The Crystal Structure of D,L-Alanyl-L,D-methionine

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The structure of L-alanyl-D-methionine and its enantiomorph has been determined by use of X-ray crystallographic methods. The space group is $P2_1/c$ with $a = 13 \cdot 320$ (6), $b = 5 \cdot 329$ (1), $c = 16 \cdot 048$ (6) Å, $\beta = 106 \cdot 87$ (2)°, $Z = 4$. Parameters for the 14 nonhydrogen and 16 hydrogen atoms in the structure were refined by least-squares techniques to a conventional R of $0 \cdot 068$ for 2489 reflections to a maximum $\sin \theta / \lambda$ of $0 \cdot 650$. The effects of refinement with data sets based on instability constants of $0 \cdot 01$ and $0 \cdot 03$ were investigated. The peptide group is significantly nonplanar with a torsion angle ω of $171 \cdot 5$ (2)°. The molecules exist as zwitterions in the crystal, and each amino end group participates in three hydrogen bonds.

Introduction

L-Alanyl-D-methionine, Fig. 1, has seen varied use as a test compound in different areas of biochemical research. It has been used in yeast metabolism studies (Schlenk & Zydek, 1968), in cell culture growth-stimulation experiments (Ito & Moore, 1969), in e.p.r. investigations of free radicals formed in the ninhydrin reaction (Yuperov, Froncish, Khartonenkov & Kalmanson; 1970), in bacteria growth response studies (Lockingen, 1958), and as a test compound used in determining the characteristics of a proteinase of *Lacti streptococci* (Cowman, Yoshimira & Swaisgood, 1968). In all of this work the compound was not chosen because of any extraordinary chemical property, but mainly because it is an easily obtainable dipeptide made up of aliphatic and sulfur-containing amino acids.

The molecule has been studied crystallographically, the unit cell and space group having been reported by Tranter (1954), but the structure was not solved. We have undertaken the structure determination as part of a program of investigating small molecules of biological interest.

Experimental

Table 1 contains data concerning the crystal and data collection. (D, L)-α-Alanyl-(D, L)-methionine was ob-
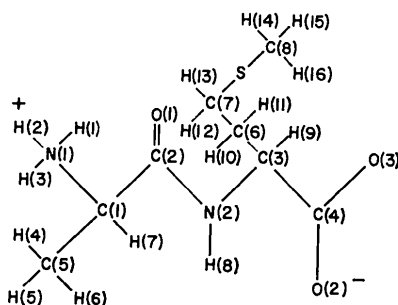

Fig. 1. Structural formula for L-alanyl-D-methionine.

Table 1. *Crystal and data-collection information*

Space group $P2_1/c$	Crystal dimensions: $0 \cdot 1 \times 0 \cdot 06 \times 0 \cdot 5$ mm
$a = 13 \cdot 320$ (6) Å	Mo $K\alpha$ radiation, $\lambda = 0 \cdot 71069$ Å, Nb filter
$b = 5 \cdot 329$ (1)	Max. $\sin \theta / \lambda = 0 \cdot 650$ Å^{-1}
$c = 16 \cdot 048$ (6)	$\omega/2\theta$ scan, $2°$/min in 2θ
$\beta = 106 \cdot 87$ (2)°	Scan range $1 \cdot 2°$ with adjustment for $\alpha_1 - \alpha_2$
Unit-cell volume: $1090 \cdot 1$ Å^3	20 s backgrounds at limits of scan
Formula $C_8H_{16}N_2O_3S$	3 monitor reflections every 200
M.W. $220 \cdot 6$	13 standard reflections every 400
4 molecules per unitcell	2489 unique reflections
$F_{000} = 472$	1986 greater than $\sigma(I)$ or $2\sigma(F)$

tained from Sigma Chemical Company and crystals were obtained by the method reported by Tranter (1954). Preliminary photographic work confirmed his unit-cell parameters and space group, $P2_1/c$.

From the identification of the starting material, there are four possible molecular conformations: D–L, L–D, L–L, and D–D. In space group $P2_1/c$, optically active molecules must be paired with their enantiomers, and with four molecules in the unit cell, only one of the two possible pairs of molecules can be present in the crystal for the space-group determination.

Data were collected from a small crystal on a four-circle diffractometer. The unit-cell parameters determined in aligning the crystal on the diffractometer and listed in Table 1 agree to within $0 \cdot 8\%$ with those determined by Tranter (1954).

Because of the small crystal size, most of the reflections used to monitor the crystal and equipment stability were of medium or weak intensity. Initially, much of their variation in intensity was ascribed to statistical fluctuations and the data were processed with no deterioration correction. The standard deviations in intensities were calculated from the expression

$$\sigma(I) = [N_{pk \; scan} + (T_{scan}/T_{bkg})^2 N_{bkg} + (CN_{pk \; net})^2]^{1/2}$$

where C is the instability constant which was initially given a value of $0 \cdot 01$. It was experimentally determined

that no absorption correction was necessary, and because the crystal used was a relatively small one, coincidence losses were negligible.

Structure solution and refinement

A sharpened Patterson map was computed which showed a single large sulfur–sulfur peak on the Harker line and several peaks in the Harker section which had possible cross-vector peaks in appropriate positions. Several of the S atom positions consistent with the Patterson map were eliminated by calculating ΔF maps based on the S positions and searching for the other atoms. In the ΔF map based on the correct sulfur positions, 13 other nonhydrogen atoms were found. However, the two placed nearest the sulfur at the largest peaks in the first ΔF map were in large negative regions in the next map. Accordingly, they were deleted from the model and another map was calculated. In this one, the two atoms appeared near the sulfur but in new positions.

Three cycles of ΔF refinement of the positions and thermal parameters reduced R from 0·414 to 0·298. Initially, the atoms at the amino terminus were treated as carbons since the configuration of the molecule was not known. The ambiguity of atom type was determined by noting that the bond length C(1)–N(1), Fig. 2, was less than C(1)–C(5) and that there was residual positive density in the ΔF map at N(1). Both criteria fix the assignment of N(1) and C(5) as shown in Fig. 2.

At this point full-matrix, least-squares refinement on $|F|$ with weights $[1/\sigma(F)]^2$ was initiated. The first numerical column in Table 2 shows the results of the refinement (isotropic temperature factors for the hydrogen atoms and anisotropic temperature factors for the nonhydrogen atoms). We noted that the refinement converged to an $R(=\sum||F_o|-|F_c||/\sum|F_o|)$ of 0·069 and that the $R_w=\sqrt{\sum w(|F_o|-|F_c|)^2/\sum w|F_o|^2}$ of 0·043 was much less. Careful inspection of the monitor and standard reflections suggested that there had been deterioration (about 2% over 2500 reflections) and that 0·03 would be a more realistic value of the instability constant C in the expression for the standard deviation of the intensity.

The data were reprocessed with the correction for deterioration and the higher value of C. This new data set was used in further least-squares refinement and the

results are given in the last column of Table 2. For the nonhydrogen atoms the r.m.s. difference between the final positions and those for $R=0·298$ is 0·040 Å, suggesting that much of the decrease in R resulted from adjusting the thermal parameters (Luzzati, 1952). The final F_o, F_c, and $\sigma(F)$ are given in Table 3 and the atomic parameters in Table 4.
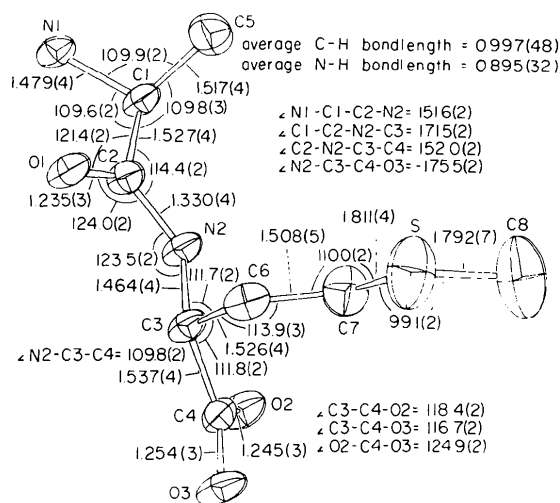

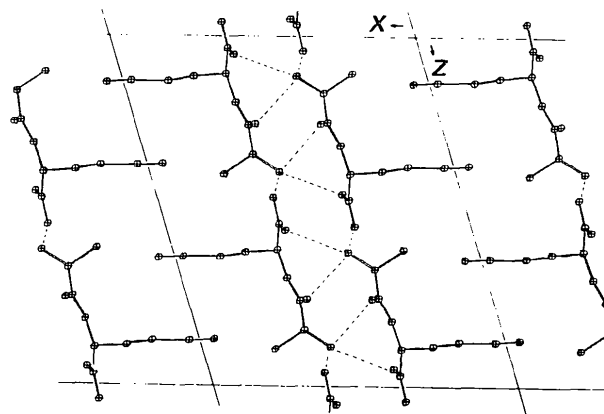Fig. 2. Bond lengths and angles and main-chain torsion angles.

Fig. 3. Packing of L, D-alanyl-D, L-methionine as viewed along $-y$.

Table 2. *Refinement results for two data sets based on different instability constants*

	1% instability	3% instability
Number of reflections greater than $2\sigma(F)$	1997	1986
Number of reflections less than $2\sigma(F)$	492	503
$R=\sum||F_o|-|F_c||/\sum|F_o|$, omitting F's $<2\sigma(F)$	0·069	0·068
$R_w=\sqrt{\sum w(|F_o|-|F_o|)^2/\sum w|F_o|^2}$, omitting F's $<2\sigma(F)$	0·043	0·060
'Goodness of fit'	1·96	1·59
Average shift/error, final cycle	0·3	0·4
Average σ in bond lengths involving nonhydrogen atoms	0·0038 Å	0·0040 Å
Average σ in bond angles involving nonhydrogen atoms	0·22°	0·24°
Average σ in bond lengths involving hydrogen atoms	0·034 Å	0·044 Å

Table 3. *Observed and calculated structure factors*

Each set of four columns contains respectively h, $10 \times F_o$, $10 \times F_c$, $10 \times \sigma(F_o)$
where $\sigma(F_o)$ is based on an instability constant of 0·03.

Table 4. *Positional and thermal parameters*

(a) Atomic parameters for carbon, nitrogen, oxygen and sulfur atoms.

Thermal parameters are defined by expression $\exp[-2\pi^2(U_{11}h^2a^{*2}+U_{22}k^2b^{*2}+U_{33}l^2c^{*2}+2U_{12}hka^*b^*+2U_{13}hla^*c^*+2U_{23}klb^*c^*)]$. Values are $\times 10^4$.

	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N(1)	4263 (2)	9494 (5)	1141 (1)	420 (14)	255 (12)	211 (11)	4 (11)	147 (10)	46 (10)
C(1)	3555 (2)	10467 (5)	1626 (2)	443 (15)	263 (14)	232 (12)	39 (12)	160 (11)	−9 (11)
C(5)	2424 (3)	10282 (8)	1066 (2)	429 (18)	716 (27)	358 (16)	81 (18)	149 (14)	149 (18)
C(2)	3703 (2)	8952 (5)	2461 (2)	370 (14)	280 (14)	242 (13)	12 (11)	107 (11)	−9 (11)
O(1)	3931 (2)	6701 (3)	2496 (1)	646 (13)	283 (10)	256 (9)	94 (9)	187 (9)	6 (8)
N(2)	3500 (2)	10215 (4)	3108 (1)	510 (14)	236 (12)	238 (11)	35 (11)	180 (10)	8 (9)
C(3)	3455 (2)	9048 (5)	3922 (1)	456 (16)	248 (13)	216 (12)	39 (12)	161 (11)	15 (10)
C(6)	2383 (2)	7876 (6)	3832 (2)	498 (17)	344 (16)	319 (15)	−57 (14)	138 (13)	−23 (14)
C(7)	1507 (3)	9762 (7)	3699 (3)	477 (19)	477 (21)	578 (22)	−28 (16)	160 (17)	−23 (19)
S	301 (1)	8189 (2)	3697 (1)	564 (6)	654 (7)	1208 (10)	−74 (5)	430 (6)	−44 (7)
C(8)	−517 (5)	10884 (13)	3656 (5)	602 (31)	1027 (41)	1478 (61)	136 (29)	386 (35)	145 (41)
C(4)	3752 (2)	10979 (5)	4665 (2)	352 (14)	294 (14)	253 (13)	51 (11)	126 (11)	−10 (11)
O(2)	3900 (1)	13194 (3)	4483 (1)	599 (13)	231 (10)	325 (10)	−16 (9)	198 (9)	−14 (8)
O(3)	3812 (2)	10190 (3)	5414 (1)	767 (15)	307 (11)	225 (9)	32 (10)	201 (10)	6 (8)

Table 4 (*cont.*)

(b) Atomic parameters for hydrogen atoms. The thermal parameter is defined by the expression $\exp[-8\pi^2 U^2(\sin^2\theta/\lambda^2)]$. Values are $\times 10^3$.

H(1)	495 (3)	979 (6)	147 (2)	47 (10)
H(2)	410 (2)	781 (7)	98 (2)	48 (10)
H(3)	419 (2)	1035 (6)	67 (2)	42 (9)
H(4)	227 (2)	851 (7)	89 (2)	62 (10)
H(5)	196 (3)	1072 (7)	139 (2)	65 (11)
H(6)	234 (2)	1133 (6)	56 (2)	58 (9)
H(7)	373 (2)	1216 (5)	177 (2)	24 (7)
H(8)	341 (2)	1180 (6)	307 (2)	34 (8)
H(9)	394 (2)	771 (5)	406 (2)	26 (7)
H(10)	242 (2)	686 (6)	438 (2)	59 (10)
H(11)	220 (2)	667 (6)	334 (2)	51 (8)
H(12)	168 (3)	1098 (7)	417 (2)	68 (11)
H(13)	139 (2)	1065 (6)	318 (2)	61 (10)
H(14)	−7 (8)	1141 (23)	445 (7)	332 (68)
H(15)	−119 (4)	1039 (9)	352 (3)	134 (19)
H(16)	−36 (7)	1152 (17)	306 (6)	161 (48)

Discussion

The results of the refinements of the two data sets with different instability constants and deterioration corrections, Table 2, are not very different. The number of reflections less than the threshold of $1\sigma(I)$ is nearly the same, and R showed only a slight decrease on increasing the instability constant; but R_w increased from 0·043 to 0·060. This is not unexpected since increasing the constant decreases the weights of the larger reflections causing the weaker, less accurate ones to contribute more to R_w. In contrast to R_w the 'goodness of fit' shows a marked decrease for the data set with the larger instability constant. This follows from the fact that the larger constant gives smaller weights to the more intense reflections, substantially reducing the numerator of the expression for the 'goodness of fit' while the denominator remains virtually unchanged.

In spite of the differences in the data caused by allowing for deterioration and using a different instability constant, we find a r.m.s. difference between the non-hydrogen atom positions after refinements based on both data sets of only 0·004 Å and an r.m.s. difference in bond lengths even smaller, 0·0015 Å. The average standard deviations in bond lengths and angles from the two sets of data are not significantly different for the nonhydrogen atoms.

The change in the instability constant would be expected to have a greater effect on the hydrogen atoms and this is, indeed, found to be so. Bond lengths involving hydrogen atoms tend to be more variable with the larger constant, and the average standard deviation is almost 30% greater. The poorer precision follows from the fact that the larger constant gives smaller weights to the intense, predominantly low-angle reflections, and it is the low-angle reflections to which the hydrogen atoms make their greatest contribution.

Although the use of 0·03 rather than 0·01 for the instability constant gives somewhat larger standard deviations, we consider them more realistic estimates of the precision. Accordingly, the σ's in Table 3 and the parameters in Table 4 are based on the higher value.

Initially, there was a possibility that the pair of molecules in this crystal would be l-ala-l-met and its enantiomer. However, the crystal structure determination shows the molecules to be l-ala-d-met and its enantiomer. Since the molecules exist as zwitterions in the crystal, the amino group resembles the alanyl side chain, and initially the two groups could have been

Table 5. *Hydrogen bonds*

	N···O	N–H	H···O	N–H···O	H–N···O
N(1)–H(1)···O(1)	2·983 (3) Å	0·92 (4) Å	2·14 (3) Å	150·5 (3·2)°	2·7 (2·3)°
N(1)–H(2)···O(2)	2·747 (3)	0·94 (4)	1·83 (3)	165·6 (3·2)	9·6 (2·1)
N(1)–H(3)···O(3)	2·844 (3)	0·96 (3)	1·99 (3)	170·1 (2·8)	6·9 (1·9)

interchanged. As noted above, nitrogen was assigned on the basis of residual density in a ΔF map. The choice was confirmed by the C(1)–C(5) bond length of 1·517 (4) Å compared with the C(1)–N(1) bond length of 1·479 (4) Å. Conclusive evidence is found in the fact that all three hydrogen atoms of the group identified as the amino group are involved in hydrogen bonds.

The bond lengths, bond angles, and peptide main-chain torsion angles, uncorrected for thermal motion, are given in Fig. 2. The values obtained for this dipeptide agree well with those obtained for the corresponding amino acids (Mathieson, 1952; Simpson & Marsh, 1966; Dunitz & Ryan, 1966), except for the C(1)–N(1) bond length which appears to be shortened by about 0·015 Å. The usual C–NH$_3^+$ bond length is 1·494 Å, but in this case it is closer to the usual single C–N bond length of 1·472 Å. Bond lengths and angles involving the S atom are close to normal although the terminal atoms of the methionine side chain exhibit considerable thermal motion, Fig. 2.

Fig. 3 is a packing diagram of the crystal structure. The molecules are arranged with the peptide main-chains oriented head-to-tail along the z axis.

The molecules are bound together by hydrogen bonds forming a double layer parallel to the yz plane. The methionine and alanine side chains are packed between the double layers in such a way as to form the rungs of a ladder-like structure.

All three hydrogen atoms of the free amino group participate in hydrogen bonding with all three possible oxygen acceptors. Table 5 lists the distances and angles of the hydrogen bonds. The N(1)–H(1)$\cdots$O(1) bond is rather long and distorted, but it is well within the limits for an acceptable hydrogen bond. There is another close contact in the crystal between N(1) and O(3), the distance being 3·057 (3) Å. The O(3)$\cdots$H(3) distance, however, is 2·44 (3) Å and the N–H$\cdots$O angle is 129·0 (2·8)°, suggesting that any interaction is weak.

As in many of the peptide structures determined by crystallographic techniques, crystal packing forces cause the peptide group in this molecule to be significantly uonplanar. The r.m.s. deviation of the five atoms comprising the peptide 'plane' from the least-squares plane through them is 0·042 Å and the torsion angle about the C(2)–N(2) bond is 171·5 (2)°.

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