265·33
Space group $P2_1$
$a=7{\cdot}171$ (2), $b=6{\cdot}129$ (2), $c=15{\cdot}051$ (5) Å, $\beta=90{\cdot}83$ (2)°
$V=661{\cdot}5$ Å^3
$D_m=1{\cdot}330$, $D_x=1{\cdot}332$ g cm^{-3} for $Z=2$
μ(Mo $K\alpha$) = 2·52 cm^{-1}
$F(000)=284$

The integrated intensities were collected at 20 ± 2 °C on a Rigaku automatic four-circle diffractometer with Zr-filtered Mo $K\alpha$ radiation. A single crystal with dimensions $0{\cdot}31\times0{\cdot}38\times0{\cdot}35$ mm was mounted with its b axis parallel to the φ axis of the goniostat. The θ–2θ scan mode with a constant scan speed of 4° min^{-1} (2θ) and a scan range of $(0{\cdot}80+0{\cdot}36\tan\theta)°$ in θ was employed. The background intensities were measured for 10 s at each end of the scan. Nickel foil attenuators were inserted as required to keep the maximum counting rate below 8000 c.p.s. Intensities of three standard reflexions were monitored every 50 reflexions to check the stability of the counting equipment. No systematic drift

was observed in three standards during data collection; the maximum variation in structure amplitudes was ±1·8 %. 2624 independent reflexions with 2θ less than 65° were scanned, of which 163 had zero intensities. The data were corrected for Lorentz and polarization factors, but no absorption correction was applied.

Structure determination and refinement

Trial positional parameters for the sulphur atom were obtained from analysis of a sharpened Patterson map, and a subsequent minimum function gave a pair of enantiomers with plausible structures. The enantiomer with correct absolute configuration was derived in the first Fourier map phased by the sulphur and the non-hydrogen atoms of the imidazole ring, and was confirmed by successive Fourier syntheses.

The structure was then refined by block-diagonal least-squares techniques to minimize the quantity $\sum w(|F_o|-|F_c|)^2$. When R reached 0·088, a difference Fourier synthesis revealed the positions of all hydrogen atoms. These hydrogen atoms were included in the subsequent cycles of refinement with variable isotropic thermal parameters. Convergence was achieved after an additional five cycles. The final R and $R2=[\sum w(|F_o|-|F_c|)^2/\sum w|F_o|^2]^{1/2}$ were 0·066 and 0·072, respectively, for all reflexions. R for 2461 non-zero reflexions was 0·057. The maximum parameter shifts in the last cycle were $0{\cdot}04\sigma$ for non-hydrogen atoms and $0{\cdot}08\sigma$ for

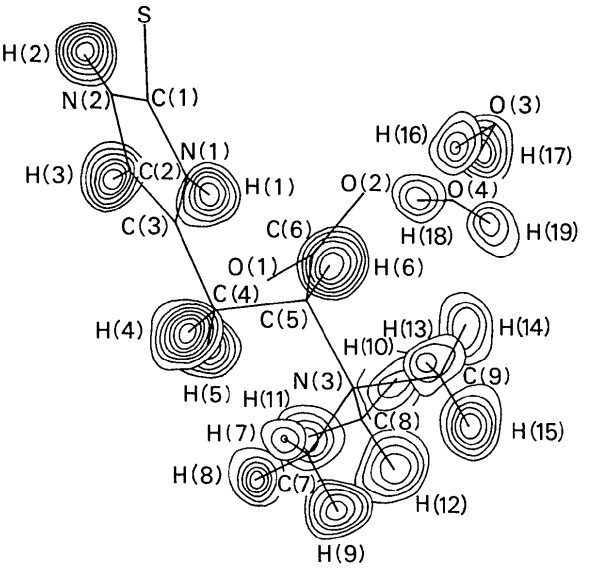

Fig. 2. Difference map showing the hydrogen atoms. Contours are drawn at intervals of 0·1 e Å^{-3}, starting at 0·3 e Å^{-3}.

Table 2. *Fractional atomic coordinates and thermal parameters with their e.s.d.'s*

(*a*) Non-hydrogen atoms. All values are $\times 10^4$. Thermal parameters are of the form

$$T=\exp[-(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+\beta_{12}hk+\beta_{13}hl+\beta_{23}kl)]\,.$$

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
S	9050 (1)	−2500 (2)	877 (1)	153 (1)	218 (2)	47 (1)	72 (3)	51 (1)	23 (2)
O(1)	3629 (3)	6221 (4)	2141 (2)	176 (4)	184 (6)	59 (1)	−27 (9)	−1 (4)	58 (5)
O(2)	5545 (3)	4854 (5)	3173 (2)	159 (4)	257 (7)	51 (1)	−75 (10)	−33 (3)	−1 (5)
O(3)	7014 (4)	2478 (7)	4548 (2)	246 (6)	378 (10)	70 (2)	−115 (18)	−27 (5)	87 (9)
O(4)	5386 (8)	−1328 (7)	4106 (2)	692 (16)	289 (11)	58 (2)	−219 (24)	2 (9)	−34 (8)
N(1)	5789 (3)	−524 (4)	1311 (2)	124 (4)	153 (5)	31 (1)	−18 (8)	28 (3)	9 (4)
N(2)	7548 (3)	1515 (5)	520 (2)	129 (4)	194 (6)	33 (9)	−32 (9)	37 (3)	20 (4)
N(3)	1366 (3)	2479 (5)	3044 (2)	113 (3)	167 (5)	33 (1)	−12 (9)	21 (3)	8 (4)
C(1)	7449 (3)	−476 (5)	898 (2)	121 (4)	159 (6)	28 (1)	−23 (9)	20 (3)	−14 (4)
C(2)	5958 (4)	2716 (5)	690 (2)	150 (4)	164 (7)	34 (1)	−6 (10)	20 (3)	14 (5)
C(3)	4850 (3)	1424 (5)	1180 (2)	124 (4)	156 (6)	28 (1)	2 (9)	16 (3)	−1 (4)
C(4)	2985 (4)	1839 (5)	1576 (2)	116 (4)	204 (7)	30 (1)	25 (9)	7 (3)	−1 (4)
C(5)	3226 (3)	2475 (5)	2551 (2)	96 (3)	143 (5)	30 (1)	21 (9)	14 (3)	11 (5)
C(6)	4202 (3)	4735 (5)	2625 (2)	114 (4)	172 (7)	34 (1)	−18 (9)	33 (3)	−6 (5)
C(7)	−85 (4)	3777 (7)	2555 (2)	116 (5)	304 (10)	52 (2)	79 (13)	12 (4)	29 (7)
C(8)	689 (5)	160 (7)	3133 (3)	195 (6)	213 (9)	57 (2)	−107 (13)	63 (6)	23 (7)
C(9)	1652 (5)	3400 (8)	3951 (2)	177 (6)	381 (13)	33 (1)	−88 (16)	50 (4)	−45 (7)
O(4-1)	5047 (10)	−1307 (10)	4104 (4)	381 (17)	203 (15)	61 (3)	89 (29)	−23 (12)	20 (12)
O(4-2)	5753 (10)	−1373 (14)	4113 (4)	398 (19)	403 (24)	57 (3)	269 (39)	7 (12)	44 (15)

(*b*) Hydrogen atoms. Coordinates are $\times 10^3$.

	x	y	z	B (Å^2)		x	y	z	B (Å^2)
H(1)	536 (5)	−158 (7)	155 (2)	3·6 (8)	H(10)	154 (6)	−52 (9)	348 (3)	5·3 (10)
H(2)	839 (5)	185 (7)	25 (2)	3·2 (7)	H(11)	29 (6)	−32 (9)	257 (3)	5·2 (10)
H(3)	576 (5)	421 (7)	51 (2)	3·2 (7)	H(12)	−39 (5)	24 (8)	346 (3)	4·4 (9)
H(4)	245 (4)	291 (6)	129 (2)	2·4 (6)	H(13)	191 (7)	512 (12)	395 (3)	7·4 (14)
H(5)	217 (5)	533 (8)	150 (2)	3·8 (8)	H(14)	267 (6)	286 (11)	423 (3)	6·0 (11)
H(6)	399 (4)	149 (7)	285 (2)	2·6 (6)	H(15)	50 (5)	329 (7)	424 (3)	3·7 (8)
H(7)	41 (9)	524 (15)	234 (4)	10·4 (20)	H(16)	656 (7)	324 (10)	415 (3)	6·5 (12)
H(8)	−57 (5)	282 (8)	201 (2)	3·9 (8)	H(17)	645 (5)	143 (8)	441 (2)	3·7 (8)
H(9)	−121 (5)	360 (8)	289 (2)	3·7 (8)	H(18)	551 (8)	−221 (17)	368 (4)	10·6 (20)
					H(19)	465 (8)	−166 (13)	455 (4)	8·6 (17)

hydrogen. The weighting scheme finally adopted was $w=0{\cdot}11$ for $|F_o|=0$, $w=1{\cdot}0$ for $0<|F_o|\leq 14$ and $w=1{\cdot}0/[1{\cdot}0+(|F_o|-14{\cdot}001)\times 1{\cdot}125]$ for $|F_o|>14$. Fig. 2 shows the final difference map which provides evidence that the present structure corresponds to tautomer (II) of Fig. 1. The residual electron density did not exceed $\pm 0{\cdot}32$ e Å^{-3}.

The large standard deviations of the coordinates of the O(4) atom and its high thermal parameters suggested the presence of positional disorder. An attempt was then made to refine the parameters of the atom after splitting it into two sites, O(4-1) and O(4-2), each with half weight. Although the refinement converged at the same R and $R2$ as those of the ordered model, the thermal parameter shifts of the split atoms could not be made less than their estimated standard deviations. The final positional and thermal parameters of the ordered model are listed in Table 2, with those of the split atoms of the disordered model. The atomic scattering factors for the non-hydrogen atoms were taken from *International Tables for X-ray Crystallography* (1968), and those for hydrogen from Stewart, Davidson & Simpson (1965). For the sulphur atom the anomalous dispersion corrections of Cromer (1965) were used.

The calculations were performed on a NEAC 2200–700 at the Computer Center of Osaka University with the programs in the *Universal Crystallographic Computing System–Osaka* (1973).*

Discussion

Fig. 3 shows a stereoscopic view of the molecule. The principal bond lengths and angles are given in Table 3. They are in good agreement with values found in related compounds. The mean C–H distances are 0·96 Å. The observed value (1·691 Å) for S–C(1) is intermediate between the S–C single-bond distance of 1·82 Å and double-bond value of 1·56 Å. The π-bond order is estimated to be 0·50 according to the equation presented by Trinajstić (1968). This partial double-bond character is in agreement with the canonical resonance forms of the thiourea system and is a normal feature of these compounds (Valle, Cojazzi, Busetti & Mammi, 1970; Pérez-Garrido, López-Castro & Márquez, 1973; Jiménez-Garay, López-Castro & Márquez, 1974). Although the carboxyl group is deprotonated the bond distance C(6)–O(2) is significantly longer than C(6)–O(1). This could be explained by the fact that O(1) is involved in one hydrogen bond and O(2) in two. The variation in angles at C(6) corresponds to that of the bond lengths mentioned above. As in amino acids the angle C(5)–C(6)–O(1) is slightly greater than C(5)–C(6)–O(2), and the values agree quite well with the averages for amino acids (118·0 and 116·4°) given by Marsh & Donohue (1967). The five atoms of the imidazole ring are on the same plane described by $-0{\cdot}4170X-0{\cdot}3630Y-0{\cdot}8333Z+3{\cdot}2416=0$, where X, Y and Z in Å refer to the set of orthogonal axes a, b and c^*. Deviations of the atoms from the mean plane are: N(1) −0·004; N(2) 0·001; C(1) 0·002; C(2) −0·003; C(3) 0·005 Å. The S and C(4) atoms also lie on the plane, the deviations being 0·000 and −0·022 Å, respectively. Inspection of bond lengths and angles as well as the planarity of the ring reveals that the imidazolethione moiety has a plane of symmetry perpendicular to the C(2)–C(3) bond, passing through the midpoint of this bond and C(1).

Fig. 4 shows the arrangement of the molecules in the crystal and the hydrogen bonding scheme. The hydrogen bond distances and angles, and the shortest intermolecular contacts are listed in Table 4. All the hydrogen atoms bonded to nitrogen and oxygen are involved in O–H···O, N–H···O and N–H···S hydrogen bonds. Since the H···S distance in N–H···S is significantly shorter than the sum of their van der Waals radii and the angle N–H–S is 170°, the N–H···S interaction may be classified as a hydrogen bond ac-

Table 3. *Bond distances* (Å) *and angles* (°) *with their e.s.d.'s*

S——C(1)	1·691 (3)	C(5)–C(6)	1·555 (4)
C(1)–N(2)	1·349 (4)	C(6)–O(1)	1·233 (4)
N(2)–C(2)	1·384 (4)	C(6)–O(2)	1·261 (4)
C(2)–C(3)	1·349 (4)	C(5)–N(3)	1·536 (4)
C(3)–N(1)	1·384 (4)	N(3)–C(7)	1·494 (5)
N(1)–C(1)	1·351 (4)	N(3)–C(8)	1·508 (5)
C(3)–C(4)	1·493 (4)	N(3)–C(9)	1·489 (6)
C(4)–C(5)	1·526 (4)		

S——C(1)–N(1)	126·6 (2)	C(4)–C(5)–N(3)	112·2 (3)
S——C(1)–N(2)	128·0 (2)	N(3)–C(5)–C(6)	110·9 (3)
N(1)–C(1)–N(2)	105·4 (3)	C(5)–C(6)–O(1)	118·0 (3)
C(1)–N(2)–C(2)	110·8 (3)	C(5)–C(6)–O(2)	115·9 (3)
N(2)–C(2)–C(3)	106·4 (3)	O(1)–C(6)–O(2)	126·1 (3)
C(2)–C(3)–C(4)	131·0 (3)	C(5)–N(3)–C(7)	111·5 (3)
C(2)–C(3)–N(1)	107·1 (3)	C(5)–N(3)–C(8)	108·9 (3)
N(1)–C(3)–C(4)	121·9 (3)	C(5)–N(3)–C(9)	109·5 (3)
C(3)–N(1)–C(1)	110·3 (3)	C(7)–N(3)–C(8)	108·9 (3)
C(3)–C(4)–C(5)	109·7 (3)	C(7)–N(3)–C(9)	109·6 (3)
C(4)–C(5)–C(6)	109·9 (3)	C(8)–N(3)–C(9)	108·4 (3)

* A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 31175 (13 pp., 1 microfiche). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

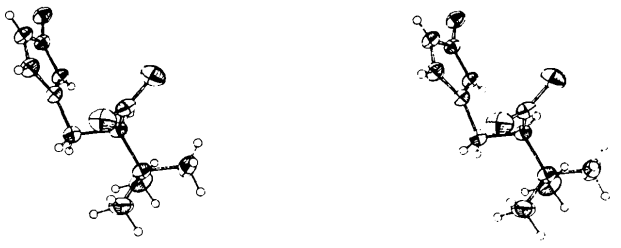

Fig. 3. Stereoscopic view of the L-ergothioneine molecule. Thermal ellipsoids enclose 50% probability. Hydrogen atoms are shown as spheres of arbitrary radius.

Table 4. *Hydrogen bonds and shortest intermolecular contacts*

Symmetry code

(i)	$1+x, \quad y, \quad z$	(iv)	$1-x, \quad \frac{1}{2}+y, \quad -z$
(ii)	$x, \quad 1+y, \quad z$	(v)	$2-x, \quad \frac{1}{2}+y, \quad -z$
(iii)	$x, \quad -1+y, \quad z$	(vi)	$1-x, \quad -\frac{1}{2}+y, \quad 1-z$

Hydrogen bonds

D–H···*A*	*D*–H (Å)	H–*A* (Å)	*D*–*A* (Å)	*D*–H···*A*(°)
N(1)–H(1)····O(1^{iii})	0·81 (4)	2·04 (4)	2·829 (4)	164 (4)
N(2)–H(2)····S^{v}	0·76 (4)	2·54 (3)	3·299 (3)	170 (4)
O(3)–H(16)···O(2)	0·82 (6)	1·91 (6)	2·729 (5)	176 (6)
O(3)–H(17)···O(4)	0·79 (5)	1·91 (5)	2·687 (7)	172 (5)
O(4)–H(18)···O(2^{iii})	0·85 (8)	1·95 (8)	2·732 (6)	152 (7)
O(4)–H(19)···O(3^{vi})	0·87 (9)	1·91 (9)	2·776 (7)	176 (9)

Shortest intermolecular non-bonding contacts

O(2)···C(7^{i})	3·348 (5) Å	O(2)····H(9^{i})	2·49 (5) Å
C(2)···N(1^{iv})	3·419 (4)	O(3)····H(15^{i})	2·59 (6)
S·····H(5^{i})	3·04 (5)	N(2)····H(8^{i})	2·72 (8)
S·····H(11^{i})	3·00 (5)	H(19)···H(17^{vi})	2·1 (1)
C(6)···H(18^{ii})	2·61 (8)	H(19)···H(16^{vi})	2·2 (1)

cording to the criteria given by Donohue (1969). The carboxyl group is twisted around the C(5)–C(6) bond in such a way that the two oxygen atoms are involved in as many hydrogen bonds as possible. Thus the carboxyl group accepts three hydrogen bonds. The situation is different from those of histidine (Madden, McGandy & Seeman, 1972) and betaine hydrochloride (Fischer, Templeton & Zalkin, 1970). The dihedral angle between the planes defined by C(6), O(1), O(2), and by C(5), C(6), N(3) is 77·6° in ergothioneine. The corresponding values in histidine and betaine hydrochloride are 25·9 and 6·8°, respectively. The orientation around the four C–N(3) bonds is very nearly staggered as is the case in betaine hydrochloride.

At present the biological function of ergothioneine is still uncertain, though several activities are ascribed to it (Melville, 1959). Nevertheless it will be of interest to compare the molecular conformation of ergothioneine with those of histamine and histidine in the manner of Veidis, Palenik, Schaffrin & Trotter (1969). Fig. 5 illustrates the view down the C(4)–C(5) bond in ergothioneine, histamine (Bonnet & Ibers, 1973) and histidine free bases. It is apparent that the conformation of ergothioneine around the C(4)–C(5) bond, with the quaternary nitrogen atom N(3) *trans* to the imidazole ring, is similar to that of histamine, and is markedly different from the *gauche* conformation of histidine. The torsion angles around the bond in ergothioneine, histamine and histidine are 168·6, 170·8 and 58·3°, respectively. A major difference between ergothioneine and histamine is present in the angle around the C(3)–C(4) bond. The imidazole ring is rotated about the bond by nearly 180°, thus interchanging N(1) and C(2) in relation to C(5). According to the MO study of the conformation of histamine the variation in total energy with the rotation of the imidazole ring is of the order of hydrogen bond energy for *trans* conformation around the C(4)–C(5) bond (Kier, 1968; Ganellin, Pepper, Port & Richard, 1973). This result, is supported by comparison of the crystal structures of histamine derivatives (Prout, Critchley & Ganellin, 1974). This seems to be the case also in ergothioneine and the conformation around the bond will be greatly affected by the hydrogen-bond scheme.

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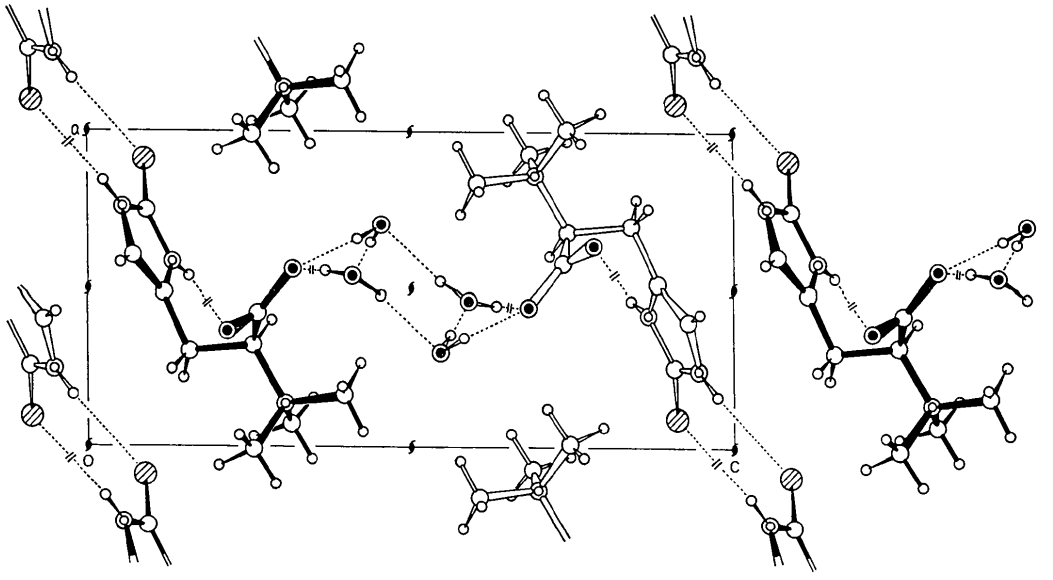
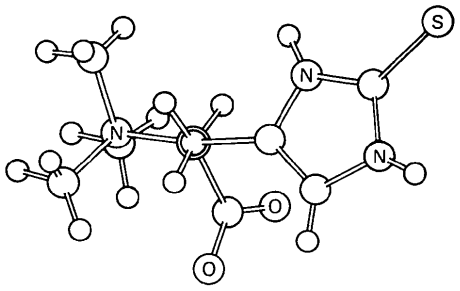


Fig. 4. Projection of the crystal structure along **b**. Hydrogen bonds are indicated by dashed lines.



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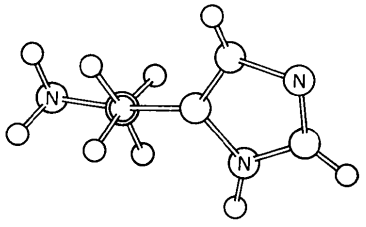

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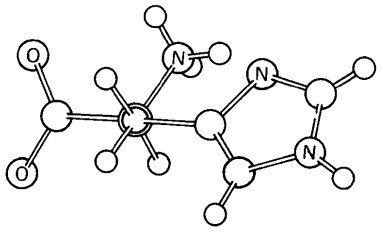

(III)

Fig. 5. Projection of the structures along the C(4)–C(5) bond of ergothioneine, histamine and histidine free bases.

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