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X-ray Studies on Crystalline Complexes Involving Amino Acids. I. Crystal Structure of L-Lysine L-Aspartate*

BY T. N. BHAT AND M. VIJAYAN†

Department of Physics and Molecular Biophysics Unit, Indian Institute of Science, Bangalore-560012, India

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Lysine aspartate, $C_6H_{15}N_2O_2.C_4H_6NO_4$, a crystalline complex of lysine and aspartic acid, crystallizes in the monoclinic space group $P2_1$ with two formula units in a unit cell of dimensions $a=5{\cdot}539$ (3), $b=7{\cdot}848$ (4), $c=15{\cdot}324$ (15) Å, $\beta=99{\cdot}1$ (1)°. The structure was solved by direct methods and Fourier techniques and refined to an R value of 0·069 for 1134 photographically observed reflexions. The dimensions and the conformation of the lysine molecule are similar to those observed in lysine monohydrochloride dihydrate. The side-chain carboxyl group of the aspartate ion is deprotonated unlike that in L- and DL-aspartic acid. The bond lengths and angles in the aspartate ion, except those in the side-chain carboxylate group, are comparable with those in L- and DL-aspartic acid. Considerable differences, however, exist in the conformation of the molecule in these structures. The crystal structure of lysine aspartate consists of alternating layers, one layer comprising lysine molecules and the other, aspartate ions. The two layers are interconnected primarily by hydrogen bonds between the side-chain amino group of lysine and carboxylate oxygen atoms belonging to neighbouring aspartate ions.

Introduction

Non-covalent interactions involving amino acid residues play a crucial role in the structure, assembly and function of proteins. Crystalline complexes involving amino acids and short peptides would serve as good model systems for studying the atomic details of these interactions which cannot be understood from protein crystallographic studies alone on account of the limited resolution of protein electron-density maps. The X-ray structure analysis of such a complex, namely L-lysine L-aspartate is reported here. A preliminary note on the analysis has been published (Bhat & Vijayan, 1974).

Experimental

Crystals of lysine aspartate were grown by slow evaporation at room temperature of an aqueous solution of the compound, obtained commercially. The unit-cell dimensions and the space group were determined from oscillation and Weissenberg photographs. The cell dimensions were subsequently refined using a few high-angle $0kl$ and $h0l$ reflexions after allowance was made for the thickness of the film envelope. The density was measured by flotation in a mixture of benzene and carbon tetrachloride.

Crystal data

L-Lysine L-aspartate, $C_6H_{15}N_2O_2.C_4H_6NO_4$. Monoclinic, $P2_1$ (systematic absences: $0k0$, k odd), $a=5{\cdot}539$ (3), $b=7{\cdot}848$ (4), $c=15{\cdot}324$ (15) Å; $\beta=99{\cdot}1$ (1)°; $V=657{\cdot}74$ Å^3; $D_m=1{\cdot}412$ (5), $D_c=1{\cdot}410$ g cm^{-3}; $Z=2$, μ(Cu $K\alpha$) = 10·02 cm^{-1}.

Intensity data were collected with Cu $K\alpha$ radiation ($\lambda=1{\cdot}5418$ Å) about the a axis (layer lines 0 through 4) and the b axis (layer lines 0 through 2) with the multiple-film equi-inclination Weissenberg technique. The crystals used for data collection were of nearly cylindrical shape with average radii of 0·15 mm and 0·13 mm respectively. The intensities were estimated visually and corrected for Lorentz and polarization factors and for spot shape. No absorption correction was applied as the mass absorption coefficient of the crystals was small. Of the 1767 independent reflexions in the copper sphere, 1436 were recorded of which 1134 were in the measurable range. The overlapping data present in both the a axis and the b axis data sets were used to put the intensities of all the reflexions on a single scale.

The structure was solved by the non-centrosymmetric direct method (Karle & Karle, 1966) followed by conventional difference Fourier techniques. The atomic parameters of the non-hydrogen atoms were refined, first isotropically and then anisotropically, to an R value of 0·092 on the IBM 360/44 computer at the Institute with a block-diagonal structure factor least-squares program originally written by Dr R. Shiono and modified by Dr B. Swaminatha Reddy for the IBM 360/44 system. The anisotropic temperature factors were of the form $\exp[-(b_{11}h^2+b_{22}k^2+b_{33}l^2+2b_{12}hk+2b_{23}kl+2b_{13}hl)]$. The positional parameters, determined from a difference Fourier map and geometrical considerations, and the isotropic temperature factors of the H atoms were also included at this stage in the least-squares calculations. The refinement was terminated at $R=0{\cdot}069$ for 1134 observed reflexions when the average and the maximum least-squares shifts were $0{\cdot}25\sigma$ and $0{\cdot}98\sigma$ respectively. The weighting scheme used in the final cycles had the form $1/(a+bF_o+cF_o^2)$ where $a=0{\cdot}747$, $b=-0{\cdot}0056$, $c=0{\cdot}0024$.

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† Author to whom correspondence should be addressed at the Molecular Biophysics Unit.

Table 1. *Final positional coordinates* ($\times 10^4$) *and anisotropic thermal parameters* ($\times 10^4$) *of the non-hydrogen atoms*

The standard deviations are given in parentheses.

	x	y	z	b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}
N(1)	7211 (9)	2125 (0)	9018 (3)	86 (16)	42 (7)	23 (2)	25 (10)	13 (5)	2 (4)
C(2)	6069 (11)	3703 (8)	9351 (4)	122 (20)	19 (8)	24 (3)	11 (11)	15 (6)	−7 (4)
C(1)	3446 (11)	3260 (9)	9511 (4)	112 (20)	52 (9)	24 (3)	−11 (12)	12 (6)	−2 (4)
O(1)	2331 (8)	2106 (7)	9031 (4)	85 (14)	89 (8)	49 (3)	−20 (10)	17 (5)	−25 (4)
O(2)	2558 (9)	4133 (7)	10064 (3)	20 (17)	72 (8)	38 (2)	−5 (10)	51 (5)	−13 (4)
C(3)	5944 (12)	5159 (9)	8688 (4)	152 (23)	62 (10)	24 (3)	−3 (13)	22 (6)	10 (5)
C(4)	8401 (12)	5904 (9)	8554 (5)	124 (21)	48 (9)	35 (3)	0 (13)	15 (7)	11 (5)
C(5)	8191 (13)	7355 (10)	7879 (5)	151 (21)	77 (12)	30 (3)	−17 (14)	11 (6)	20 (5)
C(6)	10567 (13)	8321 (10)	7945 (5)	154 (23)	73 (11)	33 (3)	6 (14)	5 (7)	24 (5)
N(7)	10755 (10)	9360 (7)	7127 (4)	121 (18)	52 (8)	32 (3)	−25 (11)	18 (5)	10 (4)
N(11)	4412 (9)	3382 (7)	5069 (4)	80 (17)	41 (8)	29 (2)	0 (10)	12 (5)	16 (4)
C(12)	4273 (11)	3526 (8)	6035 (4)	130 (21)	37 (8)	21 (3)	5 (12)	10 (6)	4 (4)
C(11)	5418 (11)	1936 (9)	6509 (4)	112 (20)	36 (8)	24 (3)	−5 (12)	−5 (6)	−3 (4)
O(11)	6888 (9)	1088 (6)	6115 (3)	170 (16)	54 (7)	30 (2)	27 (9)	6 (5)	−4 (3)
O(12)	4899 (9)	1599 (7)	7246 (3)	254 (19)	100 (9)	20 (2)	−32 (12)	9 (5)	6 (4)
C(13)	1682 (12)	3860 (9)	6164 (5)	108 (21)	60 (11)	41 (3)	−2 (13)	29 (7)	10 (5)
C(14)	720 (11)	5641 (9)	5872 (5)	77 (19)	52 (10)	41 (3)	−29 (13)	37 (6)	0 (5)
O(15)	2170 (10)	6863 (7)	6009 (4)	226 (18)	56 (8)	52 (3)	−2 (11)	49 (6)	−9 (4)
O(16)	−1479 (10)	5737 (8)	5528 (5)	183 (19)	79 (9)	90 (5)	46 (12)	10 (7)	38 (6)

The form factors of the non-hydrogen atoms were taken from Cromer & Waber (1965) and those of H from Stewart, Davidson & Simpson (1965). The final positional and thermal parameters of the non-hydrogen and H atoms are listed in Tables 1 and 2 respectively.

Table 2. *Final positional coordinates* ($\times 10^3$) *and isotropic temperature factors of hydrogen atoms*

The standard deviations are given in parentheses.

		x	y	z	B
H(11)	N(1)	676 (21)	949 (19)	940 (7)	7 (3)
H(12)	N(1)	671 (11)	191 (10)	842 (4)	1 (1)
H(13)	N(1)	885 (14)	223 (12)	898 (5)	2 (2)
H(71)	N(7)	921 (14)	990 (11)	691 (5)	2 (2)
H(72)	N(7)	1117 (18)	905 (17)	654 (7)	6 (3)
H(73)	N(7)	1194 (16)	1001 (13)	723 (6)	3 (2)
H(111)	N(11)	626 (16)	308 (14)	508 (6)	4 (2)
H(112)	N(11)	310 (81)	278 (14)	481 (6)	4 (2)
H(113)	N(11)	394 (16)	453 (15)	478 (6)	4 (2)
H(21)	C(2)	687 (15)	372 (14)	988 (6)	4 (2)
H(31)	C(3)	499 (14)	487 (11)	806 (5)	2 (2)
H(32)	C(3)	502 (17)	611 (14)	892 (6)	4 (2)
H(41)	C(4)	929 (14)	638 (12)	913 (5)	2 (2)
H(42)	C(4)	946 (15)	498 (12)	834 (5)	3 (2)
H(51)	C(5)	768 (13)	695 (12)	726 (4)	2 (2)
H(52)	C(5)	676 (15)	805 (13)	792 (5)	3 (2)
H(61)	C(6)	1061 (15)	907 (13)	846 (5)	3 (2)
H(62)	C(6)	1186 (15)	759 (13)	800 (6)	3 (2)
H(121)	C(12)	514 (12)	459 (10)	626 (4)	1 (1)
H(131)	C(13)	53 (12)	292 (10)	58 (4)	1 (1)
H(132)	C(13)	169 (14)	368 (12)	688 (5)	2 (2)

Table 3. *Bond lengths* (Å) *and angles* (°) *involving hydrogen atoms*

	Number	Maximum value	Minimum value	Average value
C–H	12	1·06 (7)	0·86 (8)	0·99 (6)
N–H	9	1·15 (14)	0·83 (9)	0·97 (3)
C–C–H	22	125 (6)	103 (4)	109 (5)
H–C–H	5	112 (8)	106 (7)	107 (5)
C–N–H	9	132 (6)	101 (5)	111 (8)
H–N–H	9	128 (8)	92 (9)	107 (11)

The bond lengths and valency angles involving non-hydrogen atoms are shown in Fig. 1 and those involving H atoms are summarized in Table 3.*

* A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 31328 (7 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

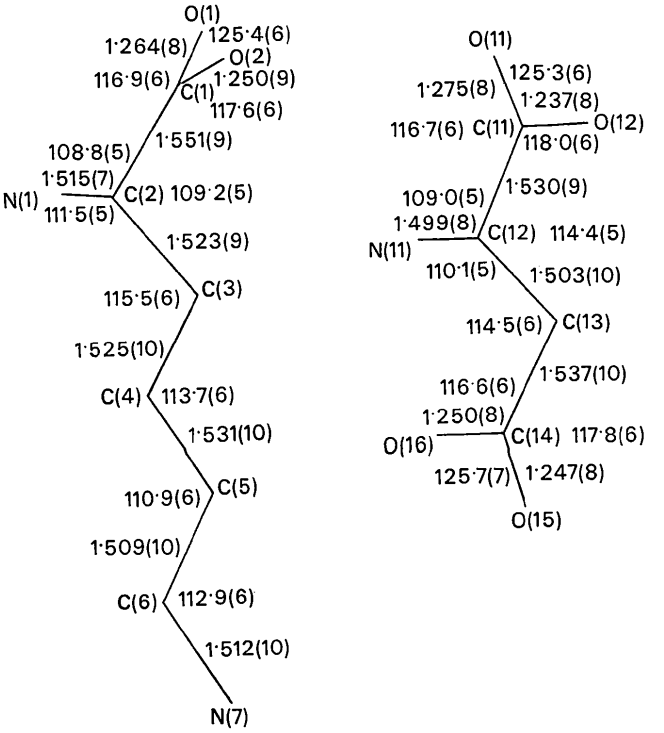

Fig. 1. Bond lengths (Å) and angles (°) involving non-hydrogen atoms. The standard deviations are given in parentheses.

Discussion

The lysine molecule

The lysine molecule in the structure is a positively charged zwitterion in which the two amino groups carry a positive charge each, whereas the carboxylate group carries a negative charge. The bond lengths and angles are comparable with those found in lysine monohydrochloride dihydrate (Wright & Marsh, 1962; Koetzle, Lehmann, Verbist & Hamilton, 1972; Bugayong, Sequeira & Chidambaram, 1972). The conformations of the molecules in the two structures are also similar as can be seen from Fig. 2. The major difference between the two structures involves the two C–N bond lengths. The C^{α}–N distance of $1{\cdot}515 \pm 0{\cdot}007$ Å in the present structure is significantly longer ($\Delta l > 3\sigma$) than that ($1{\cdot}484 \pm 0{\cdot}006$ Å) found in lysine monohydrochloride dihydrate (Wright & Marsh, 1962). The difference between the observed lengths of the C^{ε}–N^{ζ} bond ($1{\cdot}512 \pm 0{\cdot}010$ Å in lysine aspartate and $1{\cdot}480 \pm 0{\cdot}006$ Å in lysine monohydrochloride dihydrate), though large, is not statistically significant (Cruickshank, 1949). No satisfactory explanation could be found, however, for the increased C–N bond lengths in the present structure.

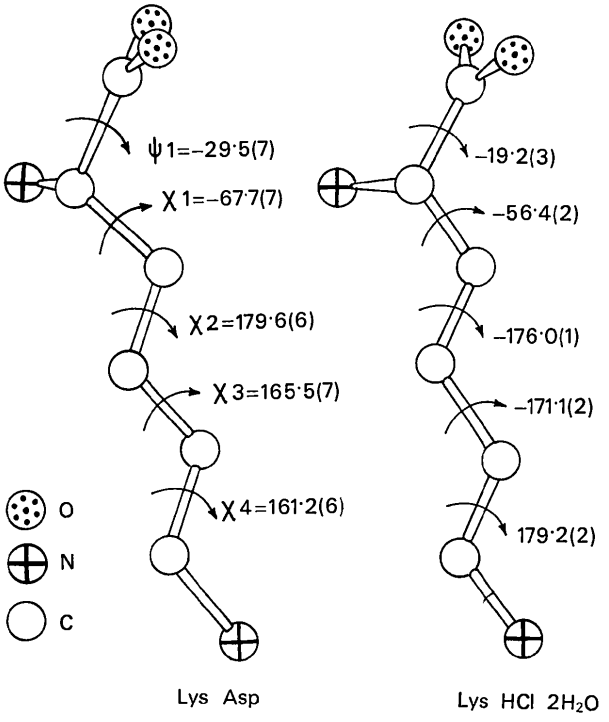

Fig. 2. Structure of the lysine molecule in lysine aspartate and lysine monohydrochloride dihydrate as viewed perpendicular to the mean plane of the carbon skeleton. The torsional angles are indicated.

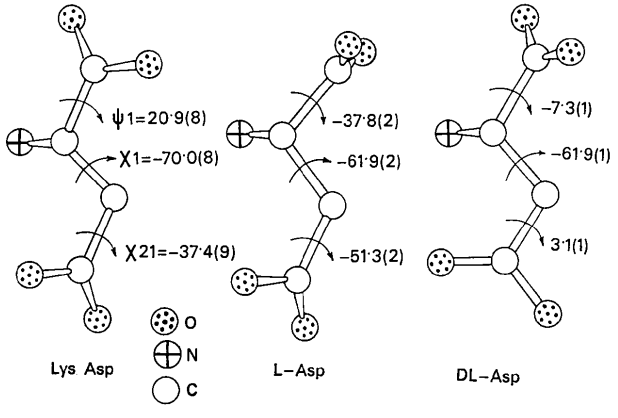

Fig. 3. Structure of the aspartate ion (or aspartic acid molecule) in lysine aspartate, L-aspartic acid and DL-aspartic acid as viewed perpendicular to the mean plane of the carbon skeleton. The torsional angles are indicated.

The aspartate group

The aspartate group in the structure is also zwitterionic and carries a net negative charge with two negatively charged carboxylate groups and a positively charged amino group. Among the amino acids and their salts studied so far by X-ray diffraction this is perhaps the first in which an ionized side-chain carboxylate group is encountered. The alanine part of the aspartate group in the present structure has bond lengths and angles similar to those found in L-aspartic acid (Derissen, Endeman & Peerdeman, 1968) and DL-aspartic acid (Rao, 1973) except for the two C···O lengths. The C···O lengths, however, are known to vary substantially from structure to structure presumably on account of the differences in hydrogen-bonding environment. In L-aspartic acid and DL-aspartic acid the α-carboxyl group is deprotonated whereas the side-chain carboxyl group is neutral, and the C^{α}–C bond is considerably longer than the C^{β}–C^{γ} bond. However, in the present structure both the α and side-chain carboxyl groups are deprotonated, and the C^{α}–C and C^{β}–C^{γ} bonds have similar lengths. In this context, it is interesting to note that the average C^{α}–C bond length (1·533 Å) in amino acids with negatively charged α-carboxylate groups is larger than that (1·520 Å) in amino acids with neutral α-carboxyl groups (Vijayan, 1975). Thus it appears that, in general the C–C length in a neutral carboxyl group is shorter than that in a negatively charged carboxylate ion.

Yet another difference between the side-chain carboxyl groups in L-aspartic acid and DL-aspartic acid on the one hand and the present structure on the other involves the C–C–O angles. As is expected, in the former the C–C–O angles are not equal whereas in the latter they are nearly equal.

Considerable differences exist between the conformations of the molecule in L-aspartic acid and DL-aspartic acid. As can be seen from Fig. 3, the conformation of the aspartate group in the present structure is different from the conformation of the

aspartic acid molecule in the above two compounds. $C, C^{\alpha}, C^{\beta}, C^{\gamma}$ are nearly coplanar in all the three structures. The differences in the conformation arise mainly from the different orientations of the terminal carboxylate (or the carboxyl) groups with respect to the plane of the carbon skeleton. The ψ_1 angle of 20·9° observed in the present structure is perhaps the largest positive ψ_1 angle encountered so far in the crystal structures of non-glycine amino acids and their salts.

Crystal structure and hydrogen bonding

The crystal structure is stabilized by ionic interactions and hydrogen bonds involving amino and carboxylate groups. The hydrogen-bond parameters are given in Table 4. All the amino H atoms and all the carboxylate O atoms are involved in hydrogen bonds. There are ten crystallographically independent hydrogen-bonded N···O interactions in the structure, as shown in the packing diagram (Fig. 4). Of these, eight are normal hydrogen bonds with one amino H atom interacting with one carboxylate O atom. The remaining two are made up of a bifurcated hydrogen bond in which the amino N atom, N(11), interacts with two O atoms through one of its H atoms, H(111). No specific ion-pair interaction exists in the structure since in no instance do the two O atoms in a carboxylate group interact with the same N atom. However, it is interesting to note that the structure consists of alternating layers of lysine molecules packed about the xy plane at $z=0$ and aspartate groups packed

Table 4. *Hydrogen-bond parameters and their standard deviations*

	N···O	H···O	H–N···O	H···O–C
N(1)—H(12)····O(12)–C(11)*A*	2·843 (7) Å	1·93 (6) Å	9 (4)°	131 (2)°
N(1)—H(13)····O(1)—C(1)*C*	2·833 (7)	1·92 (8)	7 (5)	112 (3)
N(1)—H(11)····O(2)—C(1)*H*	2·730 (6)	1·66 (14)	16 (6)	136 (4)
N(7)—H(73)····O(12)–C(11)*D*	2·874 (8)	2·05 (10)	7 (6)	115 (3)
N(7)—H(72)····O(15)–C(14)*C*	2·795 (8)	2·01 (12)	31 (7)	121 (3)
N(7)—H(71)····O(11)–C(11)*B*	2·789 (7)	1·87 (7)	15 (5)	112 (2)
N(11)–H(113)···O(11)–C(11)*E*	2·810 (7)	1·84 (10)	14 (6)	127 (3)
N(11)–H(112)···O(16)–C(14)*F*	2·705 (8)	1·87 (11)	19 (6)	124 (3)
N(11)–H(111)···O(16)–C(14)*C*	2·930 (8)	2·47 (10)	54 (5)	119 (2)
N(11)–H(111)···O(15)–C(14)*G*	2·953 (8)	2·22 (10)	38 (5)	122 (3)

(*A*) x,y,z; (*B*) $x,y+1,z$; (*C*) $x+1,y,z$; (*D*) $x+1,y+1,z$; (*E*) $-x+1,y+\frac{1}{2},-z+1$; (*F*) $-x,y-\frac{1}{2},-z+1$; (*G*) $-x+1,y-\frac{1}{2},-z+1$; (*H*) $-x+1,y-\frac{1}{2},-z+2$.

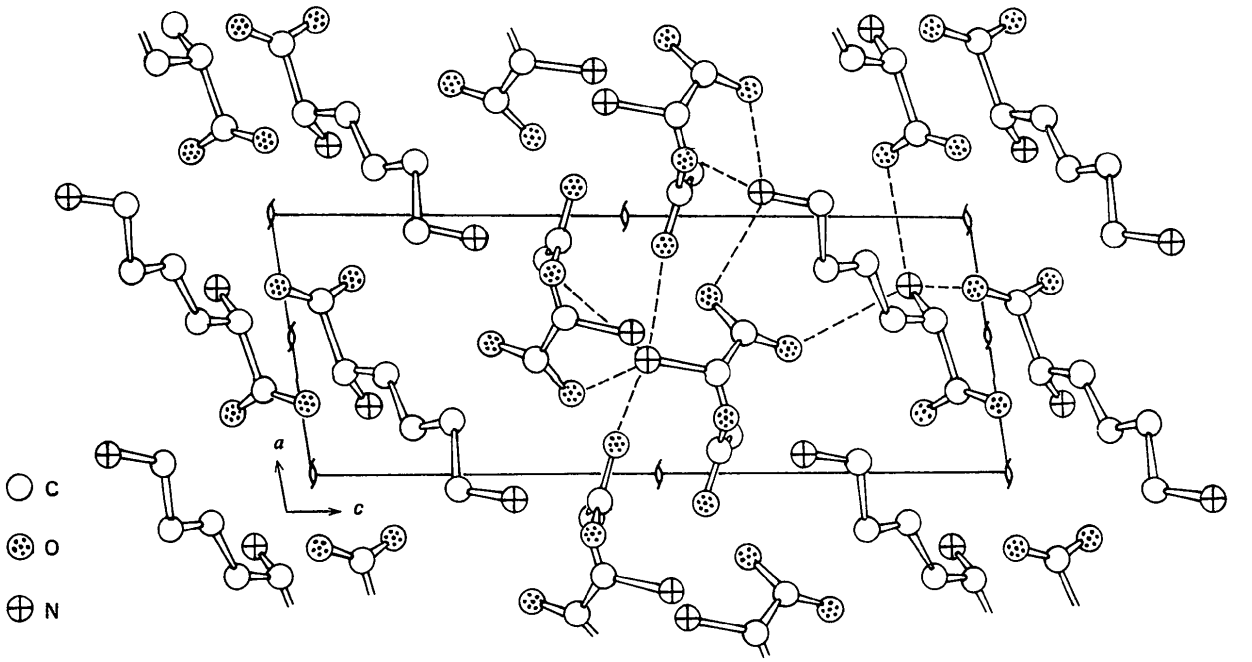

Fig. 4. The crystal structure as viewed along the *b* axis. The broken lines indicate a set of crystallographically non-equivalent hydrogen bonds.

about the xy plane at $z=\frac{1}{2}$. The lysine molecules in the former are held together by hydrogen bonds involving the α-amino and the α-carboxylate groups belonging to them whereas the latter are stabilized by hydrogen bonds between the α-amino group and the α- and side-chain carboxylates belonging to the aspartate groups in the layer. The two layers are interconnected primarily by three hydrogen bonds made by the side-chain amino group of lysine with O atoms belonging to the neighbouring aspartate groups. There is also an additional hydrogen bond between the α-amino N atom of lysine and an α-carboxylate O atom of the aspartate group.

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The Crystal Structures of Two Tetracyclic Spirodilactams Containing Non-Planar Amide Bonds

BY S. E. EALICK, D. M. WASHECHECK AND DICK VAN DER HELM*

Department of Chemistry, University of Oklahoma, Norman, Oklahoma 73069, *U.S.A.*

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The crystal structures of 1,4-diaza-5,12-dioxotetracyclo[5,5,1,$0^{4,13}$,$0^{10,13}$]tridecane, $C_{11}H_{14}N_2O_2$, and 1,5-diaza-6,13-dioxotetracyclo[6,5,1,$0^{5,14}$,$0^{11,14}$]tetradecane, $C_{12}H_{16}N_2O_2$, have been determined and refined by three-dimensional least-squares techniques. Both molecules crystallize in space group $C2/c$ and have similar unit-cell dimensions. Unit-cell dimensions for the former are $a=13{\cdot}6008$ (4), $b=8{\cdot}3177$ (2), $c=10{\cdot}0596$ (2) Å and $\beta=120{\cdot}563$ (2)° while those for the latter are $a=13{\cdot}7523$ (3), $b=8{\cdot}9985$ (2), $c=10{\cdot}2316$ (2) Å and $\beta=120{\cdot}527$ (1)°. The final R values are 3·7% for the tridecane derivative and 3·9% for the tetradecane derivative. Each molecule lies on the twofold rotation axis of space group $C2/c$. The amide group in each molecule is non-planar even though the conversion of a five-membered ring to a six-membered ring in the tetradecane derivative releases a certain amount of the strain.

Introduction

The geometry of the non-planar amide group (or the non-planar peptide group in biological systems) has been the subject of several theoretical and experimental studies in the last few years (Warshel, Levitt & Lifson, 1970; Winkler & Dunitz, 1971; Ramachandran, Lakshminarayanan & Kolaskar, 1973; Ramachandran & Kolaskar, 1973; Dunitz & Winkler, 1975). The present study deals with two compounds of a series of polycyclic spirodilactams. The syntheses and infrared spectra of these compounds have been published (Smolikova, Koblicova & Bláha, 1973). The first compound of this series, a tricyclic spirodilactam, and a preliminary study of the present tetracyclic compounds have been reported (Ealick & van der Helm, 1975; van der Helm, Ealick & Waschecheck, 1975). The occurrence of both *cis* and *trans* non-planar peptide groups has been observed in cyclic and acyclic molecules (*e.g.* Sletten, 1970; Winkler & Dunitz, 1971; Pedone, Benedetti, Immirzi & Allegra, 1970). One must also consider the occurrence of the non-planar peptide group in protein molecules. In the present paper we present the description of the structures of two molecules containing non-planar amide bonds.

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