

Precision Neutron Diffraction Structure Determination of Protein and Nucleic Acid Components.

XI. Molecular Configuration and Hydrogen Bonding of Serine in the Crystalline Amino Acids

L-Serine Monohydrate and DL-Serine*

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(Received 27 October 1972; accepted 27 December 1972)

The precise molecular configurations of the amino acid serine ($C_3H_7NO_3$) in crystals of L-serine monohydrate and DL-serine have been refined by neutron diffraction techniques. The neutron diffraction data collected for DL-serine were used in conjunction with earlier X-ray results to refine the atomic structural parameters. The unknown structure of L-serine $\cdot H_2O$ was solved by direct methods, thus confirming that the negative scattering length for hydrogen in neutron diffraction is not an obstacle to the use of such methods even with a comparatively high percentage of scattering by hydrogen atoms

[$\sum b_H^2 / \sum_{\text{all atoms}} b_i^2 = 0.26$]. Both compounds show the characteristics of most of the crystalline amino acids previously studied: The serine molecules are zwitterionic. There are three-dimensional networks of hydrogen bonds. The calculated barriers to rotation of the ammonium groups are 7.3 kcal mole⁻¹ in L-serine $\cdot H_2O$ and 10.9 kcal mole⁻¹ in DL-serine. The molecular packing in the two crystals is remarkably similar. There are structurally identical layers parallel to the main faces of the two crystals. In L-serine $\cdot H_2O$ these layers are tied together by hydrogen bonds formed through interleaving sheets of water molecules. A previous X-ray study of anhydrous L-serine [Benedetti, Pedone & Sirigo (1972). *Cryst. Struct. Commun.* **1**, 35-37] shows that the packing in this structure is quite different. Statistical tests indicate that the portion of the serine molecule not involved in hydrogen bonding has the same geometry in both crystals we have studied and in anhydrous L-serine, to the limits of experimental accuracy. In L-serine $\cdot H_2O$, $\sigma(X-X)$ and $\sigma(X-H)$ are respectively 0.003, 0.006 Å and in DL-serine 0.001, 0.001 Å.

Introduction

Neutron diffraction studies of the crystalline amino acids L-serine monohydrate and DL-serine have been carried out to provide definitive stereochemical information on hydrogen atom positions and possible effects of packing and hydrogen bonding on the geometry of the serine molecule. This work is part of a series of studies of hydrogen bonding in naturally occurring amino acids.

Crystal data

L-Serine $\cdot H_2O$

$C_3H_7NO_3 \cdot H_2O$. Orthorhombic $P2_12_12_1$, $a = 9.365(7)$, $b = 12.239(9)$, $c = 4.835(7)$ Å, $D_x = 1.475$ g cm⁻³, $Z = 4$, $D_m = 1.482$ g cm⁻³ (floatation). The unit-cell dimensions

were obtained by least-squares techniques from the orientation angles of 21 reflections from a small crystal centered on a four-circle diffractometer using Mo $K\alpha_1$ radiation ($\lambda = 0.70926$ Å).

DL-Serine

$C_3H_7NO_3$. Monoclinic $P2_1/a$, $a = 10.719(3)$, $b = 9.136(4)$, $c = 4.833(2)$ Å, $\beta = 106.43(3)^\circ$, $D_x = 1.537$ g cm⁻³, $Z = 4$, $D_m = 1.537$ g cm⁻³ (Albrecht, Schnakenberg, Dunn & McCullough, 1943). The unit-cell dimensions were determined as above, using 30 reflections.

Experimental

L-Serine $\cdot H_2O$

Single crystals of L-serine $\cdot H_2O$ were grown at 5°C from an aqueous solution saturated at room temperature. The plate-like crystals had {010} as the most developed form. When removed from the liquid, they tended to lose water, turning into a white powder. A single crystal with 7 boundary planes, having minimum and maximum linear dimensions of 1.0 and 5.7 mm and a volume of 13.1 mm³, was used for the data collection. The crystal was mounted with a near the goniometer head spindle axis, and was sealed under a quartz cap to prevent dehydration.

The intensities of the Bragg reflections were measured at the Brookhaven National Laboratory High

* Research performed under the auspices of the U.S. Atomic Energy Commission; Part X, 'A Comparison between the Crystal and Molecular Structures of L-Tyrosine and L-Tyrosine Hydrochloride', by Frey, Koetzle, Lehmann & Hamilton (1973). *J. Chem. Phys.* In the press.

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‡ On leave from Kemisk Institut, Århus Universitet, Denmark and supported in part by a grant from Statens Naturvidenskabelige Forskningsråd, Copenhagen, Denmark.

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|| Estimated standard deviations here and throughout this paper are given in parentheses in units of the last digit.

Flux Beam Reactor, using a four-circle diffractometer under the Multi-Spectrometer Control System (Beaucage, Kelley, Ophir, Rankowitz, Spinrad & van Norton, 1966). The θ - 2θ step-scan technique was employed with a scan range $\Delta 2\theta = 1.2^\circ (1 + 8 \tan \theta)$ and the step size was varied in order to obtain about 40 points in each scan. Data were collected for 1267 hkl and $hk\bar{l}$ reflections having $d^* < 1.36 \text{ \AA}^{-1}$, and two standard intensities were monitored every 60 measurements. There was no significant change in the intensity of the standard reflections throughout the data collection.

The integrated intensities were corrected for background by a method described by Lehmann, Hamilton & Larsen (1972). Squared observed structure factors, obtained as $F_o^2 = I \sin 2\theta$, were then corrected for absorption using Gaussian numerical integration. The incoherent scattering cross section for hydrogen was assumed to be 40 barns, and mass absorption coefficients for the other elements were taken from *International Tables for X-ray Crystallography* (1962), leading to a linear absorption coefficient $\mu = 2.63 \text{ cm}^{-1}$. Transmission coefficients ranged from 0.55 to 0.77. Finally, the

two symmetry-related forms were averaged, giving 803 unique reflections, with an agreement index $R_c = \sum |F_o^2 - \bar{F}_o^2| / \sum F_o^2 = 0.049$, where \bar{F}_o^2 is the mean value for symmetry-related reflections.

DL-Serine

Large crystals of DL-serine were grown at room temperature from a solution saturated at 65°C . A single crystal with 11 bounding planes, having minimum and maximum linear dimensions of 1.4 and 4.8 mm and a volume of 17.2 mm^3 , was used for data collection. The crystal was mounted with the c axis nearly parallel to the goniometer head spindle axis, and data were collected under the same conditions as for L-serine. H_2O . In all, 2598 reflections with $d^* \leq 1.36 \text{ \AA}^{-1}$ and $l \geq 0$ were measured. Squared structure factors for the 1248 unique reflections were obtained as described above. The linear absorption coefficient of $\mu = 2.46 \text{ cm}^{-1}$ led to transmission coefficients in the range 0.58 to 0.72; and the agreement index between symmetry-related reflections was $R_c = 0.020$.

Structure solution

L-Serine. H_2O

The scattering length for hydrogen in neutron diffraction is negative, and one of the major assumptions underlying the usual theory for direct methods, namely that the scattering density is nowhere negative, is therefore not fulfilled in applications of these methods to the solution of structures on the basis of neutron diffraction data. However, Sikka (1969) suggested that the

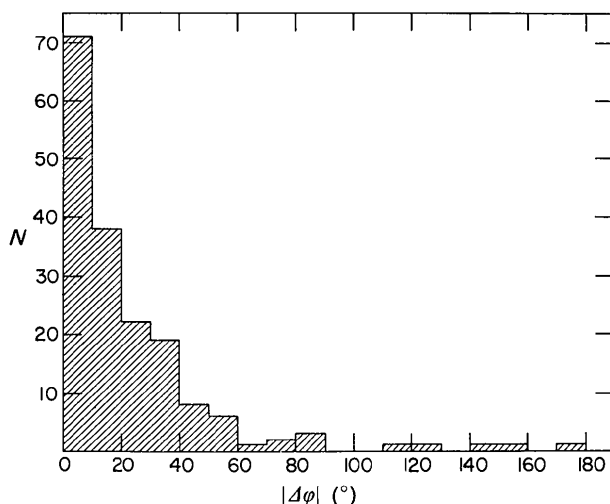


Fig. 1. Histogram of the deviations ($^\circ$) between phases obtained by *MULTAN* and the phases from the final least-squares refinement for L-Serine. H_2O .

Table 1. Mean deviation ($^\circ$) between phases obtained by *MULTAN* and the phases from the final refinement for L-serine. H_2O as a function of $w_h E_h$ (Germain, Main & Woolfson, 1971)

Each entry contains 35 observations.

$\langle w_h E_h \rangle$	$\langle \Delta\phi_h \rangle$
1.20	28.0
1.35	23.0
1.46	25.4
1.64	19.9
2.01	13.2

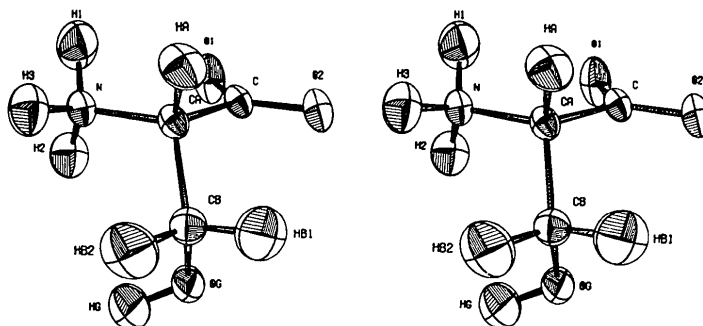


Fig. 2. Stereoscopic drawing of L-serine. H_2O . The thermal ellipsoids include areas with an atomic density greater than 50%, $A = \alpha$, $B = \beta$, $G = \gamma$, $D = \delta$.

Table 2. Fractional coordinates ($\times 10^4$) and temperature parameters ($\times 10^4$) for L-serine·H₂O

The form of the anisotropic Debye-Waller factor is

$$\exp[-2\pi^2(U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}l^2c^{*2} + 2U_{12}ha^*kb^* + 2U_{13}ha^*lc^* + 2U_{23}kb^*lc^*)].$$

	x	y	z	U ₁₁	U ₂₂	U ₃₃	U ₁₂	U ₁₃	U ₂₃
O ¹	5377 (3)	1548 (2)	4788 (6)	275 (15)	469 (17)	490 (18)	-32 (14)	-125 (14)	223 (14)
O ²	6354 (3)	2971 (2)	2670 (5)	214 (12)	434 (14)	262 (12)	-64 (11)	5 (12)	29 (12)
O ^y	3258 (3)	3945 (2)	4529 (6)	275 (15)	377 (16)	416 (17)	-48 (14)	84 (13)	-141 (14)
N	2882 (2)	1686 (1)	2155 (4)	194 (8)	281 (9)	271 (9)	-7 (7)	-27 (7)	0 (8)
C	5346 (2)	2323 (2)	3113 (4)	160 (10)	300 (10)	199 (10)	8 (9)	-4 (9)	13 (10)
C ^α	3997 (2)	2499 (2)	1396 (4)	182 (10)	271 (11)	182 (9)	11 (9)	-7 (9)	2 (9)
C ^β	3445 (2)	3659 (2)	1714 (5)	268 (13)	260 (11)	311 (12)	13 (10)	-5 (12)	13 (10)
H ^α	4253 (5)	2357 (4)	-784 (8)	402 (23)	539 (25)	215 (18)	14 (23)	34 (18)	-41 (21)
H ¹	3295 (5)	915 (4)	1976 (12)	448 (26)	340 (26)	760 (34)	-20 (23)	-42 (27)	55 (28)
H ²	2476 (5)	1801 (4)	4105 (10)	402 (26)	473 (29)	340 (23)	-51 (24)	9 (23)	39 (23)
H ³	2068 (5)	1726 (4)	709 (10)	363 (25)	510 (29)	425 (25)	-55 (25)	-75 (24)	-11 (25)
H ^{β1}	4230 (6)	4215 (4)	819 (11)	616 (31)	402 (26)	592 (31)	-36 (26)	189 (30)	142 (26)
H ^{β2}	2444 (5)	3760 (5)	566 (10)	474 (32)	585 (37)	635 (32)	152 (27)	-230 (30)	17 (28)
H ^γ	2286 (6)	3813 (5)	5043 (10)	389 (29)	531 (35)	574 (32)	-22 (26)	119 (28)	-69 (25)
O ^w	784 (4)	-55 (3)	3585 (8)	389 (19)	321 (17)	461 (19)	4 (15)	-59 (17)	-40 (16)
H ^{w1}	439 (6)	-532 (4)	2199 (11)	587 (32)	514 (29)	466 (30)	22 (27)	-3 (29)	-87 (26)
H ^{w2}	-42 (7)	334 (4)	4194 (12)	709 (41)	443 (30)	521 (32)	81 (27)	24 (35)	-55 (27)

Table 3. Fractional coordinates ($\times 10^5$) and temperature parameters ($\times 10^4$) for DL-serine

The form of the anisotropic Debye-Waller factor is as given in Table 2.

	x	y	z	U ₁₁	U ₂₂	U ₃₃	U ₁₂	U ₁₃	U ₂₃
O ¹	16414 (7)	40220 (7)	-6901 (14)	369 (3)	244 (3)	232 (3)	-15 (2)	26 (3)	49 (2)
O ²	33205 (7)	50588 (7)	25276 (16)	420 (4)	235 (3)	289 (3)	-94 (2)	90 (3)	-21 (2)
O ^y	43155 (6)	16875 (7)	22985 (17)	247 (3)	284 (3)	449 (4)	-55 (2)	168 (3)	-103 (3)
N	15240 (3)	17000 (4)	24428 (8)	220 (2)	214 (2)	256 (2)	18 (1)	81 (1)	35 (2)
C	24976 (5)	40565 (5)	16750 (10)	283 (2)	174 (2)	197 (2)	0 (2)	89 (2)	-4 (2)
C ^α	25492 (5)	27886 (5)	37754 (10)	238 (2)	202 (2)	177 (2)	18 (2)	81 (2)	5 (2)
C ^β	38891 (5)	20731 (6)	47038 (12)	217 (2)	282 (3)	265 (3)	15 (2)	38 (2)	12 (2)
H ^α	23158 (13)	32218 (13)	55957 (26)	497 (6)	418 (6)	280 (5)	14 (5)	188 (5)	-38 (5)
H ¹	6406 (11)	22-64 (13)	18410 (30)	268 (5)	365 (5)	483 (7)	47 (4)	89 (5)	62 (5)
H ²	16730 (11)	119<3 (3)	6236 (26)	378 (6)	359 (5)	363 (6)	-10 (4)	119 (5)	-38 (5)
H ³	14830 (12)	9214 (13)	39864 (28)	421 (6)	314 (5)	417 (6)	0 (4)	147 (5)	100 (5)
H ^{β1}	45738 (13)	29032 (17)	59472 (33)	377 (6)	603 (8)	452 (7)	-70 (6)	11 (5)	-170 (6)
H ^{β2}	38902 (15)	11546 (18)	61126 (36)	481 (7)	537 (7)	571 (8)	120 (6)	130 (6)	256 (7)
H ^γ	40092 (12)	6937 (13)	16897 (31)	371 (5)	323 (6)	540 (7)	-26 (4)	139 (5)	-114 (5)

Table 4. Observed and calculated squared structure factors for L-serine·H₂OThe quantities given are $100F_o^2$, $100\sigma(F_o)^2$, $100|F_c|^2$. The extinction correction is applied to F_c^2 .

h	k	l	F _o ²	σ(F _o) ²	F _c ²	h	k	l	F _o ²	σ(F _o) ²	F _c ²	h	k	l	F _o ²	σ(F _o) ²	F _c ²
0	0	0	10000	0	10000	0	0	0	10000	0	10000	0	0	0	10000	0	10000
0	0	1	10000	0	10000	0	0	1	10000	0	10000	0	0	1	10000	0	10000
0	0	2	10000	0	10000	0	0	2	10000	0	10000	0	0	2	10000	0	10000
0	0	3	10000	0	10000	0	0	3	10000	0	10000	0	0	3	10000	0	10000
0	0	4	10000	0	10000	0	0	4	10000	0	10000	0	0	4	10000	0	10000
0	0	5	10000	0	10000	0	0	5	10000	0	10000	0	0	5	10000	0	10000
0	0	6	10000	0	10000	0	0	6	10000	0	10000	0	0	6	10000	0	10000
0	0	7	10000	0	10000	0	0	7	10000	0	10000	0	0	7	10000	0	10000
0	0	8	10000	0	10000	0	0	8	10000	0	10000	0	0	8	10000	0	10000
0	0	9	10000	0	10000	0	0	9	10000	0	10000	0	0	9	10000	0	10000
0	0	10	10000	0	10000	0	0	10	10000	0	10000	0	0	10	10000	0	10000
0	0	11	10000	0	10000	0	0	11	10000	0	10000	0	0	11	10000	0	10000
0	0	12	10000	0	10000	0	0	12	10000	0	10000	0	0	12	10000	0	10000
0	0	13	10000	0	10000	0	0	13	10000	0	10000	0	0	13	10000	0	10000
0	0	14	10000	0	10000	0	0	14	10000	0	10000	0	0	14	10000	0	10000
0	0	15	10000	0	10000	0	0	15	10000	0	10000	0	0	15	10000	0	10000
0	0	16	10000	0	10000	0	0	16	10000	0	10000	0	0	16	10000	0	10000
0	0	17	10000	0	10000	0	0	17	10000	0	10000	0	0	17	10000	0	10000
0	0	18	10000	0	10000	0	0	18	10000	0	10000	0	0	18	10000	0	10000
0	0	19	10000	0	10000	0	0	19	10000	0	10000	0	0	19	10000	0	10000
0	0	20	10000	0	10000	0	0	20	10000	0	10000	0	0	20	10000	0	10000
0	0	21	10000	0	10000	0	0	21	10000	0	10000	0	0	21	10000	0	10000
0	0	22	10000	0	10000	0	0	22	10000	0	10000	0	0	22	10000	0	10000
0	0	23	10000	0	10000	0	0	23	10000	0	10000	0	0	23	10000	0	10000
0	0	24	10000	0	10000	0	0	24	10000	0	10000	0	0	24	10000	0	10000
0	0	25	10000	0	10000	0	0	25	10000	0	10000	0	0	25	10000	0	10000
0	0	26	10000	0	10000	0	0	26	10000	0	10000	0	0	26	10000	0	10000
0	0	27	10000	0	10000	0	0	27	10000	0	10000	0	0	27	10000	0	10000
0	0	28	10000	0	10000	0	0	28	10000	0	10000	0	0	28	10000	0	10000
0	0	29	10000	0	10000	0	0	29	10000	0	10000	0	0	29	10000	0	10000
0	0	30	10000	0	10000	0	0	30	10000	0	10000	0	0	30	10000	0	10000
0	0	31	10000	0	10000	0	0	31	10000	0	10000	0	0	31	10000	0	10000
0	0	32	10000	0	10000	0	0	32	10000	0	10000	0	0	32	10000	0	10000
0	0	33	10000	0	10000	0	0	33	10000	0	10000	0	0	33	10000	0	10000
0	0	34	10000	0	10000	0	0	34	10000	0	10000	0	0	34	10000	0	10000
0	0	35	10000	0	10000	0	0	35	10000	0	10000	0	0	35	10000	0	10000
0	0	36	10000	0	10000	0	0	36	10000	0	10000	0	0	36	10000	0	10000
0	0	37	10000	0	10000	0	0	37	10000	0	10000	0	0	37	10000	0	10000
0	0	38	10000	0	10000	0	0	38	10000	0	10000	0	0	38	10000	0	10000
0	0	39	10000	0	10000	0	0	39	10000	0	10000	0	0	39	10000	0	10000
0	0	40	10000	0	10000	0	0	40	10000	0	10000	0	0	40	10000	0	10000
0	0	41	10000	0	10000	0	0	41	10000	0	10000	0	0	41	10000	0	10000
0	0	42	10000	0	10000	0	0	42	10000	0	10000	0	0	42	10000	0	10000
0	0	43	10000	0	10000	0	0	43	10000	0	10000	0	0	43	10000	0	10000
0	0	44	10000	0	10000	0	0	44	10000	0	10000	0	0	44	10000	0	10000
0	0	45	10000	0	10000	0	0	45	10000	0	10000	0	0	45	10000	0	10000
0	0	46	10000	0	10000	0	0	46	10000	0	10000	0	0	46	10000	0	10000
0	0	47	10000	0	10000	0	0	47	10000	0	10000	0	0	47	10000	0	10000
0	0	48	10000	0	10000	0	0	48	10000	0	10000	0	0	48	10000	0	10000
0	0	49	10000	0	10000	0	0	49	10000	0	10000	0	0	49	10000	0	10000
0	0	50	10000	0	10000	0	0	50	10000	0	10000	0	0	50	10000	0	10000
0	0	51	10000	0	10000	0	0	51	10000	0	10000	0	0	51	10000	0	10000
0	0	52	10000	0	10000	0	0	52	10000	0	10000	0	0	52	10000	0	10000
0	0	53	10000	0	10000	0	0	53	10000	0	10000	0	0	53	10000	0	10000
0	0	54	10000	0	10000	0	0	54	10000	0	10000	0	0	54	10000	0	10000
0	0	55	10000	0	10000	0	0	55	10000	0	100						

difference between the two sets of phases, $\langle |\Delta\phi| \rangle$, for blocks of 35 values arranged in decreasing order of weighted E 's as defined by Germain, Main & Woolfson (1971). The total mean difference is 21.9° . Fig. 1 shows in histogram form the distribution of the $|\Delta\phi|$ values.

The ease with which this structure and the two previously reported structures were solved clearly indicates that the negative scattering power of hydrogen is no obstacle to a successful solution of a structure by direct methods based on neutron diffraction data, and demonstrates that these methods can be used to attack successfully problems of about the same complexity as in the X-ray case.

Refinement

L-Serine. H₂O

Full-matrix least-squares refinement was carried out until the shifts in the parameters were less than 10% of the estimated standard deviations. The quantity minimized was $\sum w|F_o^2 - |F_c|^2|^2$ with weights $w = 1/\sigma^2(F_o^2)$, where $\sigma^2(F_o^2) = \sigma_{\text{count}}^2(F_o^2) + (0.02 F_o^2)^2$, and $\sigma_{\text{count}}(F_o^2)$ is based on counting statistics. The 640 independent reflections with $F_o^2 > 3\sigma_{\text{count}}(F_o^2)$ were included in the refinement, and the parameters refined were a scale factor, atomic coordinates, anisotropic thermal parameters and an extinction parameter g (Zachariasen, 1967). Scattering lengths used were $b_o = 0.575$, $b_N = 0.940$, $b_C = 0.6626$ and $b_H = -0.3723$ (all $\times 10^{-12}$ cm). The final value for the extinction parameter was $g = 6.1(3) \times 10^3$ corresponding to a maximum correction on F_o of 1.49 for the 020 reflection. The final agreement between observed and calculated squared structure factors was $R_2 = \sum |F_o^2 - |F_c|^2| / \sum F_o^2 = 0.055$. The corresponding weighted value $R_{w2} = (\sum w|F_o^2 - |F_c|^2|^2 / \sum w F_o^4)^{1/2}$ was 0.054 while the conventional R index, $R = \sum |F_o - |F_c|| / \sum F_o$, was 0.039.

DL-Serine

The structure was refined in a manner identical to L-serine. H₂O. The coordinates given by Shoemaker, Baricau, Donohue & Lu (1953) were used as starting

parameters, and 1100 reflections with $F_o^2 > 3\sigma_{\text{count}}(F_o^2)$ were included in the refinement. The weights used in the refinement were based on standard deviations obtained from comparison between symmetry-related reflections as $\sigma_e = \{\sum (F_o^2 - F_o^2)^2\} / (n-1)$, where the sum is over n symmetry-related reflections having mean value F_o^2 .

The data were categorized according to values of $\sin \theta/\lambda$ and F_o^2 , each datum being identified by indices i and j such that

$$F_i^2 < F_o^2 < F_{i+1}^2 \quad i = 1, 12$$

$$(\sin \theta/\lambda)_j < \sin \theta/\lambda < (\sin \theta/\lambda)_{j+1} \quad j = 1, 22$$

with approximately equal numbers of reflections in each F_o^2 and $(\sin \theta/\lambda)$ class. Values of $\sigma_i(F_o^2)$ and $\sigma_j(\sin \theta/\lambda)$ were determined by a least-squares procedure that tried to fit each standard deviation by

$$\sigma_e = \sigma_i + \sigma_j.$$

The resulting 34 parameter weight function was employed in the usual way: for any reflection belonging to i th intensity class and j th diffraction angle class

$$w = (\sigma_i + \sigma_j)^{-2}.$$

The final value for the extinction parameter was $g = 4.9(1) \times 10^3$ corresponding to a maximum correction on F_o of 1.25 for the 011 reflection. The final R values were $R_2 = 0.024$, $R_{w2} = 0.039$ and $R = 0.020$.

The final structural parameters for the two crystal are presented in Tables 2 and 3, and the squared structure factors in Tables 4 and 5.

Molecular structures

The molecular configurations of the L-serine molecule in L-serine. H₂O and in DL-serine are shown in Figs. 2 and 3, respectively. The intramolecular bond distances and angles and the torsion angles are given in Tables 6 and 7. The atom and the torsion angle labeling is in accordance with the IUPAC-IUB conventions (IUPAC-IUB Commission on Biochemical Nomenclature, 1970).

Table 6. Intramolecular distances (Å) and bond angles (°)

Distances	Corrected for		Corrected for		L-Serine* (X-ray)
	L-Serine. H ₂ O	thermal motion	DL-Serine	thermal motion	
C—O ¹	1.248 (3)	1.256	1.248 (1)	1.252	1.235 (5)
C—O ²	1.251 (3)	1.254	1.257 (1)	1.261	1.258 (5)
C—C ^α	1.527 (3)	1.534	1.531 (1)	1.537	1.523 (5)
C ^α —N	1.488 (2)	1.493	1.487 (1)	1.491	1.490 (5)
C ^α —H ^α	1.095 (5)	1.099	1.101 (1)	1.105	
N—H ¹	1.023 (6)	1.031	1.037 (1)	1.039	
N—H ²	1.026 (6)	1.029	1.045 (1)	1.047	
N—H ³	1.035 (6)	1.038	1.041 (1)	1.044	
C ^α —C ^β	1.519 (3)	1.529	1.518 (1)	1.524	1.530 (5)
C ^β —H ^{β1}	1.090 (6)	1.097	1.095 (1)	1.101	
C ^β —H ^{β2}	1.096 (6)	1.104	1.095 (2)	1.103	
C ^β —O ^γ	1.413 (4)	1.422	1.414 (1)	1.420	1.424 (5)
O ^γ —H ^γ	0.958 (7)	0.959	0.981 (1)	0.983	
O ^w —H ^{w1}	0.946	0.947			
O ^w —H ^{w2}	0.955	0.957			

* The hydrogen atom positions were determined but not refined.

Table 6 (cont.)

Bond angles	L-Serine. H ₂ O	DL-Serine
O ¹ —C—O ²	125.1 (2)	125.8 (1)
O ¹ —C—C ^α	118.6 (2)	117.9 (1)
O ² —C—C ^α	116.3 (2)	116.3 (1)
C—C ^α —N	110.6 (1)	109.6 (1)
C—C ^α —C ^β	110.9 (2)	111.5 (1)
C—C ^α —H ^α	108.6 (3)	108.2 (1)
N—C ^α —C ^β	111.2 (2)	111.7 (1)
N—C ^α —H ^α	106.6 (3)	106.8 (1)
C ^β —C ^α —H ^α	108.7 (3)	108.8 (1)
C ^α —N—H ¹	109.3 (3)	107.7 (1)
C ^α —N—H ²	113.2 (3)	113.0 (1)
C ^α —N—H ³	108.6 (3)	108.8 (1)
H ¹ —N—H ²	110.1 (4)	108.8 (1)
H ¹ —N—H ³	105.4 (4)	108.1 (1)
H ² —N—H ³	109.9 (4)	110.4 (1)
C ^α —C ^β —O ^γ	111.8 (2)	111.4 (1)
C ^α —C ^β —H ^{β1}	108.3 (3)	108.1 (1)
C ^α —C ^β —H ^{β2}	110.1 (3)	110.3 (1)
O ^γ —C ^β —H ^{β1}	108.1 (4)	107.8 (1)
O ^γ —C ^β —H ^{β2}	110.7 (4)	111.2 (1)
H ^{β1} —C ^β —H ^{β2}	107.8 (4)	107.3 (1)
C ^β —O ^γ —H ^γ	109.2 (4)	109.3 (1)
H ^{w1} —O ^w —H ^{w2}	106.6 (5)	

All features of the heavy atom backbone agree well with the results of the earlier X-ray studies of DL-serine (Shoemaker *et al.*, 1953) and of pure L-serine (Benedetti *et al.*, 1972). In both L-serine.H₂O and DL-serine the serine molecule is a zwitterion. The carboxyl group and the C^α—N bond are coplanar; one hydrogen atom of the ammonium group is very close to the plane of the carboxyl group and has a nearly eclipsed configuration with respect to O¹. Table 7 shows that the conformation of the serine molecule remains nearly the same in the three structures.

Hydrogen bonding and packing

The distances and angles of the hydrogen bonds are given in Table 8, and the packing and hydrogen bond networks are shown in Figs. 4 and 5.

In L-serine monohydrate there are six hydrogen bonds per asymmetric unit in the crystal structure. One hydrogen of the ammonium group is donated to a water molecule and the remaining two hydrogens form bonds to carboxyl oxygens (O²) of neighboring serine molecules. The hydrogen atom of the hydroxyl group is donated to a carboxyl oxygen (O¹) of a neighboring molecule, and the oxygen accepts a hydrogen bond from a water molecule. The N—H distances are inversely correlated with the corresponding hydrogen bond distances H···O, and the A—H···B and C—B···H angles differ significantly from 180 and 120°, respectively. There is no evidence of an intramolecular hydrogen bond if we assume a van der Waals radius of 1.0 Å for hydrogen (Baur, 1972). A short intermolecular contact worth noting is C···H^γ = 2.456 Å.

The hydrogen bond picture proposed by Shoemaker *et al.* (1953) for DL-serine is confirmed. The three N—H···O hydrogen bonds involving the ammonium group are very similar in geometry and are all much weaker than the bond O^γ—H^γ···O¹. Again the characteristic angles A—H···B and H···B—C differ significantly from 180 and 120°. There are two short intermolecular contacts C···H² = 2.529 and C···H^γ = 2.450 Å.

The molecular packing of DL-serine has been described as a stacking of sheets of serine molecules parallel to the (100) face (Shoemaker *et al.*, 1953). A sheet of L-serine molecules is bonded to a neighboring sheet

Table 7. Torsion angles (°)

The IUPAC—IUB conventions have been used.

Angle	Atoms involved	L-Serine. H ₂ O	DL-Serine	L-Serine (X-ray)
φ ¹	C—C ^α —N—H ¹	-54.5 (4)	-55.3 (1)	-56.6
φ ²	C—C ^α —N—H ²	+68.7 (3)	+64.8 (1)	+63.7
φ ³	C—C ^α —N—H ³	-165.9 (3)	-172.2 (1)	-176.3
ψ ¹	N—C ^α —C—O ¹	+2.1 (3)	+1.4 (1)	+1.7
ψ ²	N—C ^α —C—O ²	-179.3 (2)	-179.7 (1)	-178.7
χ ¹	N—C ^α —C ^β —O ^γ	+70.2 (2)	+70.0 (1)	+60.2
χ ²	C ^α —C ^β —O ^γ —H ^γ	-94.6 (4)	-88.0 (1)	-104.8

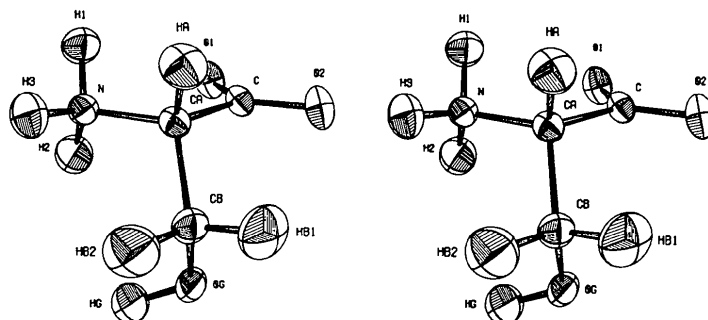


Fig. 3. Stereoscopic drawing of DL-serine. The thermal ellipsoids include areas with an atomic density greater than 50%, A = α, B = β, G = γ, D = δ.

of D-serine molecules by $H^1 \cdots O^{\gamma}$ bonds. The three remaining hydrogen bonds bind the molecules within each sheet. In L-serine monohydrate there is a remarkably similar pattern, except that there the sheets of L-serine molecules lie parallel to (010). The internal structure of these sheets is nearly the same as in DL-serine as may be seen by comparing the left-hand sides of Figs. 4 and 5. In L-serine.H₂O the sheets are tied together by hydrogen bonds through water molecules. The repeat period along the **b** axis consists of two sheets of L-serine molecules separated by layers of water molecules, while in DL-serine the period along the corresponding **a** axis consists of adjacent layers of L- and D-serine molecules.

This similarity in packing is reflected in the near equality of the short **c** axes in the two structures and the close correspondence of hydrogen bond geometries involving the H², H³ and O² atoms (Table 8).

The hydrogen bonding and packing in pure L-serine (Benedetti *et al.*, 1972) differs completely from those described above. The hydroxyl groups are exclusively hydrogen bonded with neighboring hydroxyl groups and all hydrogens of the ammonium group are donated to neighboring carboxyl groups.

Thermal motion

The thermal motion parameters of the non-hydrogen atoms were fitted to a rigid-body model, the motion of which was described in terms of a translation tensor **T**, a libration tensor **L** and a screw tensor **S** (Schomaker & Trueblood, 1969). The fit $\overline{\Delta u_{ij}^2}^{1/2}$ between observed and calculated thermal parameters is quite satisfactory: 0.0015 Å² in L-serine.H₂O and 0.0013 Å² in DL-serine. The screw components are very small. Bond lengths within the non-hydrogen backbone were corrected for thermal motion using the calculated rigid-body librations.

To correct the bond lengths involving hydrogen for thermal motion we used the 'minimum correction' of Busing & Levy (1964) which has been shown to lead to reasonable corrected C-H distances in L-lysine.HCl.2H₂O (Koetzle, Lehmann, Verbist & Hamilton, 1972) as well as in other amino acids. Corrected bond distances are included in Table 6.

Additional rigid-body calculations were carried out to analyze the thermal motion of the ammonium groups. The rigid-body consisted of the atoms C^α, N, H¹, H², H³. The fit was again satisfactory, $\overline{\Delta u_{ij}^2}^{1/2} =$

Table 8. Distances (Å) and angles (°) in the hydrogen bonds

A-H ¹ ⋯B-C	A⋯B	H⋯B	∠A-H⋯B	∠C-B⋯H
L-Serine.H ₂ O				
N-H ¹ ⋯O ^w	2.919 (4)	2.130 (7)	132.4 (4)	
N-H ² ⋯O ² -C	2.913 (4)	1.901 (6)	168.1 (4)	111.7 (2)
N-H ³ ⋯O ² -C	2.769 (4)	1.804 (6)	153.4 (4)	124.4 (2)
O ^γ -H ^γ ⋯O ¹ -C	2.785 (4)	1.843 (6)	166.8 (4)	103.5 (2)
O ^w -H ^w ⋯O ¹ -C	2.809 (5)	1.868 (6)	173.2 (5)	154.4 (3)
O ^w -H ^{w2} ⋯O ^γ -C ^β	2.877 (5)	1.822 (7)	177.4 (5)	108.7 (3)
DL-Serine				
N-H ¹ ⋯O ^γ -C ^β	2.772 (1)	1.787 (1)	157.3 (1)	114.3 (1)
N-H ² ⋯O ² -C	2.876 (1)	1.844 (2)	168.8 (1)	107.8 (1)
N-H ³ ⋯O ² -C	2.821 (1)	1.814 (1)	160.7 (1)	120.4 (1)
O ^γ -H ^γ ⋯O ¹ -C	2.671 (1)	1.692 (2)	175.1 (1)	112.1 (1)
L-Serine (X-ray)				
N-H ¹ ⋯O ¹ -C	2.833	1.889	156.6	147.7
N-H ² ⋯O ² -C	2.856	1.932	153.4	123.0
N-H ³ ⋯O ² -C	2.874	1.949	152.3	92.8
O ^γ -H ^γ ⋯O ^γ -C ^β	2.916	1.992	155.9	107.6

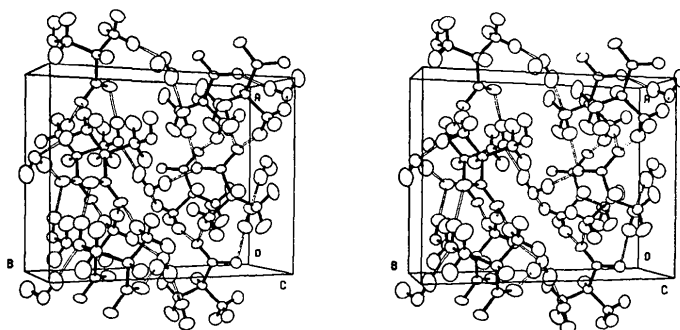


Fig. 4. Stereoscopic view of the molecular packing of L-serine.H₂O. The thermal ellipsoids include areas with an atomic density greater than 80%. Molecular bond, thick; hydrogen bond, thin.

0.003 Å² and 0.002 Å² for L-serine.H₂O and DL-serine respectively. The largest principal axis of libration is inclined at 19° to the N-C^α direction in L-serine.H₂O and 13° in DL-serine. The corresponding libration amplitudes are 8.6 (9) and 7.3 (6)°. If we assume a threefold cosine potential (Schlemper, Hamilton & La Placa, 1971) these amplitudes correspond to estimated barriers to rotation of the α-ammonium group of 7.3 kcal mole⁻¹ in L-serine.H₂O and 10.9 kcal mole⁻¹ in DL-serine, values which are typical of previously derived barriers for hydrogen bonded NH₃⁺ groups in other amino acids.

Comparisons between the molecular geometries of serine in L-serine.H₂O and DL-serine

The molecular geometries in the two crystals are nearly identical. To evaluate the significance of small changes in bond lengths and valence angles between the two structures, the sum of the squares of the weighted deviations* between various sets of values were tested at the 0.05 probability level as χ² (Hamilton, 1969). The results are shown in Table 9. If we omit the atoms not directly involved in hydrogen bonds and the angle C-C^α-N, we find no significant differences. Comparing the whole molecule, however, the test does show small

* The weighted deviation is defined as

$$\Delta_i = \frac{p_i^1 - p_i^2}{[(\sigma_i^1)^2 + (\sigma_i^2)^2]^{1/2}} = \frac{\Delta p_i}{\sigma_i}$$

where p_i^j is the parameter value and σ_i^j is its associated standard deviation. The pooled standard deviation is denoted by σ_i .

but significant differences between the two sets of distances and angles. Thus hydrogen bonding and crystal packing do influence internal geometry to some degree.

The distribution of the weighted deviations between the 'identical' parts of the serine molecule as described above was analyzed further by the method of normal probability plots (Abrahams & Keve, 1971; Hamilton & Abrahams, 1972). For both distances and angles the Δ_i between the two sets were compared with the values expected for a normal distribution function with zero mean and unit variance.

For the uncorrected distances, a straight line was obtained with slope slightly less than one. This indicates that the errors are normally distributed but that they are a bit overestimated. The line does not pass exactly through the origin, because the L-serine.H₂O distances show a tendency to be slightly shorter than those in DL-serine. These anomalies disappear if we take into account the thermal motion corrections.

A straight line passing through the origin was obtained for the angles. The slope is 1.4, which corresponds to an underestimation of the pooled standard deviation of the same order of magnitude.

We conclude that the values of the pooled standard deviations of the bond distances and angles obtained from our neutron diffraction studies of DL-serine and L-serine.H₂O are approximately correct. The major contribution to the pooled standard deviations is from the e.s.d.'s for L-serine.H₂O, so the errors in this structure refinement have apparently been estimated properly. In DL-serine, we dealt with the very favorable

Table 9. Statistical comparisons between the molecular geometries of serine in L-serine.H₂O and DL-serine (χ² tests)

Hypothesis Two sets equal if they include	Results					
	Distances			Angles		
	N	χ _{obs} ²	χ _{N=0.05} ²	N	χ _{obs} ²	χ _{N=0.05} ²
1. All atoms	13	37.7	22.4	22	170.7	37.7
2. Atoms not hydrogen bonded (C, C ^α , H ^α , N, C ^β , H ^{β1} , H ^{β2})	6	4.0	12.6	9	65.1	16.9
3. Same as above and O ^γ	7	4.0	14.1	12	70.3	21.0
				11	20.3	19.7*

* ∠ C-C^α-N excluded.

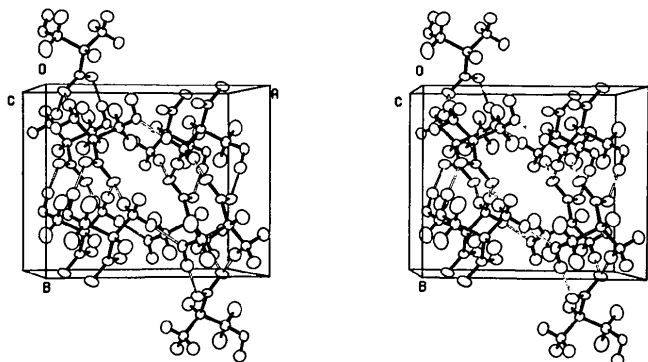


Fig. 5. Stereoscopic view of the molecular packing of DL-serine. The thermal ellipsoids include areas with an atomic density greater than 80%. Molecular bond, thick; hydrogen bond, thin.

case of an extremely high-quality crystal and a centrosymmetric structure with a small number of parameters. The accuracy of this structure is characteristic of the best attainable by present-day neutron diffraction techniques.

We wish to thank Dr Betty Davis for technical assistance and for useful and helpful discussions.

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Metal Binding to Nucleic Acid Constituents. The Crystal Structure of Trichloroadeniniumzinc(II)

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(Received 24 October 1972; accepted 18 December 1972)

The crystal structure of a zinc(II) complex of adenine, crystallized from hydrochloric acid solution, has been determined. The compound, trichloroadeniniumzinc(II), $C_5H_6N_5Cl_3Zn$, crystallizes in the space group $P2_1/c$ with unit-cell parameters $a = 10.847(2)$, $b = 5.934(1)$, $c = 15.726(2)$ Å, $\beta = 90.95(2)$, $Z = 4$. Three-dimensional data were collected from two crystals using a Supper two-circle diffractometer. The structure was refined by full-matrix least-squares calculations, minimizing $\sum w(F_o - sF_c)^2$, to a final R of 0.035. The zinc atom is coordinated to the three chlorine atoms and to N(7) of the adenine moiety in a distorted tetrahedral arrangement. This indicates that N(7) is a possible coordination site for bivalent metal ion interaction with nucleic acids. The six-membered ring of the adenine moiety, which is protonated at N(1), is significantly non-planar. There is an extensive network of intra- and intermolecular hydrogen bonds.

Introduction

The importance of bivalent metal ions in biochemical processes is well known, and their interactions with the bases of nucleotides and nucleic acids have been the subject of numerous studies (*e.g.* Weser, 1968; Eichhorn, Berger, Butzow, Clark, Rifkin, Shin & Tarien, 1971, and references therein). Shin & Eichhorn (1968) have shown that zinc(II) ions can be used to unwind and rewind double-helical DNA reversibly by heating and cooling respectively. It has also been shown that zinc(II)

and other transition metal ions affect the single-helix \rightleftharpoons random-coil equilibrium in polyriboadenylic acid (Shin, Heim & Eichhorn, 1972). In many of these, and other similar phenomena, binding of the transition metal ions to the bases has been postulated.

As an aid in the interpretation of these results it is important to establish which sites on the bases are the primary coordination sites for bivalent metals under a variety of conditions, and to obtain a quantitative measure of the electronic changes that occur within the bases as a consequence of metal-binding. Accord-