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The Crystal Structure of *S*-Benzyl-L-cysteinyl-*S*-benzyl-L-cysteine

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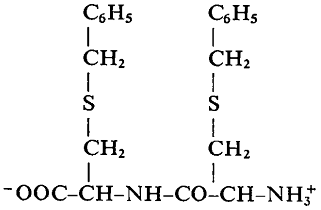
The crystal structure of *S*-benzyl-L-cysteinyl-*S*-benzyl-L-cysteine has been determined from X-ray three-dimensional data. The space group is $P2_12_12_1$ with $a=27{\cdot}70$ (3), $b=15{\cdot}00$ (2), $c=4{\cdot}86$ (1) Å, $Z=4$. The structure was solved by direct methods and refined by least-squares calculations to a conventional R of 0·061 for 956 independent reflexions to a maximum $\sin\theta/\lambda$ of 0·50 Å^{-1}. The molecule exists as a zwitterion, and the amino terminal group participates in two hydrogen bonds. The shortest hydrogen bond (2·75 Å) occurs between the nitrogen of the amide group and a carboxyl oxygen of a translated molecule.

Introduction

The X-ray structure determination of cysteine-containing peptides is part of a research programme on the structure of peptides and proteins which is being carried out in this laboratory to gain knowledge on the side-group conformation in small model compounds.

The sequence of two half cystines has been found in some proteins, and recently it has been suggested that cysteinylcysteine is involved in the biosynthetic pathway of luciferins, responsible for the light emission in many bioluminescent systems (McCapra & Razavi, 1975). In this paper the molecular features and conformation of the cysteine dipeptide with the sulphur atoms blocked by benzyl groups (Fig. 1) are presented.



$$^{-}OOC-\overset{\begin{array}{c}C_6H_5\\|\\CH_2\\|\\S\\|\\CH_2\\|\end{array}}{CH}-NH-CO-\overset{\begin{array}{c}C_6H_5\\|\\CH_2\\|\\S\\|\\CH_2\\|\end{array}}{CH}-NH_3^+$$

Fig. 1. Molecular formula.

Experimental

S-Benzyl-L-cysteinyl-*S*-benzyl-L-cysteine was prepared according to the method of Izumiya & Greenstein (1954). Crystals were grown by slow cooling from ethanol as thin colourless prisms elongated along the c axis, which diffracted X-ray intensity up to a θ angle of $\sim 50°$ (copper radiation). Crystals are orthorhombic, space group $P2_12_12_1$ from systematic absences ($h00$, $0k0$ and $00l$ reflexions absent for odd indices). Lattice constants were obtained from a least-squares refinement of the setting angles of 17 reflexions on a Siemens AED automatic diffractometer with Cu $K\alpha$ radiation. A summary of the crystal data is given in Table 1.

Table 1. *Crystal data*

Formula	$C_{20}H_{24}N_2O_3S_2$	Space group	$P2_12_12_1$
M.W.	404·3	Z	4
a	27·70 ± 0·03 Å	D_c	1·335 g cm^{-3}
b	15·00 ± 0·02	D_m*	1·338 g cm^{-3}
c	4·86 ± 0·01	F_{000}	856
		μ(Cu $K\alpha$)	25·0 cm^{-1}

* Measured by flotation in a mixture of bromobenzene and chlorobenzene.

Intensity data were collected from a small crystal employing the θ–2θ scan technique. A standard reflexion was measured at regular intervals to monitor crystal and equipment stability. 956 observed reflexions [$I \geq 1{\cdot}5\sigma(I)$] were measured out of a total of 1260 present within the limit $\sin\theta/\lambda=0{\cdot}5$ Å^{-1}. Their intensities were corrected for Lorentz and polarization effect in the usual way. No correction for absorption was applied because of the small dimensions of the crystal.

Structure determination and refinement

The structure was solved by the multisolution method with the program *MULTAN* (Germain, Main & Woolfson, 1970). The 265 strongest E values ($E > 1{\cdot}2$) were used to produce eight solutions, the two with the best figures of merit being almost coincident. The best phase set was used to calculate an E map from which 15 of the 27 atoms were located. The remaining non-hydrogen atoms (those belonging to the two phenyl rings) were located from a Fourier map based on the known atom positions.

Refinement proceeded by the block-diagonal least-squares method, using unit weights and isotropic temperature factors. Later on the weighting scheme $w = 1/[16+|F_o|+0{\cdot}01|F_o|^2]$ and anisotropic thermal parameters for sulphur atoms only were introduced. At convergence the R index was 0·082. At this stage a three-dimensional difference Fourier map was calculated, which showed peaks near the regions where the hydrogen atoms were expected to be located. All the hydrogen atoms appeared unambiguously in this map with peak heights ranging from 0·2 to 0·4 e Å^{-3}. As the stereochemistry of the hydrogen atoms was not very good their coordinates were calculated according to standard parameters, the hydrogens of the $-NH_3^+$ group being placed in a staggered conformation around the bond C(3)–C(2) on the basis of the difference Fourier results.

The inclusion of the hydrogen atoms in the structure-factor calculation, assigning to them the isotropic temperature factor of the heavy atom to which they are bonded, gave a final R index 0·061.* Further refinement did not significantly change the positional parameters of the non-hydrogen atoms. As the number of observed reflexions was relatively small, the refinement of anisotropic thermal parameters of carbon, nitrogen and oxygen was not attempted.

In Table 2 the final fractional coordinates and thermal factors are reported together with the correspond-

Table 2. *Atomic coordinates* (× 10^4) *and thermal vibration parameters* (× 10^2) *with estimated standard deviations in parentheses*

	x	*y*	*z*	*B*
S(1)	6077 (1)	2284 (2)	8094 (6)	
S(2)	3476 (1)	5051 (2)	2480 (6)	
O(1)	4547 (2)	2310 (4)	2906 (14)	481 (13)
O(2)	4750 (2)	5597 (4)	2695 (15)	543 (14)
O(3)	4932 (2)	4594 (4)	−388 (16)	571 (15)
N(1)	4944 (2)	1469 (5)	7413 (19)	440 (15)
N(2)	4646 (2)	3661 (4)	5034 (15)	383 (14)
C(1)	5591 (3)	2439 (6)	5675 (19)	393 (18)
C(2)	5092 (3)	2419 (5)	6860 (19)	381 (17)
C(3)	4735 (2)	2772 (5)	4750 (18)	363 (17)
C(4)	4345 (3)	4167 (5)	3064 (20)	378 (16)
C(5)	4702 (3)	4829 (5)	1681 (19)	384 (16)
C(6)	3933 (3)	4586 (6)	4680 (21)	465 (19)
C(7)	6125 (3)	3416 (6)	9449 (23)	534 (21)
C(8)	6316 (3)	4078 (6)	7395 (21)	444 (18)
C(9)	6000 (3)	4699 (6)	6266 (21)	476 (19)
C(10)	6170 (4)	5309 (8)	4265 (27)	676 (26)
C(11)	6648 (4)	5304 (8)	3543 (28)	680 (26)
C(12)	6968 (4)	4690 (8)	4720 (28)	694 (27)
C(13)	6795 (3)	4071 (7)	6643 (25)	561 (22)
C(14)	3191 (3)	4051 (7)	1194 (24)	562 (22)
C(15)	2986 (3)	3441 (6)	3326 (22)	475 (19)
C(16)	3260 (3)	2718 (7)	4345 (23)	542 (22)
C(17)	3065 (4)	2166 (7)	6380 (26)	680 (26)
C(18)	2615 (4)	2336 (8)	7398 (28)	694 (26)
C(19)	2341 (4)	3029 (8)	6404 (28)	698 (27)
C(20)	2523 (3)	3576 (7)	4322 (25)	591 (23)

The anisotropic temperature factors for S(1) and S(2) in the form $-\frac{1}{4}(B_{11}a^{*2}h^2+B_{22}b^{*2}k^2+B_{33}c^{*2}l^2+2B_{12}a^*b^*hk+2B_{13}a^*c^*hl+2B_{23}b^*c^*kl)$ are as follows.

	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
S(1)	634 (12)	405 (10)	449 (12)	−4 (9)	−18 (12)	148 (11)
S(2)	694 (13)	386 (10)	582 (15)	96 (10)	−54 (13)	29 (12)

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* A list of structure factors is available from the authors and has also been deposited with the British Library Lending Division as Supplementary Publication No. SUP 31061 (5 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

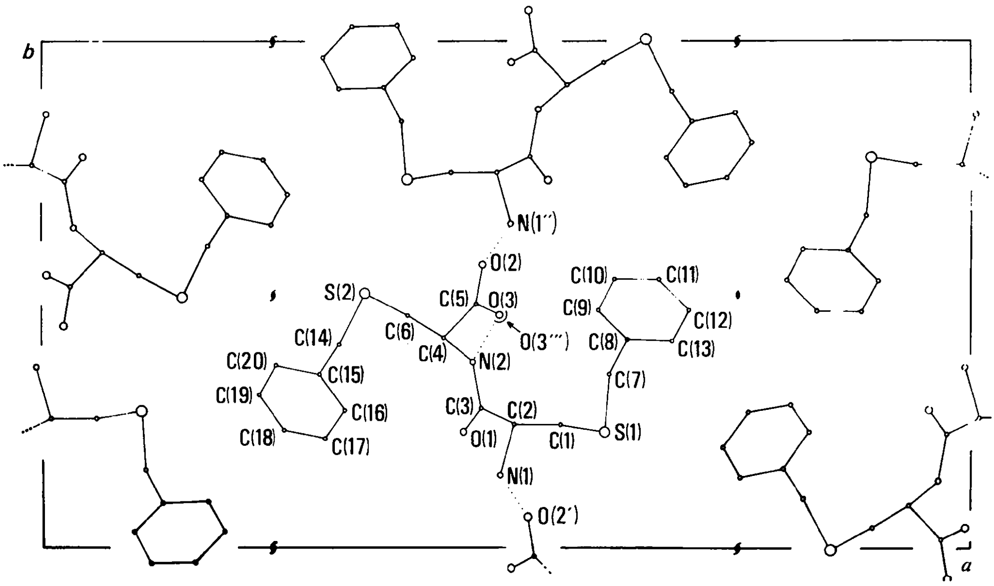

Fig. 2. Molecular packing projected on the *ab* plane: the hydrogen bonds are shown by dotted lines.

ing standard deviations. Atomic scattering factors for the non-hydrogen atoms were taken from Cromer & Mann (1968), and for the hydrogen atoms from Moore (1963).

Table 3. *Molecular dimensions*

The standard deviations are given in parentheses

S(1)—C(1)	1·802 (9) Å	C(15)–C(16)	1·413 (13) Å
C(1)—C(2)	1·498 (11)	C(16)–C(17)	1·398 (16)
C(2)—N(1)	1·507 (11)	C(17)–C(18)	1·365 (16)
C(2)—C(3)	1·520 (12)	C(18)–C(19)	1·375 (17)
C(3)—O(1)	1·247 (11)	C(19)–C(20)	1·397 (17)
C(3)—N(2)	1·363 (10)	C(20)–C(15)	1·386 (13)
N(2)—C(4)	1·479 (11)	S(1)—C(7)	1·826 (10)
C(4)—C(5)	1·554 (12)	C(7)—C(8)	1·504 (14)
C(5)—O(2)	1·260 (10)	C(8)—C(9)	1·391 (13)
C(5)—O(3)	1·241 (11)	C(9)—C(10)	1·416 (16)
C(4)—C(6)	1·521 (12)	C(10)–C(11)	1·370 (16)
C(6)—S(2)	1·798 (10)	C(11)–C(12)	1·400 (17)
S(2)—C(14)	1·807 (10)	C(12)–C(13)	1·402 (17)
C(14)–C(15)	1·494 (14)	C(13)–C(8)	1·376 (13)

S(1)—C(1)—C(2)	115·8 (3)°
C(1)—C(2)—N(1)	109·8 (3)
N(1)—C(2)—C(3)	105·8 (3)
C(1)—C(2)—C(3)	109·5 (3)
C(2)—C(3)—O(1)	124·3 (4)
O(1)—C(3)—N(2)	122·7 (3)
C(2)—C(3)—N(2)	113·0 (3)
C(3)—N(2)—C(4)	122·6 (4)
N(2)—C(4)—C(5)	104·4 (3)
C(5)—C(4)—C(6)	115·9 (3)
N(2)—C(4)—C(6)	107·5 (3)
C(4)—C(5)—O(3)	119·7 (4)
C(4)—C(5)—O(2)	118·8 (4)
O(2)—C(5)—O(3)	121·5 (4)
C(4)—C(6)—S(2)	112·4 (2)
C(6)—S(2)—C(14)	101·0 (2)
S(2)—C(14)–C(15)	115·8 (4)
C(14)–C(15)–C(16)	120·6 (5)
C(14)–C(15)–C(20)	120·3 (5)
C(16)–C(15)–C(20)	119·1 (4)
C(15)–C(16)–C(17)	119·6 (5)
C(16)–C(17)–C(18)	119·9 (5)
C(17)–C(18)–C(19)	121·2 (5)
C(18)–C(19)–C(20)	120·0 (5)
C(19)–C(20)–C(15)	120·1 (5)
C(1)—S(1)—C(7)	99·8 (2)
S(1)—C(7)—C(8)	113·6 (4)
C(7)—C(8)—C(9)	118·8 (4)
C(7)—C(8)—C(13)	120·7 (5)
C(9)—C(8)—C(13)	120·5 (4)
C(8)—C(9)—C(10)	119·6 (5)
C(9)—C(10)–C(11)	119·6 (5)
C(10)–C(11)–C(12)	120·7 (5)
C(11)–C(12)–C(13)	119·4 (5)
C(12)–C(13)–C(8)	120·1 (4)

N(1)–C(2)—C(3)—N(2)	148·0°
C(2)–C(3)—N(2)—C(4)	174·4
C(3)–N(2)—C(4)—C(5)	−114·0
N(2)–C(4)—C(5)—O(2)	−92·4
N(2)–C(4)—C(5)—O(3)	87·7
N(2)–C(4)—C(6)—S(2)	−169·3
C(4)–C(6)—S(2)—C(14)	72·9
C(6)–S(2)—C(14)–C(15)	58·2
S(2)–C(14)–C(15)–C(20)	85·8
N(1)–C(2)—C(1)—S(1)	−77·5
C(2)–C(1)—S(1)—C(7)	−81·8
C(1)–S(1)—C(7)—C(8)	−69·0
S(1)–C(7)—C(8)—C(9)	107·5

Discussion

Bond lengths and angles, calculated from the final coordinates of the non-hydrogen atoms, are listed in Table 3. The bond lengths and angles in the benzene rings are normal, with average values of 1·390 Å and 120·0° respectively. The r.m.s. variation about the mean of the bond lengths in the benzene rings is 0·016 Å, in good agreement with the e.s.d.'s from the least-squares analysis. The distances and angles involving the peptide group compare well with those of a standard peptide unit, the C(3)–N(2) and N(2)–C(4) distances being somewhat longer than the expected values and the valency angles on C(4) slightly anomalous. Valency angles and bond lengths involving sulphur atoms are normal.

A molecular packing diagram projected on the (001) plane is shown in Fig. 2. The structure, as is frequently found in most amino acids and peptides, is organized in alternate layers of hydrophobic and polar regions normal to the *a* axis. In the polar region the molecules are held together by a network of hydrogen bonds. The hydrogen bond parameters are shown in Table 4. Although amino nitrogen N(1) has four intermolecular contacts to O($2^{\rm i}$), O($3^{\rm i}$), O($2^{\rm iv}$), O($1^{\rm iii}$) at 2·93, 3·00, 2·84 and 3·15 Å respectively, only the two carboxyl oxygens O($2^{\rm i}$) and O($2^{\rm iv}$) are in a position to act as acceptors in hydrogen bonds to N(1). The shortest hydrogen bond, however, involves the nitrogen N(2) of the peptide group and the carboxyl oxygen O($3^{\rm iii}$) of a molecule translated of one unit along the *c* axis [N(2)···O($3^{\rm iii}$) = 2·75 Å, C(3)–N(2)–O($3^{\rm iii}$) = 122°, C(4)–N(2)–O($3^{\rm iii}$) = 115°], the oxygen atom being 0·5 Å out of the plane passing through the atoms of the peptide group. In peptide structures the N–H···O–C distances are usually longer than bonds from terminal NH_3^+ groups (Marsh & Donohue, 1967), the distance found in this structure, however, is shorter and agrees with the value suggested by Corey & Pauling (1953). The disposition of the hydrogen atoms about the N(1) atom and nearly equivalent carboxyl C–O bond lengths indicate that the molecule exists in the zwitterion form.

Table 4. *Hydrogen bond distances* (Å) *and angles* (°)

D	*A*	*D*···*A*	C–*D*···*A*
C(2)–N(1)···	O(2′)	2·84 Å	120·2°
C(2)–N(1)···	O(2′ᵛ)	2·93	101·1
C(3)–N(2)···	O(3‴)	2·75	121·9
C(4)–N(2)···	O(3‴)		115·2

Symmetry code

i	$1-x,\ -\frac{1}{2}+y,\ \frac{1}{2}-z$	ii	$1-x,\ \frac{1}{2}+y,\ \frac{3}{2}-z$
iii	$x,\ y,\ 1+z$	iv	$1-x,\ -\frac{1}{2}+y,\ \frac{3}{2}-z$

The least-squares planes through the benzene rings, the carboxyl and the peptide groups are listed in Table 5. The aromatic rings are planar within the experimental errors; the r.m.s. deviation of the six atoms from the corresponding planes is 0·008 and 0·011 Å

Table 5. *Least-squares planes and atomic deviations* (Å × 10^3)

The equations of the planes are expressed in the form $Ax+By+Cz=D$ and referred to crystallographic axes.

Benzene rings				Carboxyl group		Peptide group	Plane I	Plane II
C(7)	−24*	C(14)	56*	C(4)	0	C(2)	−2	−28
C(8)	−5	C(15)	14	C(5)	0	C(3)	7	16
C(9)	13	C(16)	0	O(2)	0	O(1)	−3	3
C(10)	−11	C(17)	−12	O(3)	0	N(2)	−2	43
C(11)	0	C(18)	10			C(4)	−118*	−34
C(12)	8	C(19)	5			O(3′′′)	469*	522*
C(13)	−6	C(20)	−17					
A	5·732		10·630		20·419		−21·044	−21·410
B	9·702		9·022		−5·048		−3·163	−2·779
C	3·567		3·405		2·848		2·990	2·949
D	10·220		7·397		7·641		−9·428	−9·523

* Not included in the calculation of the plane.

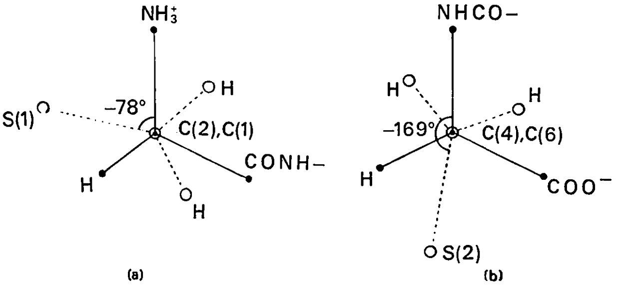

Fig. 3. Torsional angles down bonds: (*a*) C(2)–C(1); (*b*) C(4)–C(6).

respectively. The α-carbon atom C(4) is planar with the carboxyl group. The peptide group is slightly nonplanar (plane II, in Table 5), with the α-carbon atom C(4) 0·12 Å out of the plane comprising C(2), C(3), O(1) and N(2) (plane I). Similar distortion of the peptide group has been found in many peptide structures determined by crystallographic techniques, and has been ascribed mainly to the influence of hydrogen bonding involving the carboxyl group (Naganathan & Venkatesan, 1972). The torsion angle about the C(3)–N(2) bond is 174·0°.

The molecular conformation and torsion angles about various bonds are reported in Table 3, following the convention of the IUPAC-IUB Commission on Biochemical Nomenclature (1970). The torsion angle φ [C(3)–N(2)–C(4)–C(5)] is −114° and ψ [N(1)–C(2)–C(3)–N(2)] is 148°, close to values frequently found in many dipeptides, particularly those involving bulky side groups (Marsh & Donohue, 1967).

In amino-acid side chains the torsion angle about the C^{α}–C^{β} bond usually assumes values around −60, 180 or 60°, with a surprising preference for the last value when the γ-atom is sulphur (Lakshminarayanan, Sasisekharan & Ramachandran, 1967). In our case the values found are −169° for [S(2)–C(6)–C(4)–N(2)] and −78° for [S(1)–C(1)–C(2)–N(1)], the two sulphur atoms S(1) and S(2) being in both cases antiperiplanar to the peptide group (Fig. 3).

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