Acta Cryst. (1965). **19**, 860

Intermolecular complexes. III. L-Cysteine ethyl ester hydrochloride–urea (1:1).

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(*Received* 1 *March* 1965 *and in revised form* 13 *April* 1965)

The study of crystalline complexes of protein denaturants with model compounds has been in progress in this laboratory for some time (Bello & Haas, 1965). The present paper deals with the hydrogen bonded complex of L-cysteine ethyl ester hydrochloride, $CH_3CH_2OCOCH(NH_3)^+CH_2SHCl^-$ and urea, $CO(NH_2)_2$.

Crystals of the complex were obtained in the following manner: a saturated aqueous solution of urea (25°C) was warmed (100°C) and L-cysteine ethyl ester hydrochloride was added to near saturation. The solution was cooled and crystals with different morphologies were observed in the mass of crystals precipitated. It proved impossible to separate the different kinds of crystals by a kerosine—carbon tetrachloride gradient density column. The density obtained by this means for the single layer of mixed crystals is 1·34 g.cm^{-3}; the densities of urea and L-cysteine ethyl ester hydrochloride are 1·335 g.cm^{-3} and 1·348 g.cm^{-3} respectively.

Crystals with different shapes were picked out of the mass of mixed crystals and examined by X-ray methods. Several of these proved to be identical and different from either L-cysteine ethyl ester hydrochloride or urea. The crystallographic data for these crystals are:

Space group	$P2_1$	
Lattice constants	$a = 9{\cdot}97_4$ Å	
	$b = 12{\cdot}09_1$	(all $\pm 0{\cdot}005$ Å)
	$c = 5{\cdot}12_7$	
	$\beta = 97{\cdot}00°$	($\pm 0{\cdot}05°$)
Density	Obs. approx. 1·34 g.cm^{-3}	
	Calc. 1·324	

From the unit-cell volume, density, and space group, a formula weight of 248 was obtained. This agreed well with the computed formula weight, 245, of a 1:1 complex.

Initially, it could not be proven that a chemical reaction had not occurred between the urea and the S–H group to give $-S-C(NH2)_2OH$. Since there was only a limited supply of crystals, no chemical tests were performed, and the X-ray analysis was continued on the assumption that the crystals were a 1:1 complex.

Three-dimensional X-ray intensities were initially collected only to a resolution of 1 Å on the General Electric XRD5 with the Single Crystal Orienter using Cu $K\alpha$ radiation and Ni–Co balanced filters. A total of 744 intensities were measured, of which 214 were less than twice the background. (Attempts to collect the complete copper sphere were unsuccessful as the available crystals decomposed within a week, and no new crystals were obtained.)

The crystal structure was determined by the heavy atom method. The chloride-chloride and sulfur-sulfur peaks were located in the Harker section $U\frac{1}{2}W$ of the Patterson function, and the difference in the y coordinates was obtained from the chloride–sulfur cross vector.

The chloride ion was placed at $y = \frac{1}{4}$. (This can be done arbitrarily in this space group.) The substantially different y coordinates of the chloride ion and the sulfur atom prevented any false symmetry from being introduced by the use of their phases for the first electron-density synthesis. The calculation of two electron-density syntheses in addition to a difference electron-density synthesis established the positions of all twelve non-hydrogen atoms. Five cycles of block diagonal least-squares refinement with isotropic temperature factors and unit weights for all the observed structure factors reduced the R value to 0·092 (the unob-

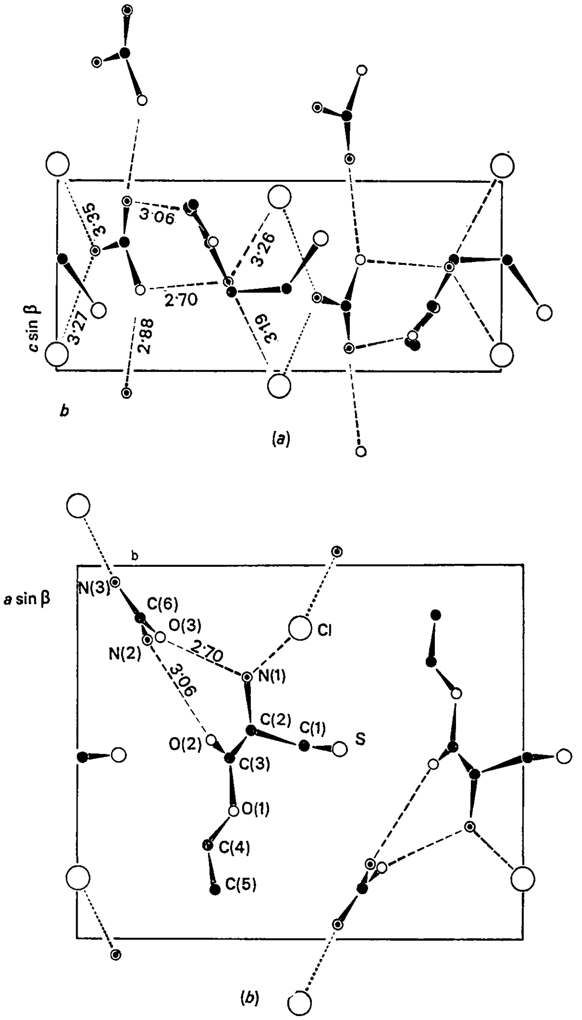

Fig. 1. (*a*) View along the *a* axis showing chains of hydrogen-bonded urea molecules, the positions of N(1) and N(3) between the chloride ions of adjacent cells, and the planar N(1)–C(2)–C(3)–O(2) group of the amino acid. (*b*) View along the *c* axis showing the urea hydrogen-bonded to the cysteine ethyl ester molecule. The short spaced broken lines show contacts that cross the (100) plane.

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served reflections were not included in this refinement). The coordinates and temperature factors obtained from this refinement are given in Table 1*.

Table 1. *Coordinates and temperature factors of the atoms*

Atom	*x*	*y*	*z*	*B*
Cl	0·1646	0·5011	0·9230	3·31
S	0·4885	0·5971	0·6921	3·69
O(1)	0·6511	0·3466	0·6494	4·07
O(2)	0·4654	0·2950	0·8386	3·51
O(3)	0·1876	0·1757	0·4292	4·32
N(1)	0·2972	0·3805	0·4459	2·82
N(2)	0·2065	0·1633	0·8741	3·91
N(3)	0·0251	0·0772	0·6109	3·95
C(1)	0·4812	0·5169	0·4010	2·69
C(2)	0·4451	0·3914	0·4249	2·81
C(3)	0·5210	0·3385	0·6630	1·96
C(4)	0·7424	0·2851	0·8501	6·69
C(5)	0·8609	0·3282	0·8852	13·62
C(6)	0·1366	0·1441	0·6423	3·71

Estimated standard deviations (Å)	$\sigma(x)$	$\sigma(y)$	$\sigma(z)$
Chlorine and sulfur atoms	0·006	0·007	0·006
Light atoms except C(4) and C(5)	0·02	0·02	0·02
C(4)	0·03	0·03	0·03
C(5)	0·05	0·06	0·05

The crystal is held together mainly by electrostatic attractions and hydrogen bonds between the $-NH_3^+$ and Cl^- ions and by two other sets of hydrogen bonds: urea-urea and urea–cysteine ethyl ester (Fig. 1). The short *c* axis allows us to describe the structure as alternating 'layers' of chloride ions and hydrogen-bonded urea–cysteine ethyl ester complexes. Each chloride ion is separated from two neighboring ions in adjacent cells in the **c** direction by four nitrogen atoms, N(3) and N(1) from two urea and two cysteine ethyl ester molecules, respectively. The CH_2–S–H and ethyl ester groups complete the coordination sphere of the chloride ion.

The urea molecules are roughly perpendicular to the (001) plane and form two chains of hydrogen-bonded urea molecules parallel to the *c* axis; only O(3) and N(2) are involved in the hydrogen bonding scheme, for N(3) coordinates two chloride ions. In addition, O(3) and N(2) are hydrogen-bonded to N(1) and O(2), respectively, of the cysteine ethyl ester molecule. The N(1)–O(3) distance, 2·70 ± 0·03 Å, is the shorter of the two; this is reasonable, since N(1) is positively charged. The N(2)–O(2) hydrogen-bond distance is 3·06 ± 0·03 Å, and O(2) is only 0·14 Å out of the plane of the urea molecule.

The sulfur atom is coordinated by a nitrogen, carbon, and two oxygen atoms in addition to the chloride ion. Little crystallographic information is available for S–H groups, but in view of the fact that none of the sulfur–oxygen or sulfur–nitrogen distances is unusually short, little can be said about specific interactions with the surrounding atoms.

The carbon atoms of the ethyl ester group appear to be in a cavity in which they have some freedom of movement; this is apparent from their high temperature factors. Besides the sulfur atom, no portion of the structure is situated so as to permit suitable stabilization by van der Waals forces for this group.

The bonding between the urea and cysteine ethyl molecule is of interest in that the -NH-C-CO- group is the structural unit of which the α helix is constructed. The present crystal structure shows that urea can be hydrogen-bonded to the charged nitrogen atom the oxygen atom of a single amino acid residue. Its interpretation in terms of protein denaturation, however, must await further experimentation.

I wish to thank Drs D. Harker, J. Bello, and H. H. Mills for many stimulating discussions and acknowledge the use of the IBM 1620 computer programs of Drs F. R. Ahmed and D. R. Harris, and the block-diagonal least-squares program of Y. Okaya and D. R. Harris. This work was supported by grants: GM-09826 from the Institute for General Medical Sciences, NIH-A-3942 from the National Institutes of Health, and NSF-GE-429 from the National Science Foundation; I am the recipient of a USPHS Traineeship, Training grant 5T1 GM-718 through the Department of Biophysics, State University of New York at Buffalo.

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* A 35 mm microfilm containing the observed and calculated structure factors has been deposited as Document Number 8394 with the ADI Auxiliary Publication Project, Photoduplication Service, Library of Congress, Washington, D.C. Microfilm copies can be obtained by an advance payment of $1·75.

Acta Cryst. (1965). **19**, 861

Location of hydrogen atoms in certain heterocyclic compounds.

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(*Received* 22 *March* 1965)

While the structures of certain pyrimidines were being investigated (Chatar Singh, 1965*a b*), it was noticed that the magnitude of the valence angle of nitrogen atoms in six-membered heterocyclic rings was dependent upon the extra-annular attachment to the nitrogen atom. The valence angle of nitrogen atoms with an extra-annular hydrogen atom was significantly larger than that of nitrogen without any attachment. To substantiate this empirical observation all six-membered heterocyclic compounds reported in *Acta Crystallographica* since 1948 were tabulated (Tables 1 and 2). In all these compounds, the authors have independently established the presence or absence of a hydrogen atom at the ring nitrogen. The histogram in Fig. 1 gives the distribution with respect to the nitrogen valence angle. The weighted mean value, with weight equal to $1/\sigma^2$, of the nitrogen valence angles with an extra-annular hydrogen atom is 125·0° ± 0·2° (from Table 1) whereas that without any attachment is 115·7° ± 0·2° (from Table 2). The differ-