

## Structure determination of L<sub>S</sub>-threonine by neutron diffraction

M RAMANADHAM, S K SIKKA and R CHIDAMBARAM

Nuclear Physics Division, Bhabha Atomic Research Centre, Trombay, Bombay 400085

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**Abstract.** The structure of the amino acid, L<sub>S</sub>-threonine [NH<sub>3</sub><sup>+</sup>CH(CHOHCH<sub>3</sub>)COO<sup>-</sup>], space group  $P2_12_12_1$ ,  $a=13.630(5)$ ,  $b=7.753(1)$ ,  $c=5.162(2)$  Å,  $z=4$ , has been determined from neutron diffraction data using direct methods. The intensities of 1148 neutron Bragg reflections were measured from a single crystal. The structural parameters were refined by the method of least squares using anisotropic temperature factors. The final  $R(F^2)$  is 0.068. The structure was also refined from the x-ray data of Shoemaker *et al* (1950 *J. Am. Chem. Soc.* **72** 2328); there is good agreement between the two sets of heavy atom parameters. The parameters of hydrogen atoms are of course more precisely determined in our neutron study. The molecular conformation and the hydrogen bonding scheme are discussed. Weighted average values of bond distances and angles from 14 amino acid structures with ionized carboxylic groups studied by neutron diffraction at Brookhaven and Trombay are also presented.

**Keywords.** L<sub>S</sub>-threonine; neutron diffraction; hydrogen bonding; direct methods.

### 1. Introduction

The x-ray diffraction structure determination of L<sub>S</sub>-threonine [NH<sub>3</sub><sup>+</sup>CH(CHOHCH<sub>3</sub>)COO<sup>-</sup>] was carried out by Shoemaker *et al* (1950). In this study, only approximate hydrogen atom positions were found. We undertook the neutron diffraction investigation of this crystal to obtain precise nuclear positions for the hydrogen atoms. This work is the fifth in a series of such studies on amino acids and their derivatives in our laboratory.\*

### 2. Experimental

Single crystals of L<sub>S</sub>-threonine were grown by slow evaporation from an aqueous solution of the compound. The crystals obtained were prisms elongated along the  $c$ -axis with {110} as the side faces. One such crystal weighing 12 mg was chosen for data collection. This was a parallelepiped of dimensions  $5.36 \times 1.62 \times 1.06$  mm with the longest dimension along the  $c$ -axis. This was mounted on the 4-circle neutron diffractometer 3D-FAD (Momin *et al* 1969) at the CIRUS reactor in Trombay. The intensity data were collected using a neutron beam of wavelength 1.178 Å, obtained by reflection from the (511) plane of a Ge single crystal. The cell constants

\*The other amino acids so far studied in our laboratory are: L-asparagine·H<sub>2</sub>O (Ramanadham *et al* 1972), L-glutamic acid·HCl (Sequeira *et al* 1972), L-lysine·HCl·2H<sub>2</sub>O (Bugayong *et al* 1972), L-cysteic acid·H<sub>2</sub>O (Ramanadham *et al* 1973) and L-cystine·2HCl (Gupta *et al* 1973).

were refined by least squares technique from optimised  $2\theta$  angles of 105 reflections, randomly distributed in reciprocal space. These refined values,  $a=13.630(5)$ ,  $b=7.753(1)$  and  $c=5.162(2)$  Å, compare well with the values  $a=13.611(20)$ ,  $b=7.738(8)$  and  $c=5.142(5)$  Å reported by Shoemaker *et al* (1950). The calculated density with four molecules in the unit cell comes out to be  $1.461 \text{ gm cm}^{-3}$  compared to the measured density of  $1.462 \text{ gm cm}^{-3}$ . The systematic absences confirmed the space group to be  $P2_12_12_1$  as determined in the x-ray study.

The integrated neutron counts for 1152 Bragg reflections† consisting of 857  $hkl$ , 211  $h\bar{k}l$ , 82  $\bar{h}kl$  and  $2\bar{h}k0$ , having  $\sin \theta/\lambda \leq 0.68 \text{ \AA}^{-1}$  were measured in the symmetrical setting of the diffractometer using  $\theta-2\theta$  coupled step scan mode ( $0.1^\circ$  step in  $2\theta$ ). The background was scanned for a minimum of  $1^\circ$  on either side of the peaks. A standard reflection was recorded after every 15 measurements. The variation in the intensity of this standard reflection during the 4 months of data collection was within 4%.

Squared observed structure factors were obtained from the integrated intensities and then corrected for absorption with the calculated linear absorption coefficient  $\mu=2.86 \text{ cm}^{-1}$ . The transmission factors ranged from 0.612 to 0.789. The symmetry related reflections were not averaged. 32 reflections were found to be having negative  $F_o^2$  values. These were omitted in Fourier calculations but included in the least squares refinement of the structure.

### 3. Structure determination by tangent formula

Sikka (1969) has shown that the direct methods involving the symbolic addition and tangent formula techniques (Karle and Karle 1966) are applicable to neutron diffraction structure determination if the relative scattering power of hydrogen atoms,  $\sigma = \sum_H b_H^2 / \sum b_i^2$  is less than 30%‡. In the case of L<sub>s</sub>-threonine  $\sigma$  is 26% near the upper

limit set by Sikka. Therefore, it was decided to determine the structure of L<sub>s</sub>-threonine by direct methods as a further test of the applicability of these methods in neutron diffraction.§

Normalized structure factors,  $E$ 's were derived from  $|F_o|$  by the formula

$$|E| = \frac{|F_o|}{[\varepsilon \langle I \rangle]^{\frac{1}{2}}} \quad (1)$$

Here  $\varepsilon$  is a factor which takes into account the enhanced  $|F_o|$  values for some reflections due to space group symmetry and  $\langle I \rangle$  is the local average intensity, which was

†Four reflections (322), (14 4 1), (11 7 0) and (393) were dropped from the data set as they were mis-set during the data collection.

‡Since this proposal three structures, L-proline·H<sub>2</sub>O (Verbist *et al* 1972), L-serine·H<sub>2</sub>O (Frey *et al* 1973) and melampodin (Bernal and Wilkins 1972), have been solved by this method from neutron data. The  $\sigma$  values for these structures are 27%, 26% and 22% respectively.

§In order to retain the objectivity of this approach, none of the authors studied the x-ray paper on L<sub>s</sub>-threonine by Shoemaker *et al* (1950) till the structure was solved from neutron data, apart from noting the crystal data.

**Table 1.** Set of initial phases for multi-solution tangent formula computations.

<i>h k l</i>	$ E $	$\phi$	weight	
3 1 4	2.628	45°	1.0	} For fixing origin and enantiomorph
17 4 0	2.109	90	1.0	
12 0 1	2.443	90	1.0	
6 2 0	2.112	180	0.837	} From $\Sigma_1$ relation
16 2 0	2.716	180	0.592	
0 8 4	1.758	180	0.515	
14 6 0	2.223	0	0.422	
0 2 4	1.743	180	0.345	
8 4 0	1.878	180	0.236	
0 8 1	2.237	0 or 180	1.0	} For multi-solution
4 0 3	1.897	$\pm 90$	1.0	

estimated by the K-plot technique of Karle and Hauptman (1953).<sup>\*</sup> The observed distribution values of  $\langle |E| \rangle = 0.85$  and  $\langle |E^2 - 1| \rangle = 0.75$  agree well with the theoretical values of 0.89 and 0.74 respectively for the non-centrosymmetric case. The phases were determined by the multisolution tangent formula method of Germain and Woolfson (1968). 175 reflections with  $|E| \geq 1.3$  were used. The starting set of reflections for generating the different tangent formula phase sets are given in table 1. These include 3 reflections for fixing the origin and enantiomorph chosen according to the rules given by Hauptman and Karle (1956), six  $\Sigma_1$  reflections and two (081) and (403) which can take 0 or  $\pi$  and  $\pi/2$  or  $-\pi/2$  respectively as their phase values. The weighted tangent formula (Germain, Main and Woolfson 1971)

$$\tan \phi_H = \frac{T}{B} = \frac{\left[ \sum_{\mathbf{K}} w_{\mathbf{K}} w_{\mathbf{H}-\mathbf{K}} |E_{\mathbf{K}} E_{\mathbf{H}-\mathbf{K}}| \sin(\phi_{\mathbf{K}} + \phi_{\mathbf{H}-\mathbf{K}}) \right]}{\left[ \sum_{\mathbf{K}} w_{\mathbf{K}} w_{\mathbf{H}-\mathbf{K}} |E_{\mathbf{K}} E_{\mathbf{H}-\mathbf{K}}| \cos(\phi_{\mathbf{K}} + \phi_{\mathbf{H}-\mathbf{K}}) \right]} \quad (2)$$

was used for calculations.

The weights were chosen as

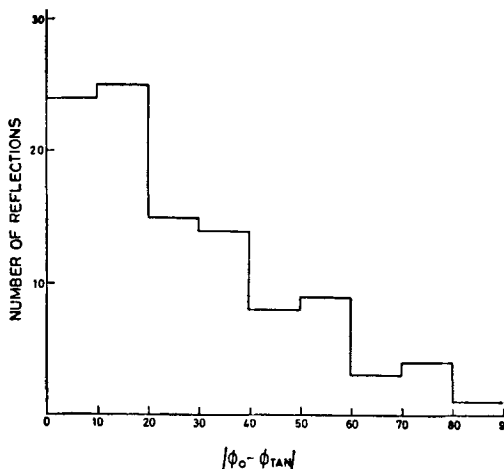
$$\begin{aligned} w &= \tanh(0.5\alpha_H) \text{ for } \alpha_H \geq 2.0 \\ &= 0 \text{ for } \alpha_H < 2.0 \end{aligned} \quad (3)$$

Here  $\alpha_H = \frac{2\beta_3}{\beta_2^{3/2}} \left[ |E_H| \sum_{\mathbf{H}} (T^2 + B^2)^{1/2} \right]$  and

$$\beta_n = \sum_{\text{all atoms}} b_j^n$$

The weights for the initial sets are given in table 1. For  $\Sigma_1$  reflections,  $w = 2P_H - 1$  where  $P_H$  is the probability that the sign is correctly determined by the  $\Sigma_1$  relation.

<sup>\*</sup>Considerable difficulty was encountered in applying the Wilson plot method (Wilson 1942) for obtaining the scale factor and the average isotropic temperature factor. This was due to extinction effects for the low angle data and curving of the linear plot for the high angle data. The latter is due to, perhaps, sharper decrease in the hydrogen contribution to the high angle reflections as a result of higher B values and lower scattering amplitude of hydrogen atoms compared to other atoms in the structure.



**Figure 1.** Frequency distribution of the error  $|\phi_0 - \phi_{\text{TAN}}|$  for non-centrosymmetric reflections in *Ls*-threonine. Five reflections had errors greater than  $90^\circ$ . Seven out of fifty-six centrosymmetric reflections had errors of  $180^\circ$ .

The set for which the phases of (081) and (403) were 0 and  $-\pi/2$ , gave the highest figure of merit and an *E* map was computed from the phases of this set. In this map, the five highest peaks which made chemical sense were chosen as nuclear sites. A structure factor calculation followed by a Fourier synthesis revealed the three remaining heavy atoms. Finally the nine hydrogen atoms were located from a difference Fourier map. The *R* value  $\left( = \frac{\sum ||F_o| - |F_c||}{\sum |F_o|} \right)$  at this stage was 0.36.

A comparison with the x-ray coordinates showed that we had obtained a set related to the former by the transformation:  $\frac{1}{2}-x$ ,  $\frac{1}{2}-y$ ,  $z-\frac{1}{2}$  and that all the atoms were correctly positioned. Figure 1 shows in histogram form, the distribution of  $|\phi_0 - \phi_{\text{TAN}}|$  for non-centrosymmetric reflections. Here  $\phi_0$  is phase calculated from the final least square parameters and  $\phi_{\text{TAN}}$  is the tangent-formula calculated phase. Only 7 out of 56 reflections whose phases were fixed by space group to be 0,  $\pi$  and  $\pm\pi/2$ , were in error by  $180^\circ$ . The average mean error in phases is  $28^\circ$ . The phase of the (314) reflection, which was used to fix the enantiomorph was found to have  $\phi_0 = -43^\circ$ . This means that enantiomorph opposite to that fixed by us was obtained by using  $\phi_{\text{TAN}}$ 's. This result confirms the observation of Hauptman and Duax (1972) that one reflection may not be sufficient for choosing the enantiomorph.

#### 4. Structure refinement

The structural parameters obtained in the last section were subjected to a series of isotropic and anisotropic full-matrix least squares refinements. The nuclear scattering lengths used were:  $b_N = 0.94$ ,  $b_C = 0.664$ ,  $b_O = 0.58$  and  $b_H = -0.374 \times 10^{-12}$  cm (Bacon 1972). All 1148 reflections were included in the refinements. The function minimized was  $\sum w |F_o^2 - |F_c|^2|^2$  where *w* is the weight. In the initial stages, *w* was taken to be  $1/\sigma(F_o^2)$  where  $\sigma(F_o^2)$  were derived from counting statistics. Near the end of the refinements, an error analysis was done and the following weighting scheme was obtained:

$$w^{-\frac{1}{2}} = \langle |\Delta F^2|^2 \rangle^{\frac{1}{2}} = 0.967 - 0.01802 |F_o|^2 + 0.0005644 |F_o|^4 \quad (4)$$

**Table 2.** Atomic parameters for  $L_s$ -threonine. First row for each atom corresponds to neutron parameters. Second row corresponds to x-ray parameters. All the parameters are multiplied by  $10^5$  except positional parameters of hydrogen atoms from x-ray refinement which are multiplied by  $10^4$ . The estimated standard deviations in units of the least significant digit are given in parentheses. The expression for the temperature factor is  $\exp [-(h^2\beta_{11} + k^2\beta_{22} + l^2\beta_{33} + 2hk\beta_{13} + 2hl\beta_{13} + 2kl\beta_{23})]$ .

	x	y	z	$\beta_{11}$	$\beta_{22}$	$\beta_{33}$	$\beta_{12}$	$\beta_{13}$	$\beta_{23}$
C	49585(19)	18436(31)	29901(49)	156(11)	615(33)	1352(84)	-95(18)	67(28)	$\beta_{33}$ -14(52)
	49573(24)	18429(37)	29795(58)	279(14)	876(42)	2155(119)	-80(26)	12(41)	-201(63)
C <sup>a</sup>	39837(16)	10601(29)	39281(51)	135(11)	498(32)	1411(88)	-51(14)	25(25)	-63(48)
	39841(21)	10618(37)	39202(60)	263(15)	899(43)	1830(113)	-48(23)	-25(34)	-69(64)
C <sup>b</sup>	31795(18)	24267(33)	43141(51)	180(11)	612(36)	1607(100)	44(16)	56(26)	-119(58)
	31803(22)	24203(39)	42954(64)	282(15)	978(44)	2426(120)	1(24)	117(35)	95(73)
C <sup>c</sup>	29119(22)	33642(46)	18388(75)	317(15)	1178(54)	2427(130)	195(24)	48(42)	567(73)
	29085(28)	33628(53)	18309(88)	419(18)	1541(61)	3460(162)	108(33)	98(52)	821(100)
N	41392(13)	1263(24)	64179(36)	183(9)	626(29)	1748(74)	3(15)	70(23)	309(42)
	41375(18)	1347(33)	64157(57)	267(13)	913(40)	2250(109)	-6(22)	33(34)	145(57)
O <sup>1</sup>	55753(22)	21641(32)	46819(66)	203(13)	1795(74)	1789(112)	-357(28)	19(34)	-101(83)
	55791(16)	21681(34)	46725(50)	331(12)	1968(54)	2631(101)	-312(21)	-46(29)	29(62)
O <sup>2</sup>	50338(24)	21127(46)	5947(56)	332(17)	1353(64)	966(108)	-276(29)	42(39)	40(63)
	50284(19)	21132(29)	5940(44)	435(13)	1509(45)	2187(89)	-263(24)	143(31)	30(52)
O <sup>3</sup>	23686(22)	14834(42)	52819(74)	165(15)	986(53)	3141(156)	94(22)	220(38)	492(79)
	23645(17)	14642(29)	52876(55)	235(11)	1254(39)	3919(115)	112(18)	223(31)	521(57)
H <sup>1</sup>	45494(42)	8551(75)	77071(121)	362(29)	1149(89)	2509(227)	-172(44)	-221(73)	292(138)
	4511(26)	899(48)	7569(74)						
H <sup>2</sup>	34762(37)	-1394(82)	72590(115)	291(26)	1261(90)	2892(219)	-24(41)	238(66)	448(149)
	3587(30)	-130(49)	7304(71)						
H <sup>3</sup>	44685(40)	-10492(74)	60432(118)	380(28)	1123(93)	2897(243)	220(44)	-59(75)	183(135)
	4426(27)	-892(49)	6031(80)						
H <sup>4</sup>	37199(39)	1111(78)	25165(104)	353(29)	1127(82)	1976(182)	-173(46)	-98(57)	-387(138)
	3743(27)	235(45)	2682(70)						
H <sup>5</sup>	34378(43)	33497(80)	58012(142)	478(34)	1035(88)	3334(249)	138(47)	-38(86)	-467(128)
	3394(29)	3354(47)	5594(83)						
H <sup>6</sup>	17797(36)	22007(68)	54960(113)	254(24)	1211(97)	2371(218)	123(43)	141(61)	244(125)
	1939(28)	2062(51)	5442(77)						
H <sup>7a</sup>	22492(62)	41359(117)	21711(215)	706(53)	2744(161)	5991(468)	849(84)	243(133)	2143(263)
	2376(35)	4113(62)	2170(104)						
H <sup>7b</sup>	34921(62)	41983(119)	11884(206)	652(47)	2585(154)	6319(474)	-31(74)	-136(140)	2815(268)
	3465(38)	4159(68)	1240(108)						
H <sup>7c</sup>	27272(65)	24428(138)	2589(178)	902(56)	2529(165)	3398(307)	331(101)	-433(119)	-140(228)
	2780(43)	2548(70)	316(100)						

Table 3. Structure Factor Table based on the refinement of the structure of L5-threonine using neutron diffraction. The five sub-columns in each column are k, l, l, 100 x F\_o^2 and 100 x |F\_C|^2 respectively.

Table with multiple columns containing numerical data representing structure factor values and indices. The table is organized into several columns, with the first column containing indices and the subsequent columns containing numerical values for k, l, l, 100 x F\_o^2, and 100 x |F\_C|^2.

Moderate extinction effects were noticed on a comparison of  $F_o^2$ 's and  $|F_c|^2$  (minimum  $F_o^2 / |F_c|^2 = 0.70$ ). The Zachariasen (1967) extinction correction term was used.

$$y = (1 + 2x)^{-\frac{1}{2}} \quad (5)$$

$$x = G Q \bar{T} \quad (6)$$

$$Q = \frac{\lambda^3 N_c^2 F_o^2 V}{\sin 2\theta} \quad (7)$$

$$\bar{T} = -\frac{1}{\mu} \frac{d\theta}{d\mu} \quad (8)$$

The parameter  $G$  was refined along with other structural parameters.

For the anisotropic refinement, the total number of variables was 155 (one scale factor,  $G$ , 51 positional and 102 temperature parameters). This refinement had to be done in two blocks with 137 parameters varied in any one cycle. The refinement was assumed to have converged when the shifts in parameters were less than 0.1 of the estimated deviations. The refined parameters are given in table 2. A list of  $F_o^2$  and  $|F_c|^2$  is shown in table 3. The final value of the extinction parameter  $G$  was  $0.366 \times 10^4$ . This corresponds to an equivalent mosaic spread of 16 sec of arc in the sample crystal. The agreement factors between  $F_o^2$  and  $|F_c|^2$  were as follows:

Number of reflections	1148	1022 ( $F_o^2 > \sigma$ )
$R(F^2) = \frac{\sum  F_o^2 -  F_c ^2 }{\sum F_o^2}$	0.068	0.063
$wR(F^2) = \left[ \frac{\sum \omega  F_o^2 -  F_c ^2 ^2}{\sum w F_o^4} \right]^{\frac{1}{2}}$	0.068	0.066

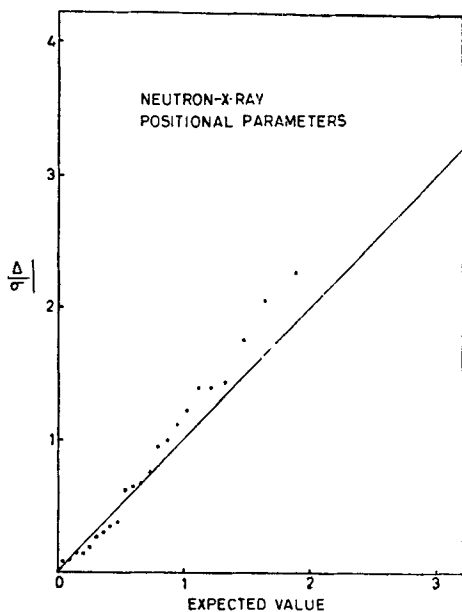
## 5. Discussion

### 5.1. Comparison with the x-ray structure

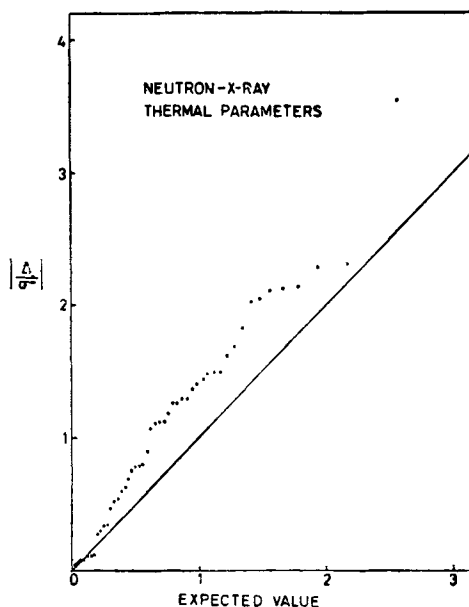
In the x-ray study of  $L_s$ -threonine, Shoemaker *et al* (1950) refined the positional parameters and a single isotropic temperature factor for the entire molecule. In order to make a quantitative comparison between the two sets of parameters from the two techniques, we carried out further refinement of the structure using 554 x-ray data listed as observed by Shoemaker *et al* (1950). The refinement procedure was similar to that described in section 4. Scattering factors for the hydrogens were those of Stewart *et al* (1965). The weighting scheme was derived from the error analysis to be

$$w^{-\frac{1}{2}} = 0.777 + 4.626 \times 10^{-2} F_o^2 + 1.017 \times 10^{-5} F_o^4 \quad (9)$$

In the isotropic refinement, the temperature factors of most of the hydrogen atoms became negative. These were then constrained to the anisotropic values obtained in our neutron study and an over all temperature factor to take into account any possible differences in the x-ray and neutron values of these was varied. The extinction correction factor  $G$  was also included. Upon convergence, the various agreement factors were:  $R(F) = 0.038$  (compared to the value 0.112 reported by Shoemaker *et al* 1950),  $R(F^2) = 0.061$  and  $wR(F^2) = 0.107$ . The refined x-ray parameters are also listed in table 1. Comparison between the two sets of positional parameters for heavy atoms shows a good agreement. Only three,  $y(N)$ ,  $y(O^{21})$  and  $z(C^8)$  have the weighted differences,  $[=\Delta p_i / \sigma(p_i)]$ , where  $\sigma(p_i)$  is the pooled standard deviation  $[=(\sigma^2 \text{ neutrons} + \sigma^2 \text{ x-rays})^{\frac{1}{2}}]$ , greater than 2. The temperature factors  $\beta_{ii}$ 's for the heavy atoms in



**Figure 2.** Half-normal probability plot comparison of positional parameters of non-hydrogen atoms in  $L_5$ -threonine.



**Figure 3.** Half-normal probability plot comparison of anisotropic thermal parameters of non-hydrogen atoms in  $L_5$ -threonine.

the x-ray case are systematically larger than those from neutrons. The average difference between the two sets is equivalent to an isotropic  $\Delta B$  of  $0.77 \text{ \AA}^2$ . Such differences between x-ray and neutron temperature factors have also been noted in aminoacids,  $L$ -lysine·HCl· $2H_2O$  (Bugayong *et al* 1972) and  $L$ -cysteic acid· $H_2O$  (Ramanadham *et al* 1973).  $\Delta B$  for these structures is  $0.91 \text{ \AA}^2$  and  $0.21 \text{ \AA}^2$  respectively.

Further comparison was done by the half normal probability plot method (Abrahams and Keve 1971). These plots for the positional and temperature parameters differences for the heavy atoms are shown in figures 2 and 3. For the latter, equivalent  $\Delta\beta_{ii}$ 's corresponding to  $\Delta B=0.77 \text{ \AA}^2$  were first subtracted from the x-ray  $\beta_{ii}$ 's. In both plots straight lines passing through the origin were obtained showing that the differences in the two sets are approximately normally distributed. The slopes of the two lines are 1.16 and 1.40. These correspond to an underestimation of the pooled  $\sigma(\beta_i)$ 's by the same factor.

## 5.2. Molecular dimensions

A stereo-view of a molecule of  $L_5$ -threonine is shown in figure 4. The bond distances and bond angles for the molecule computed from the final neutron and x-ray coordinates are given in tables 4 and 5. The molecular dimensions for the heavy atoms from the neutron and x-ray studies are nearly identical except for the  $C^\beta-O^{71}$  distance which differs by  $4\sigma$ . The corresponding C—O distances in the structures of DL-serine,  $L$ -serine· $H_2O$  (Frey *et al* 1973) and 4-hydroxy- $L$ -proline (Koetzle *et al* 1973) are 1.403, 1.414 and 1.413  $\text{\AA}$  respectively, nearer to the neutron value. The  $C^\beta-C^{72}$  bond length reported to be abnormally short (1.505  $\text{\AA}$ ) by Shoemaker *et al* (1950) has now been refined to 1.515 and 1.513  $\text{\AA}$  in the two refinements. The bond distances



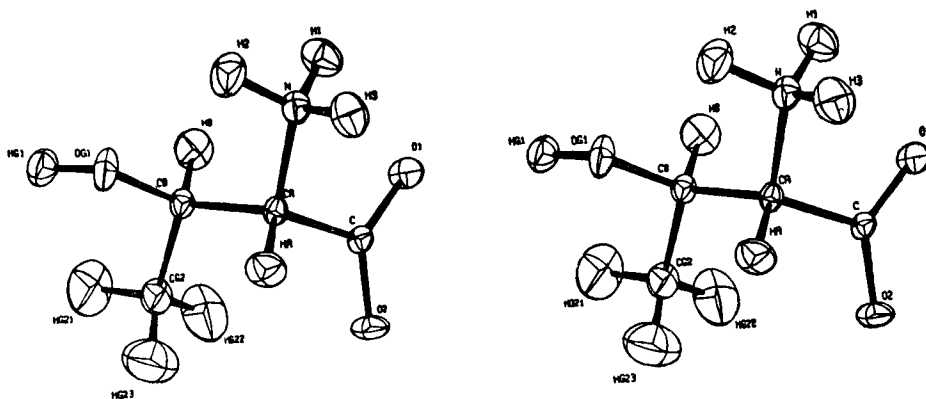


Figure 4. Stereoscopic picture of the molecule of *L*<sub>3</sub>-threonine.

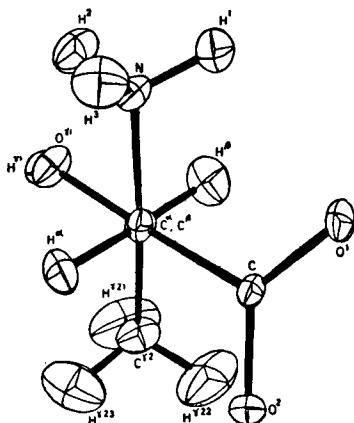


Figure 5. *L*<sub>3</sub>-threonine molecule as seen when one looks down  $C^\alpha-C^\beta$  bond.

and angles for the backbone atoms are compared in column 3 of tables 4 and 5 with the weighted average values from 14 aminoacids with ionised carboxylic group studied by neutron diffraction at Brookhaven and Trombay. The deviations are the largest for the distance  $C^\alpha-C^\beta$  and the angle  $C-C^\alpha-C^\beta$ . The latter is due to considerable variations in the values of the angle  $C-C^\alpha-C^\beta$  in different aminoacids and as Marsh and Donohue (1967) have noted may be the result of non-bonded contacts involving side chain atoms.

The threonine molecule exists as a zwitterion in the crystal with  $C^\alpha-NH_3^+$  group nearly tetrahedral. The average dimensions of the  $NH_3^+$  group are:  $\langle N^+-H^i \rangle = 1.032$  Å, angle  $\langle C^\alpha-N-H^i \rangle = 110.1^\circ$  and  $\langle H^i-N-H^j \rangle = 108.8^\circ$ . The corresponding weighted average values from other aminoacids are 1.039 Å,  $110.6^\circ$  and  $108.5^\circ$ .

The mean dimensions of the methyl group are:  $\langle C^{\gamma 2}-H^{\gamma 2i} \rangle = 1.095$  Å,  $\langle C^\beta-C^{\gamma 2}-H^{\gamma 2i} \rangle = 110.9^\circ$  and  $\langle H^{\gamma 2i}-C^{\gamma 2}-H^{\gamma 2j} \rangle = 108.0^\circ$ . It can be seen from figure 4 that the thermal motion ellipsoids for the  $CH_3$  group atoms are larger than others. This is due to the librational motion of the methyl group about the  $C^\beta-C^{\gamma 2}$  axis. A similar situation exists for the methyl group in the structure of *L*-alanine (Lehman *et al* 1972).

**Table 4.** Interatomic distance in L<sub>S</sub>-threonine\*

Bond Description	Neutron	X-ray	Weighted average values**
C — O <sup>1</sup>	1.237(4) Å	1.243(4) Å	1.246 Å
C — O <sup>2</sup>	1.258(4)	1.253(4)	1.255
C <sup>α</sup> — C	1.539(3)	1.537(4)	1.531
C <sup>α</sup> — N	1.490(3)	1.490(4)	1.487
C <sup>α</sup> — C <sup>β</sup>	1.538(3)	1.532(4)	1.524
C <sup>β</sup> — O <sup>γ1</sup>	1.416(4)	1.431(4)	
C <sup>β</sup> — C <sup>γ2</sup>	1.515(5)	1.513(6)	
N — H <sup>1</sup>	1.037(6)	0.98 (4)	
N — H <sup>2</sup>	1.024(6)	0.90 (4)	
N — H <sup>3</sup>	1.034(6)	0.91 (4)	
C <sup>α</sup> — H <sup>α</sup>	1.096(6)	0.96 (4)	1.096
C <sup>β</sup> — H <sup>β</sup>	1.107(7)	1.03 (4)	
O <sup>γ1</sup> — H <sup>γ1</sup>	0.983(6)	0.75 (4)	
C <sup>γ2</sup> — H <sup>γ21</sup>	1.097(9)	0.95 (5)	
C <sup>γ2</sup> — H <sup>γ22</sup>	1.075(10)	1.02 (5)	
C <sup>γ2</sup> — H <sup>γ23</sup>	1.113(10)	1.02 (5)	

\*Parameters listed both in tables 4 and 5 are uncorrected for thermal motion. The standard deviations of the unit cell constants were also included in estimating the standard deviations of these parameters.

\*\*The weighted average values defined as  $\left\{ \left[ \frac{\sum_i (x_i/\sigma_i^2)}{\sum_i (1/\sigma_i^2)} \right] \right\}$  are based on the neutron diffraction studies of 14 aminoacid structures studied at Brookhaven and Trombay.

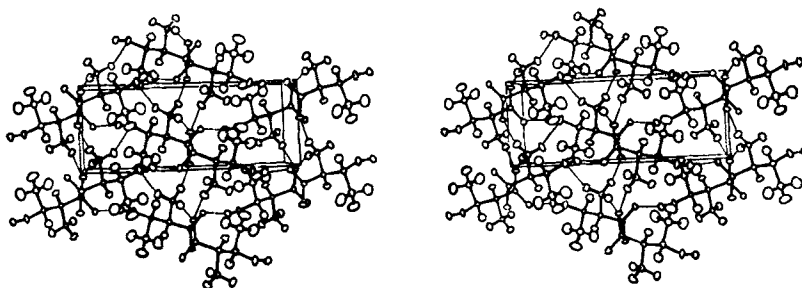
**Table 5.** Bond angles in L<sub>S</sub>-threonine

Angle description	Neutron	X-ray	Weight average values*
O <sup>1</sup> — C — C <sup>α</sup>	116.3°(3)	116.5°(3)	117.6°
O <sup>2</sup> — C — C <sup>α</sup>	116.4(2)	116.3(3)	116.6
O <sup>2</sup> — C — O <sup>1</sup>	127.2(3)	127.2(3)	125.7
C — C <sup>α</sup> — N	109.9(2)	110.0(2)	110.2
C — C <sup>α</sup> — C <sup>β</sup>	112.6(2)	112.7(2)	110.9
N — C <sup>α</sup> — C <sup>β</sup>	108.9(2)	108.8(2)	110.2
C <sup>α</sup> — C <sup>β</sup> — O <sup>γ1</sup>	104.2(2)	104.2(2)	
C <sup>α</sup> — C <sup>β</sup> — C <sup>γ2</sup>	113.1(2)	113.6(3)	
O <sup>γ1</sup> — C <sup>β</sup> — C <sup>γ2</sup>	110.9(2)	112.2(3)	
C <sup>α</sup> — N — H <sup>1</sup>	111.4(4)	108(2)	
C <sup>α</sup> — N — H <sup>2</sup>	109.8(4)	116(2)	
C <sup>α</sup> — N — H <sup>3</sup>	109.2(4)	107(3)	
H <sup>1</sup> — N — H <sup>2</sup>	108.3(5)	105(3)	
H <sup>1</sup> — N — H <sup>3</sup>	111.5(5)	116(3)	
H <sup>2</sup> — N — H <sup>3</sup>	106.6(5)	106(3)	
C — C <sup>α</sup> — H <sup>α</sup>	109.8(4)	110(2)	108.6
N — C <sup>α</sup> — H <sup>α</sup>	107.1(4)	108(2)	107.6
C <sup>β</sup> — C <sup>α</sup> — H <sup>α</sup>	108.4(4)	107(2)	108.8
C <sup>α</sup> — C <sup>β</sup> — H <sup>β</sup>	108.0(4)	111(2)	
O <sup>γ1</sup> — C <sup>β</sup> — H <sup>β</sup>	109.7(4)	111(2)	
C <sup>γ2</sup> — C <sup>β</sup> — H <sup>β</sup>	110.6(6)	106(2)	
C <sup>β</sup> — O <sup>γ1</sup> — H <sup>γ1</sup>	112.6(4)	109(3)	
C <sup>β</sup> — C <sup>γ2</sup> — H <sup>γ21</sup>	109.2(6)	109(3)	
C <sup>β</sup> — C <sup>γ2</sup> — H <sup>γ22</sup>	112.0(6)	111(3)	
C <sup>β</sup> — C <sup>γ2</sup> — H <sup>γ23</sup>	111.4(6)	113(3)	
H <sup>γ21</sup> — C <sup>γ2</sup> — H <sup>γ22</sup>	109.0(7)	105(4)	
H <sup>γ21</sup> — C <sup>γ2</sup> — H <sup>γ23</sup>	106.2(8)	113(4)	
H <sup>γ23</sup> — C <sup>γ2</sup> — H <sup>γ22</sup>	108.9(8)	106(4)	

\*Based on the neutron diffraction studies of 14 aminoacid structures studied at Brookhaven and Trombay.

**Table 6.** Torsion angles in *L*<sub>S</sub>-threonine

Angle	Description	Neutron	X-ray
$\phi^1$	C—C <sup>α</sup> —N—H <sup>1</sup>	46.4°(4)	48°(2)
$\phi^2$	C—C <sup>α</sup> —N—H <sup>2</sup>	166.4(4)	165(3)
$\phi^3$	C—C <sup>α</sup> —N—H <sup>3</sup>	-77.1(4)	-77(2)
$\psi^1$	N—C <sup>α</sup> —C—O <sup>1</sup>	-25.8(3)	-25.6(4)
$\psi^2$	N—C <sup>α</sup> —C—O <sup>2</sup>	155.3(3)	155.8(3)
$\chi^{1,1}$	N—C <sup>α</sup> —C <sup>β</sup> —O <sup>γ1</sup>	-55.1(3)	-54.8(3)
$\chi^{1,2}$	N—C <sup>α</sup> —C <sup>β</sup> —C <sup>γ2</sup>	-175.8(2)	-175.9(3)
$\chi^{1,3}$	N—C <sup>α</sup> —C <sup>β</sup> —H <sup>β</sup>	61.5(4)	64(2)
$\chi^{2,1}$	C <sup>α</sup> —C <sup>β</sup> —O <sup>γ1</sup> —H <sup>γ1</sup>	-175.8(5)	-177(3)
$\chi^{2,2,1}$	C <sup>α</sup> —C <sup>β</sup> —C <sup>γ2</sup> —H <sup>γ2,1</sup>	52.8(6)	58(3)
$\chi^{2,2,2}$	C <sup>α</sup> —C <sup>β</sup> —C <sup>γ2</sup> —H <sup>γ2,2</sup>	173.7(6)	173(3)
$\chi^{2,2,3}$	C <sup>α</sup> —C <sup>β</sup> —C <sup>γ2</sup> —H <sup>γ2,3</sup>	-64.1(6)	-68(4)

**Figure 6.** Stereoscopic picture of molecular packing and hydrogen bonding in the unit cell of *L*<sub>S</sub>-threonine crystal.

### 5.3. The molecular conformation

The conformation of the molecule is shown in figure 5 and the torsion angles are given in table 6. The torsion angle labelling is in accordance with the IUPAC-IUB commission on Biochemical Nomenclature (1970). The staggered ethane-type conformation of the threonine molecule about the C<sup>α</sup>—C<sup>β</sup> bond found by Shoemaker *et al* (1950) is confirmed. The hydrogens of the NH<sub>3</sub><sup>+</sup> group (angles  $\phi^1$ ,  $\phi^2$  and  $\phi^3$ ) are rotated about C<sup>α</sup>—N by about 14° in the counter-clockwise direction from the ideal staggered configuration with respect to the substituents on the C<sup>α</sup> atom. This departure from the ideal position appears to be due to the influence of hydrogen bonding. The difference between the angles  $\psi^1$  and  $\psi^2$  of 181° shows that the group C<sup>α</sup>—C—(O<sup>1</sup>, O<sup>2</sup>) is planar. The values ± 25° for these torsion angles show that the nitrogen atom is significantly displaced from this plane. The methyl group is also in the staggered conformation with reference to the groups on the C<sup>β</sup> atom.

### 5.4. Molecular packing and hydrogen bonding

The hydrogen bonding scheme in the crystal is depicted in the stereo drawing (figure 6) of the packing of the molecules in the unit cell. There are four hydrogen bonds per molecule — three from the NH<sub>3</sub><sup>+</sup> group and one from the O—H group. The hydrogen bond N—H<sup>1</sup>—O<sup>2</sup> links the threonine molecules to form chains along the *c*-axis of the crystal. The other three hydrogen bonds bind these chains together in a three dimensional network. All the hydrogen bond distances and angles are sum-

**Table 7.** Hydrogen bonding in L<sub>g</sub>-Threonine

X—H...Y		X—H	H...Y	X...Y	H—X...Y
N—H <sup>1</sup> ...O <sup>3</sup>	(I)	1.037(6) Å	1.900(7) Å	2.917(4) Å	9.0°(3)
N—H <sup>2</sup> ...O <sup>71</sup>	(II)	1.024(6)	2.202(7)	3.124(4)	21.2(3)
N—H <sup>3</sup> ...O <sup>3</sup>	(IV)	1.034(6)	1.791(7)	2.794(4)	11.0(4)
O <sup>71</sup> —H <sup>71</sup> ...O <sup>1</sup>	(III)	0.983(6)	1.716(6)	2.660(4)	13.0(3)
Symmetry code for the acceptor atom:					
	(I)	<i>x</i>	<i>y</i>	1 + <i>z</i>	
	(II)	$\frac{1}{2} - x$	− <i>y</i>	$\frac{1}{2} + z$	
	(III)	− $\frac{1}{2} + x$	$\frac{1}{2} - y$	1 − <i>z</i>	
	(IV)	1 − <i>x</i>	− $\frac{1}{2} + y$	$\frac{1}{2} - z$	

marized in table 7. Apart from a hydrogen bond to O<sup>71</sup> (II), the atom H<sup>2</sup> has a short intramolecular contact H<sup>2</sup>—O<sup>71</sup> of 2.214 Å. The N—H<sup>2</sup>—O<sup>71</sup> angle is 107.0°. Because of this unfavourable angle, we do not classify this as a bifurcated hydrogen bond system although the weak interaction present may explain why the N—H<sup>2</sup>—O<sup>71</sup> (II) bond is found to be the most bent as well as the longest N—H...O hydrogen bond in the structure.

## 6. Computer programs used

The computations described in this paper were done on the CDC 3600 and BESM-6 computers. The following programs were used.

No.	Program name	Purpose	Reference
1.	REFINE	Setting angles and least squares refinement of cell parameters	Srikanta and Sequeira 1968
2.	DATARED	Data reduction (absorption correction included as a subroutine)	Srikanta 1968
3.	FORDAP	Fourier calculations	Zalkin 1962
4.	TANFMULA	Tangent formula calculation of phases	Sikka 1970
5.	XFLS	Least squares program for structure refinement	Busing <i>et al</i> 1962*
6.	ORFFE	Bond distances and angles	Busing <i>et al</i> 1964*
7.	ORTEP	Stereo plotting	Johnson 1965†
8.	TORANG	Torsion angles	Ramanadham 1970

\*The BESM-6 version by H Rajagopal

†The CDC 3600 version by H Rajagopal and A Sequeira

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