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The Crystal Structure of L-Alanyl-L-alanine Hydrochloride

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(*Received* 25 *July* 1968)

The crystal structure of L-alanyl-L-alanine hydrochloride, $^{+}H_3NCH(CH_3)CONHCH(CH_3)COOH.Cl^{-}$, has been determined. The crystal is orthorhombic with space group $P2_12_12$; the unit-cell dimensions are: $a=9{\cdot}51$, $b=19{\cdot}72$, $c=5{\cdot}38$ Å. Final refinement was made by block-diagonal least-squares method to an R of 0·079 and this gave a standard deviation of about 0·012 Å in bond lengths among the non-hydrogen atoms. The bond lengths and angles agree with those found so far in amino acids and peptides. Both the carboxyl and the peptide group are planar within the limits of experimental error, and the dihedral angle between the planes is 27°. The internal rotation angles in the peptide backbone are similar to those in the antiparallel pleated sheet configuration of polypeptide.

Introduction

Accurate structure analyses by X-ray of various kinds of amino acids and peptides have been carried out at many places mainly with biochemical interest. Most of these peptides, however, contain at least one glycyl residue. The only example containing an alanyl residue is glycyl-L-alanine hydrochloride (Tranter, 1956), whereas alanine is one of the most frequently appearing components in proteins. The present work on L-alanyl-L-alanine hydrochloride has been undertaken in order to provide some fundamental knowledge on the conformations of the alanyl residues in proteins, in particular, this knowledge is of essential importance to the structure of tussah silk fibroin.

Experimental

L-Alanyl-L-alanine hydrochloride was obtained in the form of needle-like crystals elongated along the c axis by dissolving the peptide in a slight excess of 2N HCl and evaporating to dryness *in vacuo* at room temperature. Since the crystals are hygroscopic, the specimen was coated with a thin-film of collodion during the X-ray experiment.

Unit-cell dimensions were determined by the least-squares calculation, using 17 $0kl$ and 21 $hk0$ reflexions whose Bragg angles were measured on zero-layer Weissenberg photographs taken with Cu $K\alpha$ radiation and calibrated with aluminum powder lines. The density was measured by flotation in a benzene–carbon tetrachloride mixture.

Crystal data:

$a=\ 9{\cdot}51\pm0{\cdot}01$ Å, $\quad \varrho_{obs}=1{\cdot}29_0$ g.cm.$^{-3}$
$b=19{\cdot}72\pm0{\cdot}01$ Å, $\quad \varrho_{cal}=1{\cdot}29_5$ g.cm.$^{-3}$
$c=\ 5{\cdot}38\pm0{\cdot}01$ Å. $\quad Z=4$.

The systematic absence of the odd orders of $h00$ and $0k0$ reflexions were observed, hence the space group was found to be $P2_12_12$.

The three-dimensional intensity data for nickel-filtered Cu $K\alpha$ radiation were collected from multiple-film, equi-inclination Weissenberg photographs of the layer line 0–7 about the a axis and 0–4 about the c axis. The intensities were visually estimated by comparison with a standard scale. They were corrected for Lorentz and polarization factors; no absorption and extinction corrections were applied. Corrections for variation in spot-size on higher-layer photographs were made by the method presented by Phillips (1954). Since the intensity data from the c axis photographs seemed to be less reliable than those from the a axis, the former were used only for reflexions outside the region of the a axis photographs. Thus 1273 intensity data were obtained of which 183 were too weak to be observed.

Determination of the structure

The coordinates of the chloride ion were deduced from the Patterson fucntion. The electron density distribution and the minimum function were synthesized on the basis of the coordinates of the chloride ion. All the non-hydrogen atoms were identified, though

some spurious peaks appeared. Succesive Fourier syntheses did not show any spurious peak.

The positional and thermal parameters were refined by the block-diagonal least-squares method, minimizing $\Sigma w(|F_o|-|F_c|)^2$. The weighting scheme was; $w=\frac{1}{2}$ when $F_o=0$ and $w=1$ when $F_o\neq 0$. The scattering factors for H, C, N, O and Cl^- were taken from *International Tables for X-ray Crystallography* (1962). After three cycles with isotropic thermal parameters and two with anisotropic thermal parameters, the reliability index, R, was reduced to 0·114. At this stage, the seven non-methyl hydrogen atoms were assigned coordinates with reasonable bond distances and angles, and they were confirmed in the difference-Fourier synthesis. The six hydrogen atoms of the methyl groups could also be located in this synthesis. After several cycles of the least-squares calculation including all 25 atoms, the maximum shift of the non-hydrogen atom coordinates became less than the estimated standard deviations; their mean value for C, N and O was 0·007 Å (0·005–0·011 Å). The R index was 0·079 ($R=0·100$, for all 1273 reflexions).

The final atomic coordinates and thermal parameters for non-hydrogen atoms are given in Tables 1 and 2, and for the hydrogen atoms in Table 3. The absolute configuration of the molecule was not determined; the

Table 1. *The final atomic coordinates (fractional) and their standard deviations* (Å)

	x	$\sigma(x)$	y	$\sigma(y)$	z	$\sigma(z)$
C(1*A*)	−0·0080	0·007	0·3218	0·006	0·3941	0·008
C(1*B*)	−0·0640	0·008	0·3497	0·008	0·6407	0·010
C(1)	0·1402	0·007	0·3468	0·006	0·3389	0·007
C(2*A*)	0·2751	0·006	0·4398	0·007	0·1605	0·008
C(2)	0·2537	0·007	0·5168	0·007	0·1604	0·009
C(2*B*)	0·3224	0·009	0·4179	0·009	−0·1051	0·011
N(1)	−0·0030	0·006	0·2456	0·006	0·4126	0·007
N(2)	0·1434	0·006	0·4082	0·006	0·2298	0·007
O(1)	0·2442	0·005	0·3152	0·005	0·3969	0·006
O(2–1)	0·1417	0·006	0·5415	0·005	0·1508	0·009
O(2–2)	0·3680	0·006	0·5498	0·005	0·1662	0·009
Cl	0·1510	0·002	0·1999	0·002	0·9075	0·002

Table 2. *The thermal parameters and their standard deviations (multiplied by* 10^4)

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

	β_{11}	σ	β_{22}	σ	β_{33}	σ	β_{12}	σ	β_{13}	σ	β_{23}	σ
C(1*A*)	75	7	19	2	295	26	7	6	48	31	5	13
C(1*B*)	122	10	29	2	457	41	−1	8	222	39	−22	18
C(1)	64	7	21	2	307	27	−5	6	−33	28	−11	12
C(2*A*)	56	7	21	2	373	31	4	6	57	27	6	13
C(2)	58	7	25	2	451	36	−5	7	58	30	−1	15
C(2*B*)	129	11	38	3	461	40	−3	9	212	43	−61	21
N(1)	101	7	20	1	326	24	−17	6	−20	32	8	13
N(2)	60	6	23	2	373	26	1	6	−4	25	24	11
O(1)	77	5	26	1	394	22	6	5	−45	23	26	11
O(2–1)	101	7	23	1	1019	48	−2	6	155	39	4	16
O(2–2)	105	7	22	1	1098	52	−6	6	24	40	20	16
Cl	118	2	25	0	327	6	21	2	6	8	0	4

Table 3. *The hydrogen atom parameters*

	Bonded to	x	y	z	B
H(1)	N(1)	0·055	0·230	0·522	4·6
H(2)		0·051	0·227	0·262	0·8
H(3)		−0·108	0·226	0·470	2·1
H(4)	C(1*A*)	−0·091	0·332	0·261	1·6
H(5)	C(1*B*)	−0·166	0·328	0·677	4·1
H(6)		−0·086	0·395	0·630	1·0
H(7)		0·038	0·350	0·788	1·1
H(8)	N(2)	0·048	0·423	0·145	3·1
H(9)	C(2*A*)	0·365	0·435	0·277	0·9
H(10)	C(2*B*)	0·346	0·370	−0·109	6·6
H(11)		0·417	0·437	−0·161	6·5
H(12)		0·241	0·426	−0·219	4·1
H(13)	O(2–2)	0·333	0·591	0·167	4·3

$\langle\sigma(x)\rangle=0·080$ Å $\quad\langle\sigma(y)\rangle=0·075$ Å $\quad\langle\sigma(z)\rangle=0·088$ Å

$\langle\sigma(B)\rangle=2·1$ Å^2

Table 4. *Calculated and observed structure factors* ($\times 5$)

K	FO	FC	K	FO	FC	K	FO	FC	K	FO	FC	K	FO	FC	K	FO	FC	K	FO	FC	K	FO	FC
H,L= 0 0			2	107	111	20	0	3	18	16	21	4	102	107	0	0	10	2	246	233	18	29	29	0	62	46
4	92	74	3	94	104	H,L= 8 0			19	24	28	5	60	49	1	128	128	3	88	88	19	0	6	1	80	62
6	48	39	4	84	92	0	179	180	20	46	44	6	22	30	2	100	87	4	122	117	20	0	3	2	33	27
8	407	412	5	120	125	1	79	83	21	33	32	7	86	84	3	49	45	5	15	10	21	31	30	3	19	15
10	36	32	6	63	57	2	0	1	22	14	21	8	158	162	4	13	12	6	37	36	22	17	19	4	0	14
12	20	17	7	49	47	3	0	2	23	6	1	9	54	55	5	34	31	7	210	217	H,L= 5 2			5	0	10
14	56	53	8	114	116	4	51	50	24	11	15	10	64	55	6	16	17	8	105	105	0	88	88	6	18	13
16	40	37	9	49	49	5	0	1	H,L= 1 1			11	111	105	7	41	35	9	84	75	1	102	100	7	79	70
18	73	68	10	19	17	6	36	35	0	84	88	12	64	58	8	41	35	10	42	38	2	90	86	8	28	26
20	101	98	11	65	57	7	0	11	1	157	158	13	32	35	9	78	73	11	100	96	3	28	21	9	0	12
22	10	7	12	151	147	8	52	55	2	136	149	14	78	78	10	24	25	12	0	4	4	85	80	10	45	42
24	0	2	13	0	10	9	61	62	3	246	250	15	14	12	11	40	38	13	19	11	5	84	81	11	36	33
H,L= 1 0			14	45	48	10	0	6	4	266	267	16	63	62	12	46	43	14	28	33	6	0	5	12	39	35
2	107	97	15	24	23	11	20	16	5	223	234	17	57	58	13	29	24	15	0	6	7	68	63	13	14	10
3	134	135	16	87	83	12	0	6	6	291	284	18	49	46	14	21	19	16	61	66	8	37	34	14	22	18
4	120	119	17	0	10	13	40	44	7	119	119	19	38	39	15	19	18	17	49	46	9	60	54	H,L= 10 2		
5	125	121	18	21	17	14	52	56	8	71	69	20	32	30	16	33	25	18	30	31	10	21	24	0	25	23
6	309	301	19	42	41	15	19	21	9	84	77	21	38	40	17	24	23	19	52	56	11	17	26	1	44	36
7	282	277	20	76	78	16	34	32	10	32	33	22	10	10	18	20	19	20	23	25	12	50	44	2	21	21
8	119	108	21	29	35	17	0	1	11	143	131	23	9	11	H,L= 9 1			21	22	24	13	89	81	3	0	2
9	128	118	22	0	1	18	15	12	12	135	138	H,L= 5 1			0	0	6	22	13	19	14	26	30	4	24	17
10	232	215	23	0	7	19	12	20	13	63	57	0	42	40	1	0	4	23	0	12	15	17	20	5	41	32
11	48	46	24	0	24	H,L= 9 0			14	108	100	1	100	108	2	99	87	H,L= 2 2			16	46	42	6	20	14
12	61	57	H,L= 5 0			1	23	19	15	62	55	2	100	105	3	28	23	0	55	60	17	0	8	7	28	22
13	61	54	1	57	52	2	0	5	16	33	34	3	24	25	4	32	32	1	64	63	18	0	14	8	22	19
14	112	101	2	50	65	3	53	56	17	23	28	4	47	49	5	49	40	2	86	84	19	41	41	9	0	10
15	104	92	3	30	24	4	26	18	18	25	27	5	75	77	6	64	59	3	158	152	20	0	4	10	19	15
16	32	29	4	66	77	5	51	48	19	22	25	6	105	108	7	55	50	4	86	71	21	26	28	11	0	10
17	0	11	5	64	69	6	98	93	20	22	26	7	126	135	8	18	19	5	68	65	H,L= 6 2			12	25	24
18	53	57	6	257	297	7	28	24	21	15	9	8	0	3	9	45	39	6	165	158	0	30	23	H,L= 11 2		
19	0	4	7	16	17	8	0	10	22	27	26	9	55	47	10	21	16	7	100	88	1	29	26	0	20	27
20	0	10	8	12	10	9	31	23	23	19	20	10	19	23	11	35	35	8	182	173	2	54	49	1	58	47
21	0	9	9	34	33	10	29	35	24	17	17	11	46	44	12	22	21	9	14	16	3	94	81	2	14	7
22	14	11	10	26	22	11	42	41	H,L= 2 1			12	40	38	13	25	17	10	50	47	4	54	49	3	24	23
23	20	27	11	40	33	12	14	25	0	290	310	13	93	91	14	26	20	11	48	46	5	82	82	4	16	13
24	18	19	12	49	45	13	29	30	1	271	277	14	83	76	15	24	23	12	92	82	6	53	50	5	12	8
25	15	23	13	61	55	14	39	43	2	315	315	15	75	70	16	17	16	13	53	52	7	94	90	6	0	7
H,L= 2 0			14	183	199	15	17	14	3	155	157	16	30	29	H,L= 10 1			14	84	80	8	28	28	7	26	34
0	255	254	15	0	3	16	0	6	4	130	129	17	0	6	0	73	55	15	35	36	9	32	32	8	22	28
1	242	279	16	17	13	17	0	16	5	115	112	18	62	55	1	75	57	16	52	54	10	25	22	H,L= 0 3		
2	259	273	17	26	27	H,L= 10 0			6	94	85	19	30	25	2	18	14	17	13	24	11	25	29	0	92	96
3	125	140	18	13	17	0	120	114	7	80	69	20	0	6	3	0	5	18	0	3	12	18	14	1	25	22
4	15	17	19	20	15	1	16	14	8	101	93	21	14	15	4	45	36	19	18	20	13	73	72	2	166	163
5	33	35	20	11	12	2	0	7	9	124	114	22	19	21	5	25	22	20	0	6	14	52	52	3	173	162
6	49	52	21	0	2	3	16	17	10	80	72	H,L= 6 1			6	15	11	21	18	23	15	29	29	4	102	99
7	57	57	22	10	11	4	0	0	11	111	100	0	94	86	7	29	25	22	10	11	16	25	25	5	286	262
8	216	214	23	10	16	5	23	26	12	33	25	1	59	53	8	14	13	23	12	14	17	21	24	6	104	104
9	197	190	H,L= 6 0			6	40	36	13	57	51	2	93	90	9	13	10	H,L= 3 2			18	17	9	7	76	67
10	146	146	0	151	152	7	0	0	14	0	4	3	68	68	10	22	21	0	60	58	19	0	3	8	50	48
11	118	106	1	141	132	8	42	44	15	23	23	4	29	29	11	11	12	1	85	74	20	0	4	9	25	30
12	88	78	2	65	90	9	0	1	16	56	58	5	36	33	12	19	19	2	85	79	H,L= 7 2			10	19	13
13	49	47	3	53	58	10	37	37	17	33	33	6	40	42	13	18	19	3	123	114	0	103	108	11	45	46
14	50	42	4	20	20	11	0	2	18	54	51	7	36	36	H,L= 11 1			4	52	44	1	49	51	12	45	49
15	0	12	5	12	7	12	28	27	19	37	38	8	89	96	0	16	15	5	32	30	2	102	118	13	116	118
16	17	10	6	74	80	13	13	16	20	18	19	9	69	74	1	13	11	6	111	102	3	32	28	14	0	0
17	22	23	7	31	35	14	12	14	21	40	40	10	85	87	2	29	19	7	57	52	4	19	18	15	74	71
18	0	7	8	36	49	H,L= 11 0			22	36	37	11	29	35	3	20	14	8	105	98	5	12	13	16	14	17
19	66	62	9	62	64	1	0	0	23	23	27	12	63	66	4	47	38	9	52	51	6	55	55	17	0	5
20	0	6	10	25	26	2	0	0	24	8	12	13	31	31	5	26	26	10	70	71	7	54	54	18	14	5
21	60	55	11	23	26	3	0	6	H,L= 3 1			14	28	32	6	40	34	11	68	70	8	21	20	19	0	3
22	0	6	12	18	20	4	0	4	0	45	55	15	19	19	7	8	11	12	116	115	9	24	26	20	0	3
23	0	1	13	25	24	5	32	30	1	104	114	16	0	10	8	22	19	13	87	87	10	15	15	21	0	1
24	13	14	14	13	13	6	21	24	2	165	162	17	45	43	H,L= 0 2			14	86	85	11	15	20	22	0	1
H,L= 3 0			15	0	0	7	20	18	3	123	133	18	26	26	0	93	98	15	0	2	12	15	14	H,L= 1 3		
1	120	116	16	7	10	8	0	1	4	204	198	19	29	26	1	227	213	16	14	20	13	43	42	0	0	10
2	213	240	17	10	10	9	16	15	5	133	123	20	25	23	2	244	254	17	61	56	14	0	4	1	111	98
3	151	173	18	35	35	10	24	31	6	67	60	21	13	16	3	125	131	18	0	8	15	25	25	2	17	21
4	83	90	19	26	31	H,L= 12 0			7	202	193	H,L= 7 1			4	95	96	19	25	27	16	0	9	3	82	74
5	178	187	20	41	41	0	23	30	8	88	87	0	72	77	5	120	122	20	54	54	17	27	25	4	69	56
6	209	203	21	33	34	1	29	41	9	83	77	1	42	43	6	46	46	21	25	28	18	29	27	5	68	67
7	183	186	H,L= 7 0			2	26	24	10	32	32	2	53	56	7	138	138	22	32	34	19	9	7	6	55	63
8	55	44	1	46	45	H,L= 0 1			11	0	7	3	78	80	8	0	1	H,L= 4 2			H,L= 8 2			7	22	23
9	45	33	2	38	38	0	189	183	12	72	71	4	34	42	9	53	50	0	68	59	0	0	9	8	73	77
10	94	88	3	60	67	1	505	518	13	58	57	5	68	72	10	71	72	1	79	77	1	43	33	9	92	89
11	67	59	4	94	118	2	326	343	14	51	46	6	19	19	11	121	124	2	76	73	2	76	68	10	93	96
12	12	12	5	84	93	3	26	17	15	134	126	7	37	32	12	19	24	3	148	147	3	95	93	11	70	67
13	87	83	6	38	35	4	110	104	16	87	88	8	48	45	13	76	74	4	80	67	4	49	36	12	70	74
14	123	118	7	49	46	5	240	233	17	43	44	9	56	56	14	15	18	5	141	147	5	19	13	13	23	31
15	134	134	8	22	27	6	122	115	18	26	28	10	61	61	15	65	60	6	53	45	6	69	65	14	39	46
16	38	41	9	43	43	7	195	194	19	17	20	11	36	37	16	41	45	7	134	124	7	48	46	15	30	33
17	0	4	10	25	30	8	195	189	20	13	13	12	59	61	17	49	49	8	68	60	8	53	51	16	20	23
18	0	4	11	0	5	9	138	132	21	13	16	13	37	36	18	27	30	9	48	46	9	19	24	17	14	14
19	0	16	12	0	2	10	133	130	22	18	20	14	26	23	19	56	52	10	46	42	10	19	21	18	19	19
20	27	24	13	37	45	11	45	39	23	31	36	15	37	35	20	0	0	11	64	61	11	33	35	19	39	43
21	0	6	14	40	38	12	193	184	24	8	9	16	30	30	21	21	26	12	47	44	12	26	20	20	20	26
22	19	18	15	65	62	13	123	130	H,L= 4 1			17	22	21	22	13	15	13	136	141	13	21	20	21	18	21
23	15	14	16	0	2	14	77	73	0	266	295	18	25	23	23	31	34	14	35	38	14	41	44	22	13	10
H,L= 4 0			17	21	17	15	22	24	1	114	112	19	10	10	H,L= 1 2			15	35	30	15	0	7	H,L= 2 3		
0	144	156	18	0	2	16	0	13	2	171	185	20	14	17	0	184	184	16	51	53	16	29	26	0	102	104
1	161	168	19	0	1	17	16	12	3	100	112	H,L= 8 1			1	371	341	17	0	13	H,L= 9 2			1	17	20

Table 4 (*cont.*)

K	FO	FC	K	FO	FC	K	FO	FC	K	FO	FC	K	FO	FC	K	FO	FC	K	FO	FC	K	FO	FC
2	106	104	15	40	41	11	8	7	15	27	36	10	82	84	10	9	9	12	44	49	2	65	63	8	25	28
3	51	48	16	29	27	12	42	42	16	28	32	11	58	58	11	0	6	13	0	5	3	27	27	9	0	7
4	155	140	17	26	29	13	0	2	17	31	34	12	53	55	12	16	21	14	39	42	4	21	25	10	38	44
5	83	78	18	0	10	14	21	22	18	18	24	13	21	27	13	15	15	15	0	3	5	[illegible]	8	11	12	16
6	98	98	19	16	17	15	0	2	19	9	10	14	23	25	14	0	7	16	7	11	6	27	28	H,L=	1	6
7	32	29	20	0	5	16	0	9	20	18	21	15	26	30	15	18	22	H,L=	1	5	7	28	30	0	0	15
8	41	37	21	0	7	H,L=	8	3	H,L=	1	4	16	0	1	16	12	16	0	0	7	8	38	41	1	22	25
9	36	38	H,L=	5	3	0	21	18	0	69	70	17	16	20	H,L=	7	4	1	24	35	9	16	15	2	22	19
10	17	18	0	85	79	1	32	30	1	71	63	18	14	15	0	51	48	2	24	36	10	22	22	3	22	21
11	29	26	1	138	139	2	40	36	2	69	65	19	7	5	1	0	7	3	43	48	11	25	25	4	21	29
12	24	23	2	78	74	3	44	39	3	22	27	H,L=	4	4	2	34	27	4	57	62	12	[illegible]	4	5	21	20
13	23	24	3	11	12	4	43	37	4	35	35	0	19	22	3	24	23	5	32	38	13	15	24	6	21	24
14	63	62	4	0	3	5	48	42	5	9	4	1	58	51	4	49	46	6	56	58	14	12	13	H,L=	2	6
15	25	26	5	0	4	6	51	44	6	25	31	2	29	28	5	23	19	7	24	31	H,L=	5	5	0	0	7
16	55	55	6	12	18	7	0	0	7	60	58	3	78	74	6	23	18	8	24	32	0	[illegible]	5	1	26	29
17	14	8	7	59	57	8	22	21	8	28	29	4	14	16	7	23	23	9	0	5	1	14	5	2	0	9
18	22	18	8	36	40	9	43	42	9	87	93	5	91	82	8	23	21	10	0	11	2	20	14	3	16	18
19	0	6	9	111	108	10	0	10	10	35	42	6	38	41	9	29	29	11	25	32	3	20	24	4	0	18
20	0	5	10	12	16	11	30	25	11	32	34	7	21	28	10	29	26	12	29	34	4	31	31	5	16	12
21	0	10	11	65	55	12	16	15	12	30	28	8	21	18	11	21	23	13	28	32	5	14	16	6	15	19
22	15	17	12	73	71	13	26	25	13	20	23	9	14	16	12	13	12	14	30	37	6	34	30	7	14	15
H,L=	3	3	13	15	15	14	28	30	14	20	21	10	0	2	13	9	10	15	12	16	7	13	14	8	14	15
0	178	174	14	39	37	H,L=	9	3	15	23	27	11	26	28	14	15	19	H,L=	2	5	8	26	27	9	30	34
1	91	82	15	0	8	0	78	56	16	22	30	12	46	48	H,L=	8	4	0	35	31	9	[illegible]	7	10	18	22
2	160	148	16	22	21	1	37	36	17	21	23	13	21	23	0	44	34	1	55	55	10	12	15	11	19	29
3	32	32	17	26	27	2	75	51	18	23	31	14	34	35	1	27	21	2	31	34	11	20	20	H,L=	3	6
4	81	75	18	34	32	3	20	18	19	17	16	15	26	30	2	40	32	3	50	48	12	18	22	0	0	7
5	13	15	19	39	40	4	23	20	20	12	12	16	23	21	3	27	20	4	0	15	13	[illegible]	9	1	0	11
6	24	28	20	0	3	5	0	4	H,L=	2	4	17	10	8	4	53	48	5	0	13	14	9	18	2	0	8
7	52	48	H,L=	6	3	6	21	23	0	27	35	18	9	12	5	0	4	6	18	23	H,L=	6	5	3	37	37
8	59	51	0	0	0	7	16	15	1	46	45	H,L=	5	4	6	37	34	7	34	41	0	28	35	4	16	25
9	72	69	1	9	12	8	27	30	2	56	54	0	21	22	7	20	16	8	30	32	1	19	22	5	32	36
10	79	72	2	23	19	9	21	20	3	84	82	1	42	39	8	16	16	9	22	27	2	28	14	6	0	9
11	24	31	3	61	56	10	13	13	4	75	72	2	54	51	9	0	6	10	0	2	3	19	20	7	15	14
12	51	46	4	59	58	11	16	18	5	22	21	3	23	26	10	0	3	11	31	34	4	18	3	8	0	4
13	15	20	5	91	89	H,L=	10	3	6	46	46	4	32	25	11	11	13	12	18	23	5	18	13	9	0	4
14	37	38	6	44	37	0	0	8	7	45	47	5	18	19	12	7	10	13	25	28	6	18	19	10	0	10
15	39	39	7	10	12	1	0	9	8	37	39	6	68	64	H,L=	9	4	14	0	11	7	26	23	H,L=	4	6
16	33	31	8	13	17	2	23	25	9	20	26	7	0	7	0	11	12	15	0	5	8	30	33	0	16	15
17	18	20	9	35	39	3	26	24	10	32	32	8	53	51	1	13	10	16	0	4	9	17	15	1	16	16
18	40	41	10	12	12	4	24	26	11	38	38	9	39	37	2	15	14	H,L=	3	5	10	22	28	2	11	13
19	15	19	11	13	10	5	47	43	12	19	26	10	21	19	3	23	19	0	20	17	H,L=	7	5	3	19	22
20	33	37	12	16	22	6	0	6	13	21	31	11	60	53	4	9	11	1	20	22	0	21	19	4	10	6
21	0	4	13	31	35	7	19	18	14	26	32	12	33	32	5	18	15	2	26	26	1	[illegible]	18	5	19	23
H,L=	4	3	14	30	30	H,L=	0	4	15	0	11	13	14	21	6	17	20	3	30	31	2	[illegible]	9	6	0	5
0	0	19	15	35	38	0	194	202	16	28	32	14	45	43	7	13	12	4	0	7	3	25	21	7	16	11
1	49	50	16	10	13	1	45	49	17	16	21	15	12	17	8	17	23	5	31	35	4	[illegible]	6	8	15	19
2	49	44	17	17	21	2	84	75	18	15	19	16	8	8	H,L=	0	5	6	14	19	5	27	26	H,L=	5	6
3	38	37	H,L=	7	3	3	68	69	19	13	13	17	14	21	0	0	6	7	51	54	6	27	26	0	0	5
4	61	52	0	34	31	4	24	25	H,L=	3	4	H,L=	6	4	1	16	15	8	14	14	7	31	28	1	0	11
5	67	66	1	64	63	5	58	59	0	0	4	0	28	20	2	16	20	9	36	37	8	11	11	2	21	21
6	77	71	2	23	25	6	68	69	1	46	52	1	40	40	3	16	23	10	13	16	H,L=	0	6	3	0	4
7	36	37	3	35	36	7	13	20	2	63	65	2	45	43	4	0	6	11	0	11	0	47	47	4	40	47
8	65	59	4	14	7	8	73	78	3	55	55	3	21	27	5	0	10	12	0	9	1	[illegible]	3	5	0	6
9	49	47	5	26	27	9	25	35	4	17	26	4	45	44	6	0	6	13	14	20	2	55	56	6	19	21
10	21	22	6	14	9	10	12	10	5	27	29	5	58	54	7	0	13	14	0	6	3	[illegible]	2			
11	64	59	7	25	25	11	20	15	6	28	33	6	12	14	8	30	32	15	24	29	4	14	16			
12	38	40	8	54	52	12	18	15	7	64	66	7	29	28	9	0	16	H,L=	4	5	5	[illegible]	9			
13	31	32	9	41	40	13	19	13	8	48	45	8	13	16	10	46	46	0	88	85	6	[illegible]	6			
14	49	54	10	86	79	14	0	4	9	14	12	9	0	11	11	10	17	1	23	25	7	[illegible]	0			

atomic parameters in the Tables show the alanyl residues with the L-form referring to a right-handed coordinate system. The observed and calculated structure factors are given in Table 4. Finally, two Fourier synthesis were carried out. For the difference map, the coefficients were taken as the differences between the absolute values of the observed structure factors and those calculated with the non-hydrogen atoms only; the phase angles, however, included the contributions of the hydrogen atoms. A composite drawing of this synthesis is given in Fig.2, and the final electron density map is shown in Fig1. for comparison with Fig.2. All the peak heights corresponding to the hydrogen atoms in the difference map are higher than 0·55 e.Å^{-3}, and there is no other positive region exceeding 0·30 e.Å^{-3} except for two peaks of 0·40 e.Å^{-3}. There are some negative regions (as large as $-0·6$ e.Å^{-3}), but they are usually associated with the chloride ion. Parameters for the hydrogen atoms obtained from the difference map are not so significantly different from those given by the final least-squares calculation.

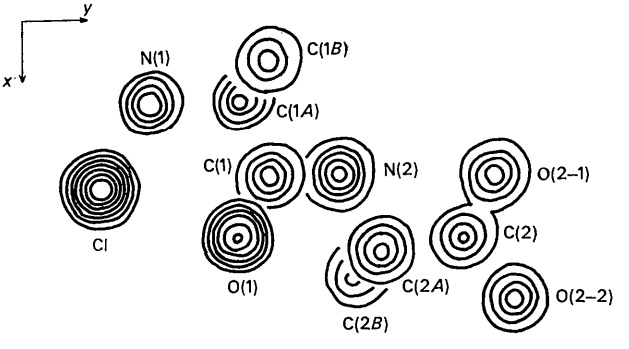

Fig. 1. A composite drawing of the final electron density map, viewed along the *c* axis. Contours are at 2, 4, 6 e.Å^{-3} . . . for the light atoms, and at 4, 8, 12 . . . e.Å^{-3} for the chloride ions.

All the numerical computations were done on the NEAC 2101 of this laboratory, the NEAC 2200 of the computation center of Osaka University and the HITAC 5020E of the computer center of the University of Tokyo.

Main programs used:

Unit cell dimension, written by A. Sugihara.
Lp and spot shape correction by N. Tanaka.
Patterson and minimum function by N. Yasuoka.
The least-squares calculation and Fourier synthesis by T. Ashida.
Bond lengths, angles and best planes by T. Ashida.

Discussion of the structure

Geometry of the molecule

The bond lengths and angles of the non-hydrogen atoms are shown in Fig. 3, while those involving the hydrogen atoms are shown in Table 5. The estimated standard deviations in the bond distances and angles among the non-hydrogen atoms are about 0·012 Å and 0·7°, respectively. In general, these structure data are in good agreement with those found so far in amino acids and peptides, and the peptide group in particular maintains good agreement with the standard conformation presented by Pauling & Corey (1953).

The two C–O bond lengths in the carboxyl group are 1·17 and 1·27 Å. The oxygen atom O(2–1) of the shorter C–O bond is an acceptor for an O···H–N hydrogen bond, while O(2–2) of the longer bond is the donor for the O–H···Cl hydrogen bond. In correlation with the bond lengths, the two O–C–C angles are different from each other, 113° and 122°.

The equations for the best planes of the carboxyl and the peptide group are given in Table 6, together with the deviations of the individual atoms from the planes. Each of the two groups is planar within the limits of error, and the dihedral angle between the planes is 27°.

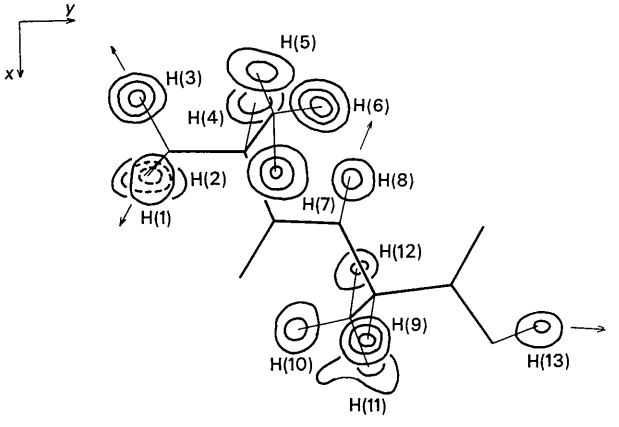

Fig. 2. A composite drawing of the final difference map, viewed along the *c* axis. The contributions of the hydrogen atoms were omitted from the amplitudes of F_c but were included in the phase angles. Contours are at intervals of 0·2 e.Å^{-3}, beginning with the 0·2 e.Å^{-3} contour. The dotted contours are in the lower section.

Table 6. *The equations of the least-squares planes through atoms*

Peptide group

$$-0{\cdot}0226X-0{\cdot}4528Y-0{\cdot}8913Z+4{\cdot}7634=0$$

Carboxyl group

$$0{\cdot}0383X-0{\cdot}0017Y-0{\cdot}9993Z+0{\cdot}7801=0$$

Peptide group		Carboxyl group	
Atom	Deviation	Atom	Deviation
C(1*A*)	0·002 Å	C(2*A*)	0·002 Å
O(1)	−0·007	C(2)	−0·007
C(1)	0·012	O(2–1)	0·003
N(2)	−0·014	O(2–2)	0·002
C(2*A*)	0·007	N(2)*	−0·417
N(1)*	0·593	C(2*B*)*	1·448
C(1*B*)*	−1·418	H(13)*	−0·017
C(2)*	−0·675		
C(2*B*)*	1·467		
H(8)*	0·282		

* These atoms were not included in the least-squares calculations.

The internal rotation angles in the peptide backbone (according to difinitions proposed by Edsall, Flory, Kendrew, Liquori, Némelty & Ramachandran, 1966) are:

ψ_1[C(1*A*)–C(1)] = 334·2°, ω[C(1)–N(2)] = 0·8°.
φ[N(2)–C(2*A*)] = 26·5°, ψ_2[C(2*A*)–C(2)] = 341·3° (or 161·3°).

Table 5. *Bond distances and angles involving the hydrogen atoms*

d(*X*–H)	
C(1*B*)–H	0·91, 1·08, 1·25 Å
C(1*A*)–H(4)	1·09 Å
C(2*A*)–H(9)	1·06 Å
C(2*B*)–H	0·97, 1·00, 1·02 Å
N(1)–H	0·87, 1·03, 1·11 Å
N(2)–H(8)	1·05 Å
O(2–2)–H(13)	0·88 Å
$\langle\sigma(d)\rangle$ = 0·09 Å	
∠(H–*X*–H)	
H–C(1*B*)–H	101, 102, 126°
H–C(2*B*)–H	98, 109, 116°
H–N(1)–H	96, 105, 123°
$\langle\sigma\rangle$ = 7·5°	
∠(C, N–*X*–H)	
C, N–C(1*A*)–H(4)	105, 105, 119°
C, N–C(2*A*)–H(9)	101, 107, 120°
C(1*A*)–C(1*B*)–H	106, 109, 112.
C(2*A*)–C(2*B*)–H	107, 111, 115°
C(1*A*)–N(1)–H	108, 110, 115°
C(1)–N(2)–H(8)	115°
C(2*A*)–N(2)–H(8)	121°
C(2)–O(2–2)–H(13)	99°
$\langle\sigma\rangle$ = 4·8°	

These are close to the expected values ($\varphi=38°$, $\psi=325°$, $\omega=0°$; (Miyazawa, 1961) for an antiparallel pleated sheet configuration and also to those found in β-poly-L-alanine ($\varphi=41{\cdot}4°, \psi=314{\cdot}7°\ \omega=1{\cdot}5°$; Arnott, Dover & Elliot, 1967).

The conformation about the two Cα–C′ bonds is similar to those of L-alanine (Simpson & Marsh, 1966) and DL-alanine (Donohue, 1950). The deviation of N(1) from the peptide plane is 0·59 Å, and that of N(2) from the carboxyl plane is 0·42 Å. Those in L- and DL-alanine are 0·44 Å and 0·38 Å, respectively.

Packing of the molecules

The drawings of the structure viewed along [001] and [100] are shown in Figs. 4 and 5, respectively. As clearly shown in Fig. 5, the molecules of L-alanyl-L-alanine make wave-like sheets parallel to the (001) joined with the hydrogen bonds, and these sheets are linked together along [001] by the chloride ions.

All five hydrogen atoms belonging to the nitrogen and the oxygen atoms take part in the hydrogen bonding. The hydrogen bond lengths and angles are given in Table 7. The terminal nitrogen atom, N(1), forms three hydrogen bonds – to two chloride ions and the oxygen atom, O(1), of the peptide group; the three acceptor atoms are approximately on the three vertices of a regular tetrahedron centered at N(1), with the carbon atom, C(1*A*), directed towards the fourth vertex. The peptide nitrogen atom, N(2), forms the

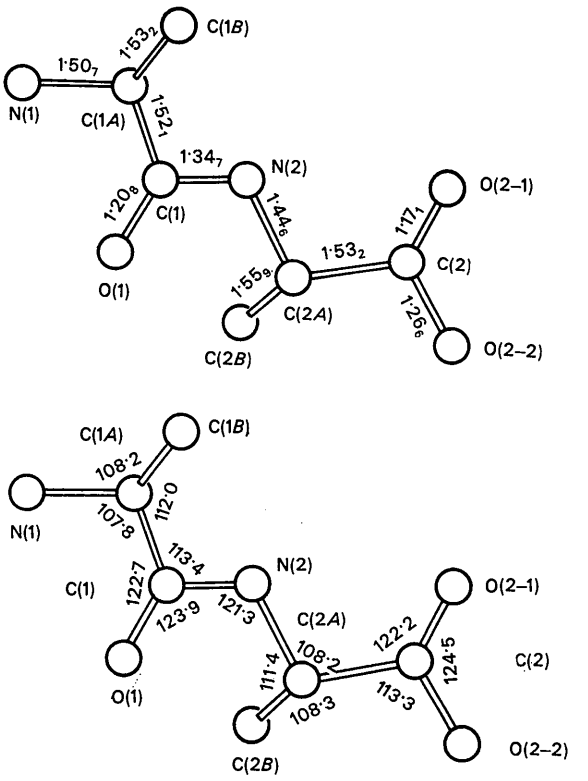

Fig. 3. Bond distances and angles.

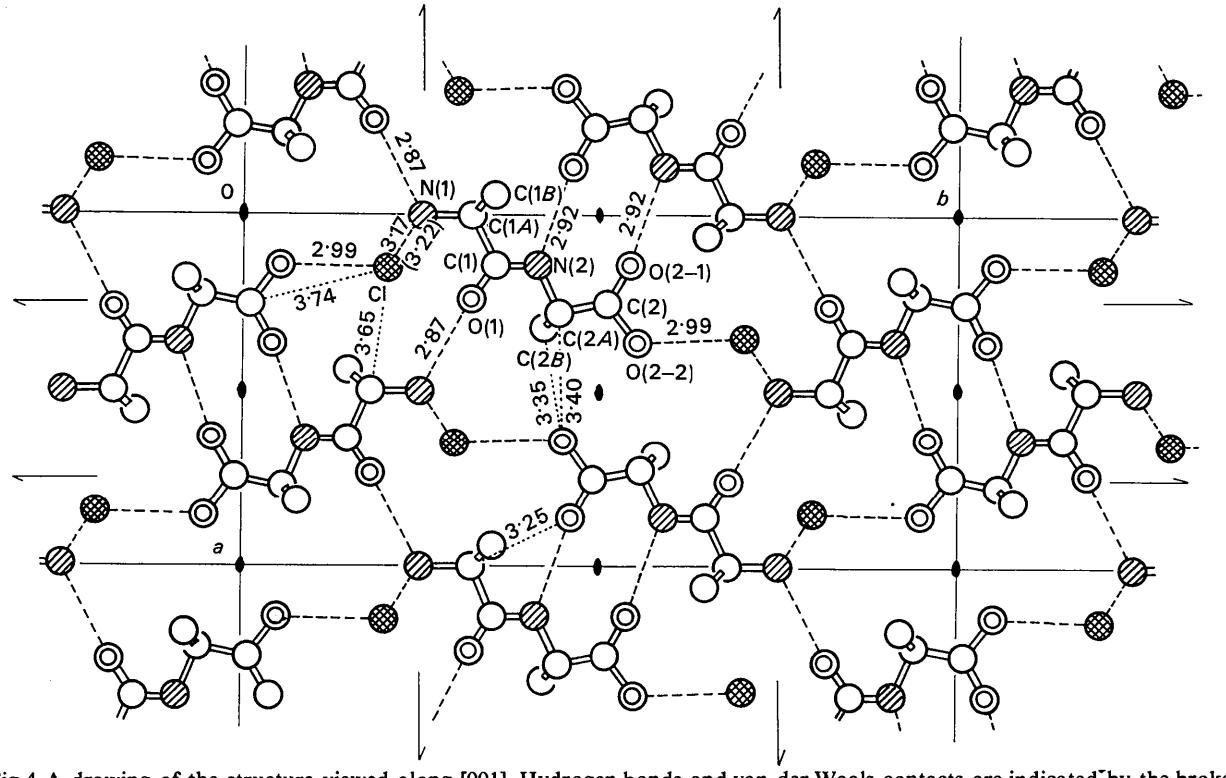

Fig. 4 A drawing of the structure viewed along [001]. Hydrogen bonds and van der Waals contacts are indicated by the broken and dotted lines, respectively.

Table 7. *Hydrogen bond lengths and angles*

Donor	H	Acceptor, at		$d(D\cdots A)$	$d(H\cdots A)$	$\angle(H\text{–}D\cdots A)$	$\angle(D\text{–}H\cdots A)$
N(1)	H(1)	Cl′	(x, y, z)	3·17 Å	2·34 Å	14°	161°
N(1)	H(2)	Cl″	$(x, y, z-1)$	3·22	2·20	6	172
N(1)	H(3)	O(1)	$(x-\frac{1}{2}, \bar{y}+\frac{1}{2}, \bar{z}+1)$	2·87	1·77	7	169
N(2)	H(8)	O(2–1)	$(\bar{x}, \bar{y}+1, z)$	2·92	1·94	17	153
O(2–2)	H(13)	Cl‴	$(\bar{x}+\frac{1}{2}, y+\frac{1}{2}, \bar{z}+1)$	2·99	2·19	20	152

$\angle(C\text{–}X\cdots A)$		$\angle(A\cdots N\cdots A)$	
C(1*A*)–N(1)···Cl′	111°	Cl′ ···N(1)···Cl″	115°
C(1*A*)–N(1)···Cl″	104	Cl″ ···N(1)···O(1)	124
C(1*A*)–N(1)···O(1)	114	O(1)···N(1)···Cl′	88
C(2*A*)–N(2)···O(2–1)	128		
C(1)–N(2) ···O(2–1)	110		
C(2)–O(2–2)···Cl‴	117		

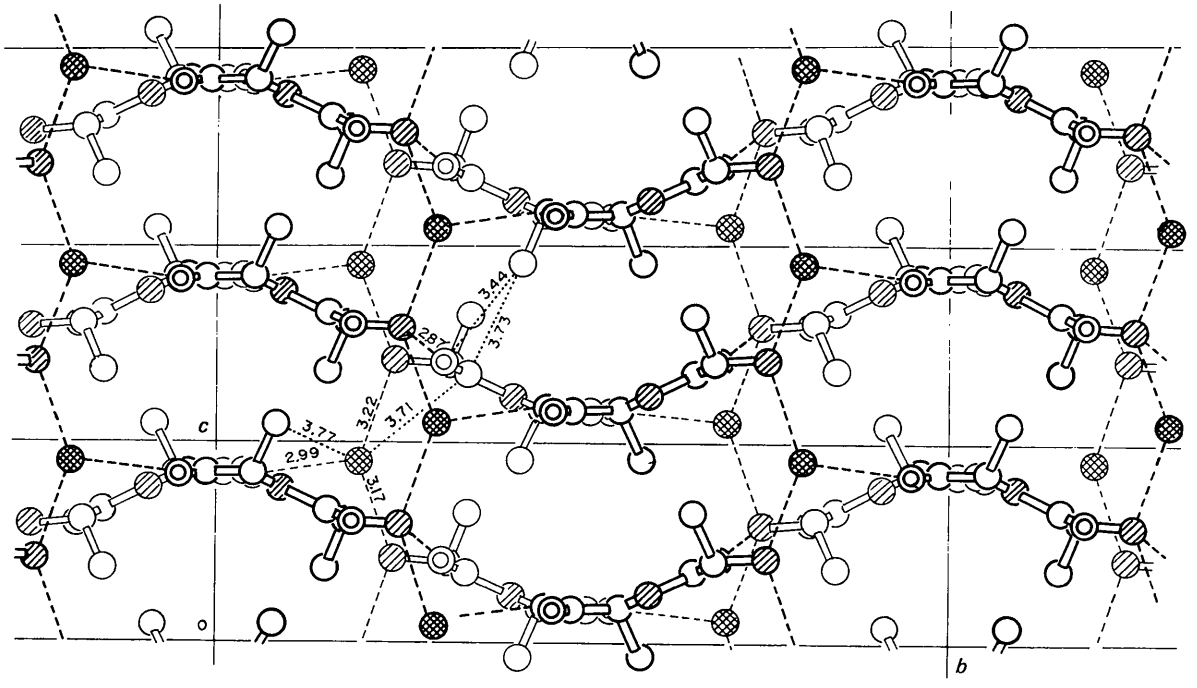

Fig.5. A drawing of the structure viewed along [100].

hydrogen bond to an oxygen atom, O(2–1), of the carboxyl group. This N(2)–H···O(2–1) hydrogen bond in the peptide group, 2·92 Å, is significantly longer (probably weaker) than the terminal N(1)–H···O(1) hydrogen bond of 2·87 Å. This is in agreement with a current survey of hydrogen bonding in peptides (Marsh & Donohue, 1967). The oxygen atom, O(2–2), of the carboxyl group forms the hydrogen bond with a chloride ion. The arrangement of these hydrogen bonds around the chloride ion is a trigonal pyramid with the chloride ion at the apex.

Some of the shorter intermolecular distances are indicated by the dotted lines in Figs.4 and 5. No unusually short contact is found in this crystal structure, and the shortest C···C, C···O and C···Cl distances are 3·73, 3·25 and 3·65 Å, respectively.

The authors thank Dr S.Sakakibara, Miss T.Nakao and Mr E.Munekata for supplying the sample. They are also indebted to Drs T.Takano and T.Ueki for their kind cooperation.

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