KEMAL, C. & BRUICE, T. C. (1976). *Proc. Natl Acad. Sci. USA*, **73**, 995–999.
KIERKEGAARD, P., NORRESTAM, R., WERNER, P., CSÖREGH, I., GLEHN, M., KARLSSON, R., LEIJONMARCK, M., RÖNNQUIST, O., STENSLAND, B., TILLBERG, O. & TORBJÖRNSSON, L. (1971). *Flavins and Flavoproteins*, edited by H. KAMIN, pp. 1–22. Baltimore: Univ. Park Press, and London: Butterworths.
MASSEY, V. & GHISLA, S. (1975). Proc. 10th FEBS Meet., pp. 145–158.
NORRESTAM, R. (1972). *Acta Cryst.* **B28**, 1713–1720.
NORRESTAM, R. & STENSLAND, B. (1972). *Acta Cryst.* **B28**, 440–447.
PAULING, L. (1948). *The Nature of the Chemical Bond*, 2nd ed. Ithaca: Cornell Univ. Press.
PORTER, D. J. T., VOET, J. G. & BRIGHT, H. J. (1973). *J. Biol. Chem.* **248**, 4400–4416.
STEWART, R. F., DAVIDSON, E. R. & SIMPSON, W. T. (1965). *J. Chem. Phys.* **42**, 3175–3187.
TAUSCHER, L., GHISLA, S. & HEMMERICH, P. (1973). *Helv. Chim. Acta*, **56**, 630–644.
WANG, M. & FRITCHIE, C. J. JR (1973). *Acta Cryst.* **B29**, 2040–2045.

Acta Cryst. (1978). B**34**, 828–836

The Crystal and Molecular Structure of *O*-Diazoacetyl-L-serine (Azaserine) at 21°C and 0°C: Model Error and Deterioration

BY A. FITZGERALD AND L. H. JENSEN

Department of Biological Structure, University of Washington, School of Medicine, Seattle, Washington 98195, *USA*

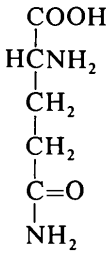
(*Received* 5 *April* 1977; *accepted* 6 *September* 1977)

The crystal structure of *O*-diazoacetyl-L-serine has been determined from the intensities of 1377 unique reflections measured with an automated diffractometer at 21(±2)°C. The compound crystallizes in space group $P2_1$ with $a = 15{\cdot}291$ (2), $b = 5{\cdot}251$ (1), $c = 9{\cdot}523$ (2) Å, and $\beta = 96{\cdot}52$ (2)° with $Z = 4$, two molecules per asymmetric unit. The structure was refined by full-matrix least-squares techniques to a conventional R value of 0·064 for the 1135 reflections greater than $2\sigma(I)$. The average deterioration, based on six monitor reflections, was ~40% with a range of ~30% over the course of the data collection. Substantial inconsistencies between the two molecules (*A* and *B*) in the asymmetric unit indicated serious problems with the model (equivalent bond lengths differing by as much as 0·099 Å, 4·5 times the standard deviation of the difference) which were thought to be related to the deterioration problem. A second data set (1352 unique reflections) was collected with a new crystal at 0 (±1)°C: $a = 15{\cdot}234$ (2), $b = 5{\cdot}2652$ (8), $c = 9{\cdot}527$ (2) Å, and $\beta = 96{\cdot}33$ (1)°, deterioration ~10% with a range of ~8%. The model refined to an R value of 0·039 for the 1216 reflections greater than $2\sigma(I)$ and precision and accuracy as judged by comparing bond lengths in molecules *A* and *B* were both substantially improved. The major difference between molecule *A*, which resembles glutamine, serine, and *O*-serine phosphate, and molecule *B* is a conformational one and is primarily due to the 76·1° difference in the χ^2[C(2)—C(3)—O(3)—C(4)] dihedral angle. The C—N and N—N distances in the diazoacetyl group are 1·312 and 1·128 Å, respectively, and the C—N—N angle is 178·7°.

Introduction

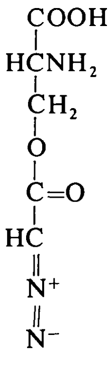
O-Diazoacetyl-L-serine (azaserine) is a glutamine antagonist which inhibits purine biosynthesis (Levenberg, Melnick & Buchanan, 1957). Buchanan and coworkers (French, Dawid, Day & Buchanan, 1963; Dawid, French & Buchanan, 1963; French, Dawid & Buchanan, 1963) have studied the reaction of azaserine with formyl-glycineamidoribonucleotide (FGAR) amidotransferase, one of the enzymes in the biosynthetic pathway, in detail. Apparently, azaserine resembles glutamine structurally and, therefore, fits into the active site of the enzyme easily. Once in the active site, azaserine alkylates an 'active' sulfhydryl group in the enzyme. A related compound, 6-diazo-5-oxo-L-norleucine (DON), resembles glutamine and inhibits FGAR amidotransferase in a manner similar to azaserine (Levenberg, Melnick & Buchanan, 1957). DON also inhibits glutamine phosphoribosyl pyrophosphate amidotransferase, another enzyme in the pathway, although azaserine is relatively inactive toward this enzyme (Hill & Bennett, 1969). Glutamine serves as an amino group donor in amination reactions catalyzed by both enzymes in the purine biosynthetic pathway (Hartman & Buchanan, 1959).

The formulas of glutamine, azaserine and DON are shown as (I), (II), and (III), respectively. The crystal



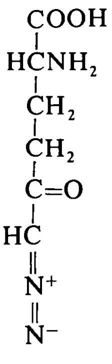
$$\begin{array}{l}\mathrm{COOH}\\ \;|\\ \mathrm{HCNH_2}\\ \;|\\ \mathrm{CH_2}\\ \;|\\ \mathrm{CH_2}\\ \;|\\ \mathrm{C{=}O}\\ \;|\\ \mathrm{NH_2}\end{array}$$

(I)



$$\begin{array}{l}\mathrm{COOH}\\ \;|\\ \mathrm{HCNH_2}\\ \;|\\ \mathrm{CH_2}\\ \;|\\ \mathrm{O}\\ \;|\\ \mathrm{C{=}O}\\ \;|\\ \mathrm{HC}\\ \;\|\\ \mathrm{N^+}\\ \;\|\\ \mathrm{N^-}\end{array}$$

(II)



$$\begin{array}{l}\mathrm{COOH}\\ \;|\\ \mathrm{HCNH_2}\\ \;|\\ \mathrm{CH_2}\\ \;|\\ \mathrm{CH_2}\\ \;|\\ \mathrm{C{=}O}\\ \;|\\ \mathrm{HC}\\ \;\|\\ \mathrm{N^+}\\ \;\|\\ \mathrm{N^-}\end{array}$$

(III)

structure of glutamine has been reported (an X-ray study by Cochran & Penfold, 1952; and a neutron study by Koetzle, Frey, Lehmann & Hamilton, 1973), and we report here the structure of azaserine.

The initial data set for azaserine collected at room temperature (~21 °C) was plagued by deterioration problems. The average deterioration as calculated from the monitor reflections was ~40% with a range of ~30%. Since there were serious problems in the final model based on substantial inconsistencies between the two molecules in the asymmetric unit, a second data set was collected at a lower temperature (~0 °C) with a second crystal. We felt that the reduced deterioration observed at the lower temperature would diminish substantially the errors in the model from the first data set.

Experimental

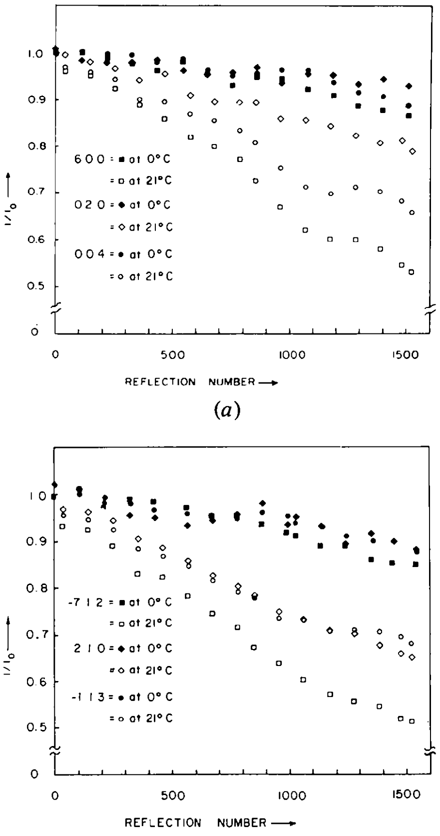
Azaserine (PL Biochemicals) was recrystallized by diffusing ethanol into an aqueous solution (~10 mg $\mathrm{cm^{-3}}$) at 4 °C in a small weighing bottle sealed with silicone grease to prevent loss of solvent. Very thin colorless platelets elongated in the **b** direction appeared in one to two weeks and grew slowly for three to six months. Several crystals were used in the initial photographic work to determine the space group and approximate unit-cell dimensions. Weissenberg and precession photographs showed only the extinction $0k0$ for k odd, indicating space group $P2_1$ or $P2_1/m$, but the reported optical activity of the azaserine sample ruled out the latter. Solution of the structure in space group $P2_1$ verified the choice of the space group. A density measurement indicated the presence of four molecules in the unit cell or two molecules per asymmetric unit.

Table 1. *Crystal data*

$C_5H_7N_3O_4$	$d_m = 1{\cdot}49\ (2)\ \mathrm{g\ cm^{-3}}$
Space group $P2_1$	$d_c = 1{\cdot}51$
$Z = 4$	$F(000) = 360$
$M_r = 173{\cdot}127$	$\mu = 5{\cdot}08\ \mathrm{cm^{-1}}$

	$t = 21\ (\pm 2)$°C	$t = 0\ (\pm 1)$°C
a (Å)	15·291 (2)	15·234 (2)
b (Å)	5·251 (1)	5·2652 (8)
c (Å)	9·523 (2)	9·527 (2)
β (°)	96·52 (2)	96·33 (1)
V (Å^3)	759·7 (4)	759·5 (4)
Crystal dimensions (mm)	0·02 × 0·63 × 0·09	0·02 × 0·63 × 0·06
$\sin\theta_{\max}/\lambda$ (Å^{-1})	0·578	0·574
Total data	1377	1352
Data > $2\sigma(I)$	1135	1216

The crystal used for collecting the 21 °C data set (RTAS, hereafter), approximately 0·02 × 0·63 × 0·09 mm, was mounted with **b** nearly coincident with the φ axis of a computer-controlled, four-circle diffractometer. Intensity data were collected in three shells to a 2θ limit of 126° with Ni-filtered Cu $K\alpha$ radiation ($\lambda = 1{\cdot}5418$ Å). The scan rate was 2° $\mathrm{min^{-1}}$, the scan width was $1{\cdot}2° + \Delta$, where Δ is the calculated separation of the $K\alpha$ doublet, and backgrounds were



(*a*)

(*b*)

Fig. 1. Plots showing the relative changes in intensity of (*a*) the axial and (*b*) the nonaxial monitor reflections over the course of the data collection for both RTAS and LTAS.

counted for 20 s on each side of the reflection. Of the 1377 unique reflections scanned to the sin θ/λ limit of 0·578 Å^{-1}, 1135 were greater than $2\sigma_I$ [$\sigma_I^2 = P + (B_1 + B_2)(T_S/2T_B)^2 + (kP)^2$, where P, B_1 and B_2 are the peak and background counts, T_S and T_B are the scan and individual background counting times, and k was set at 0·025 based on variations in individual monitor reflections].

The crystal used for the 0 °C data set (LTAS, hereafter), with approximate dimensions 0·02 × 0·63 × 0·06 mm, was mounted in the same manner as the RTAS crystal. Data were collected in four shells with the same procedure used for RTAS except the scan width was increased to 1·4°. Of the 1352 unique reflections scanned to a sin θ/λ limit of 0·574 Å^{-1}, 1216 were greater than $2\sigma_I$ (based on the same criteria as with RTAS). The temperature was maintained at 0 (±1)°C by blowing a cold dry stream of air at ~2 p.s.i. directly on the crystal.

In both cases, unit-cell dimensions were calculated from a least-squares fit of the angular settings 2θ, ω and χ for 21 reflections automatically centered by the instrument. Table 1 lists both sets of unit-cell dimensions and other pertinent data.

Six monitor reflections were measured every 100 reflections and their intensity ratios are plotted in Fig. 1. Curves were fitted to the data by least squares and the intensities corrected to zero time with the average deterioration curve. Owing to the small size of the crystals, no corrections were made for extinction, absorption or coincidence loss.

Appropriate Lorentz and polarization corrections were applied during data reduction. Scattering factors and anomalous-scattering parameters for all atoms except H were taken from *International Tables for X-ray Crystallography* (1974); all atoms were assumed to be in the zero ionization state. The H scattering factors of Stewart, Davidson & Simpson (1965) were used.

Structure solution and refinement

The RTAS structure was solved by use of the *MULTAN* program (Main, Woolfson & Germain, 1971). Some 1500 Σ_2 relationships were generated utilizing the 300 largest E values ($E_{\text{min}} = 1{\cdot}23$). The origin was specified by reflections $11\bar{3}$ ($E = 3{\cdot}20$), 136 ($E = 2{\cdot}82$) and 147 ($E = 1{\cdot}78$) and the enantiomorph was fixed by the latter. In addition, the $34\bar{3}$ and $23\bar{6}$ reflections ($E = 3{\cdot}79$, $1{\cdot}99$) were permuted ($\pm\pi/4$, $\pm 3\pi/4$) to ensure that the starting phases were within $\pi/4$ of their true values. Weighted Karle R's varied from 0·404 to 0·445 for the 32 tangent-refined sets of phases that were generated. The E map based on the set with the highest absolute figure of merit and a Karle R of 0·410 showed 23 of the 24 nonhydrogen atoms. A structure factor calculation followed by an F_o synthesis revealed the position of the remaining nonhydrogen atom in the structure.

The model was refined by least squares (unit weights) with isotropic temperature factors, the residual R ($= \Sigma||F_o| - |F_c||/\Sigma|F_o|$) decreasing from 0·363 to 0·145 in four cycles of full-matrix refinement. Two additional refinement cycles with anisotropic thermal parameters reduced R to 0·076. A difference synthesis revealed the positions of the 14 H atoms in the asymmetric unit. With the H atoms included in the model but not refined, R decreased from 0·070 to 0·056 in two cycles. In two additional cycles (blocked with one molecule per block and all parameters refined) R decreased to 0·053 with an average Δ/σ of 0·27, the largest being 1·76 for one of the H atom parameters.

Least-squares refinement of the RTAS model with a statistical weighting scheme [$w_F = 1/(\sigma_F)^2$] was unsatisfactory in the sense that convergence could not be achieved if the H atom parameters were allowed to vary. When they were fixed, however, the refinement converged to an R of 0·064. The maximum shift over error in the final pass was 0·09 with an average Δ/σ of

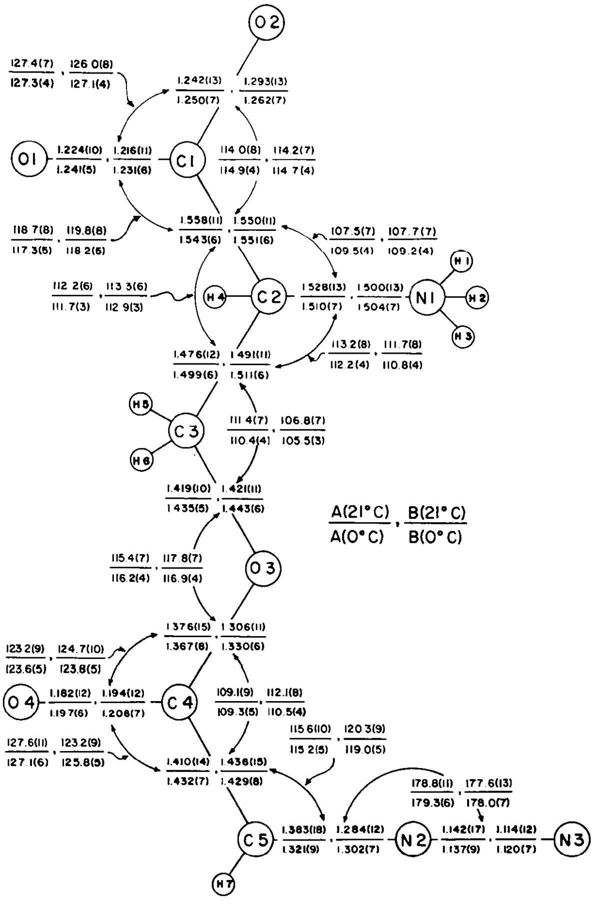


Fig. 2. Bond lengths (Å) and angles (°) for molecules *A* and *B* at 0 and 21 °C. Distances and angles involving hydrogen atoms are not shown.

Table 2. *Atomic parameters*

Thermal parameters for the heavy atoms are defined by the expression

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

Values of U_{ij} and U have been multiplied by 10^2. For hydrogen atoms the thermal parameter is defined by $\exp(-8\pi^2 U^2 \sin^2 \theta/\lambda^2)$. For each atom the first line lists the RTAS parameters, the second line the LTAS parameters.

	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O(1)A*	0·42329 (35)	0·63854	0·12403 (46)	9·0 (4)	4·2 (4)	2·4 (3)	−0·5 (4)	1·3 (3)	−0·1 (3)
	0·42323 (18)	0·63854	0·12392 (24)	6·6 (2)	3·5 (2)	2·1 (1)	−0·6 (2)	1·0 (1)	−0·3 (1)
O(2)A	0·39415 (41)	0·2395 (14)	0·17636 (52)	12·3 (6)	1·6 (3)	3·8 (3)	−0·2 (4)	2·1 (3)	−0·6 (3)
	0·39334 (21)	0·2365 (8)	0·17608 (28)	8·6 (3)	2·2 (2)	3·3 (2)	0·2 (2)	1·2 (2)	−0·4 (2)
O(3)A	0·22129 (39)	0·6182 (17)	0·21158 (63)	8·4 (5)	8·1 (6)	5·6 (4)	2·1 (5)	0·1 (3)	0·2 (4)
	0·22082 (19)	0·6185 (9)	0·21278 (32)	5·4 (2)	8·1 (3)	4·8 (2)	1·2 (2)	0·0 (2)	−0·8 (2)
O(4)A	0·17519 (44)	0·9460 (16)	0·33685 (55)	15·9 (6)	5·2 (5)	4·0 (3)	1·1 (5)	0·3 (4)	−1·0 (4)
	0·17481 (23)	0·9480 (9)	0·33822 (30)	12·2 (3)	4·4 (2)	4·5 (2)	0·9 (3)	−0·1 (2)	−0·6 (2)
N(1)A	0·39299 (43)	0·8254 (17)	0·37261 (57)	8·8 (5)	4·2 (5)	2·2 (3)	0·1 (4)	1·1 (3)	−0·8 (3)
	0·39159 (22)	0·8226 (9)	0·37372 (30)	5·5 (2)	3·1 (2)	2·1 (2)	0·3 (2)	0·4 (2)	−0·5 (2)
N(2)A	0·08689 (60)	1·1422 (25)	0·09360 (92)	8·1 (8)	11·3 (9)	4·7 (5)	−3·5 (7)	0·6 (5)	1·6 (8)
	0·08820 (31)	1·1313 (13)	0·09340 (48)	6·4 (4)	8·4 (4)	4·7 (3)	−1·5 (3)	−0·8 (2)	1·5 (3)
N(3)A	0·04710 (70)	1·3255 (22)	0·09523 (119)	16·0 (10)	6·8 (7)	10·1 (8)	1·7 (8)	−1·1 (7)	2·0 (7)
	0·04855 (34)	1·3141 (12)	0·09499 (58)	10·4 (5)	7·4 (4)	8·6 (4)	−0·1 (4)	−1·8 (3)	1·7 (4)
C(1)A	0·39683 (59)	0·4726 (21)	0·19894 (73)	8·2 (7)	4·2 (6)	1·6 (4)	0·0 (6)	0·1 (4)	0·3 (5)
	0·39707 (29)	0·4699 (11)	0·20029 (39)	5·1 (3)	3·7 (3)	1·8 (2)	0·4 (3)	−0·3 (2)	−0·3 (2)
C(2)A	0·36293 (55)	0·5519 (18)	0·34080 (73)	8·0 (6)	2·2 (5)	2·7 (4)	0·0 (4)	0·8 (4)	−0·3 (4)
	0·36429 (28)	0·5514 (10)	0·34110 (39)	5·5 (3)	2·4 (3)	2·4 (2)	0·3 (2)	0·3 (2)	−0·3 (2)
C(3)A	0·26693 (53)	0·5181 (21)	0·33764 (80)	7·4 (6)	4·8 (6)	5·1 (5)	−0·7 (6)	1·1 (5)	0·1 (5)
	0·26662 (28)	0·5139 (11)	0·33933 (42)	5·6 (3)	4·4 (3)	4·6 (3)	−0·7 (3)	1·0 (2)	−0·3 (3)
C(4)A	0·17762 (63)	0·8440 (24)	0·22668 (99)	8·4 (7)	6·6 (8)	4·8 (6)	−2·4 (7)	0·1 (6)	0·2 (6)
	0·17713 (30)	0·8420 (13)	0·22751 (52)	4·7 (3)	5·4 (4)	5·3 (3)	−1·4 (3)	0·2 (3)	0·6 (3)
C(5)A	0·13444 (60)	0·9181 (27)	0·09427 (106)	8·3 (8)	9·0 (10)	6·0 (6)	1·8 (7)	1·4 (6)	0·1 (7)
	0·13383 (31)	0·9180 (14)	0·09299 (52)	5·9 (4)	8·0 (5)	5·1 (3)	1·5 (4)	−0·3 (3)	0·4 (3)
O(1)B	0·43000 (32)	0·2420 (15)	0·60886 (44)	7·7 (4)	3·7 (3)	2·6 (3)	−0·7 (3)	1·1 (3)	−0·1 (3)
	0·43026 (16)	0·2394 (8)	0·60908 (23)	5·1 (2)	3·8 (2)	2·1 (1)	−0·7 (2)	0·4 (1)	0·1 (2)
O(2)B	0·35533 (38)	−0·1045 (15)	0·66158 (52)	11·5 (5)	2·8 (4)	3·9 (3)	−1·7 (4)	1·6 (3)	−1·1 (3)
	0·35545 (19)	−0·1055 (8)	0·66258 (27)	7·7 (2)	2·9 (2)	3·2 (2)	−1·1 (2)	1·0 (2)	−1·0 (2)
O(3)B	0·24408 (35)	0·4503 (16)	0·74694 (53)	6·4 (4)	5·6 (4)	5·5 (3)	0·2 (4)	0·6 (3)	1·8 (4)
	0·24405 (18)	0·4471 (9)	0·74737 (27)	4·8 (2)	5·2 (2)	5·0 (2)	0·1 (2)	0·7 (1)	1·3 (2)
O(4)B	0·10793 (39)	0·3977 (18)	0·80027 (68)	8·6 (5)	9·7 (7)	12·6 (6)	2·0 (5)	3·8 (4)	5·9 (6)
	0·10616 (21)	0·3918 (10)	0·80183 (36)	6·1 (2)	10·2 (4)	10·6 (3)	1·0 (3)	2·8 (2)	5·1 (3)
N(1)B	0·42240 (40)	0·4754 (18)	0·85269 (53)	8·3 (5)	4·1 (4)	1·8 (3)	−0·5 (4)	0·2 (3)	−0·8 (4)
	0·42165 (20)	0·4726 (9)	0·85358 (28)	5·5 (2)	2·9 (2)	2·1 (2)	−0·5 (2)	0·2 (2)	−0·6 (2)
N(2)B	0·05843 (51)	0·7973 (21)	0·62363 (84)	8·4 (6)	7·7 (8)	7·4 (6)	0·7 (6)	0·0 (6)	2·7 (6)
	0·05793 (29)	0·7945 (11)	0·62260 (42)	7·3 (3)	7·1 (4)	6·5 (3)	0·8 (3)	0·3 (3)	1·7 (3)
N(3)B	−0·01198 (56)	0·8529 (25)	0·59948 (96)	11·1 (7)	9·0 (8)	12·7 (8)	1·0 (8)	−0·5 (7)	3·0 (7)
	−0·01288 (31)	0·8517 (14)	0·59711 (51)	9·0 (4)	10·3 (5)	12·9 (4)	2·1 (4)	0·0 (4)	4·0 (4)
C(1)B	0·38813 (57)	0·1200 (21)	0·68672 (77)	7·9 (6)	4·8 (6)	1·9 (4)	0·7 (6)	0·8 (4)	−0·3 (4)
	0·38791 (28)	0·1126 (11)	0·68689 (41)	4·8 (3)	3·8 (3)	2·1 (2)	0·5 (3)	0·0 (2)	−0·3 (2)
C(2)B	0·37214 (53)	0·2305 (19)	0·83254 (71)	9·8 (7)	2·5 (5)	2·5 (4)	−1·0 (5)	0·1 (4)	0·3 (4)
	0·37284 (26)	0·2248 (10)	0·83285 (36)	5·7 (3)	2·3 (2)	2·1 (2)	−0·4 (2)	0·2 (2)	0·1 (2)
C(3)B	0·27707 (48)	0·2661 (20)	0·84876 (74)	5·7 (6)	4·5 (6)	4·7 (5)	−0·9 (5)	1·0 (4)	0·8 (5)
	0·27627 (25)	0·2593 (11)	0·85076 (38)	4·6 (3)	4·0 (3)	3·4 (2)	−0·2 (3)	0·7 (2)	0·3 (2)
C(4)B	0·16039 (59)	0·5063 (22)	0·73817 (90)	6·0 (6)	6·2 (8)	5·9 (6)	−0·1 (6)	1·4 (5)	1·3 (6)
	0·15876 (31)	0·5062 (12)	0·74013 (45)	5·0 (3)	5·5 (4)	4·3 (3)	0·1 (3)	0·8 (2)	0·2 (3)
C(5)B	0·13868 (56)	0·7237 (23)	0·64953 (90)	6·8 (7)	5·9 (7)	6·5 (6)	0·2 (6)	0·5 (5)	0·4 (6)
	0·13955 (30)	0·7205 (13)	0·64992 (47)	5·3 (3)	5·6 (4)	5·6 (3)	0·5 (3)	0·6 (3)	0·2 (3)

	x	y	z	U		x	y	z	U
H(1)A†	0·478 (4)	0·788 (13)	0·379 (5)	5·3 (20)	H(1)B	0·495 (3)	0·450 (12)	0·844 (5)	3·9 (14)
	0·471 (2)	0·810 (9)	0·385 (3)	11·6 (15)		0·489 (2)	0·466 (9)	0·848 (3)	9·5 (14)
H(2)A	0·381 (3)	0·838 (11)	0·433 (5)	7·5 (29)	H(2)B	0·422 (3)	0·518 (10)	0·926 (4)	4·5 (20)
	0·372 (2)	0·846 (8)	0·450 (3)	7·2 (12)		0·417 (2)	0·526 (7)	0·940 (3)	4·8 (11)
H(3)A	0·358 (7)	0·955 (29)	0·292 (11)	13·8 (34)	H(3)B	0·386 (4)	0·571 (14)	0·801 (6)	6·7 (28)
	0·365 (2)	0·922 (8)	0·305 (4)	9·1 (14)		0·388 (2)	0·573 (8)	0·791 (3)	7·4 (12)
H(4)A	0·394 (6)	0·403 (23)	0·406 (9)	9·3 (30)	H(4)B	0·391 (3)	0·130 (11)	0·879 (5)	4·5 (25)
	0·396 (2)	0·448 (7)	0·419 (3)	5·5 (11)		0·399 (2)	0·126 (7)	0·893 (3)	5·4 (11)
H(5)A	0·253 (4)	0·330 (14)	0·326 (6)	7·2 (23)	H(5)B	0·239 (4)	0·089 (13)	0·827 (6)	7·2 (23)
	0·252 (2)	0·329 (8)	0·332 (3)	7·3 (12)		0·238 (2)	0·066 (7)	0·824 (3)	6·3 (11)
H(6)A	0·248 (3)	0·566 (13)	0·434 (6)	5·8 (15)	H(6)B	0·269 (4)	0·386 (14)	0·947 (6)	6·5 (17)
	0·246 (2)	0·585 (7)	0·432 (3)	5·0 (10)		0·266 (2)	0·385 (7)	0·950 (3)	6·4 (11)

Table 2 (*cont.*)

H(7)*A*	0·136 (5)	0·841 (18)	0·012 (7)	10·7 (27)	H(7)*B*	0·189 (3)	0·824 (12)	0·624 (5)	6·1 (26)
	0·136 (2)	0·831 (8)	0·004 (3)	9·7 (14)		0·183 (2)	0·830 (8)	0·608 (3)	8·7 (14)

* The y coordinate of O(1)*A* was fixed at 0·63854 in the refinement. The e.s.d.'s of the atomic coordinates are shown to more than one place for purposes of comparison. Bond lengths and angles based on the listed positional parameters (y coordinate to four places) may be slightly but insignificantly different from those shown in Fig. 2 (calculated with the y coordinate carried to five places) due to round-off errors.

† Hydrogen atom parameters were varied with unit weights in the RTAS refinement. The LTAS data were such that hydrogen atom parameters could be varied in the refinement with statistical weights. E.s.d.'s shown in parentheses are carried to an extra place for purposes of comparison where necessary.

0·02. The weighted residual R_w $[=(\Sigma w\|F_o| - |F_c\|^2/\Sigma w|F_o|^2)^{1/2}]$ was 0·040 and R_{F^2} = 0·072 for the 1135 reflections greater than $2\sigma_I$, and the goodness of fit $\{=[\Sigma w\|F_o| - |F_c\|^2/(m-n)]^{1/2}\}$ is 3·49.

Refinement with the LTAS data set was initiated with unit weights and the model derived from the RTAS refinement. Two cycles of full-matrix refinement of this model (H atom parameters fixed) reduced R from 0·118 to 0·039. The H atom parameters were then refined in two cycles while holding all other atom parameters constant, reducing R to 0·036.

Further refinement of the anisotropic model for the LTAS structure was with statistical weights $[w_F = 1/(\sigma_F)^2]$. Two cycles in which all parameters except those for H were varied were followed by two cycles in which only H atom parameters were varied. Refinement was terminated after two final cycles in which all atomic parameters except those for H were varied. The maximum shift over error in the final pass was 0·08 with an average Δ/σ of 0·03. The conventional R was 0·039, the weighted residual R_w was 0·025, and R_{F^2} = 0·045 for the 1216 reflections greater than $2\sigma_I$. The goodness of fit is 2·61, a substantial decrease from the value obtained for the RTAS data set.

At the conclusion of the refinement a ΔF synthesis showed no significant peaks.

Results and discussion

Discussion of the details of the crystal and molecular structure of azaserine will be based on the model

Table 3. *Summary of distances* (Å) *and dihedral angles* (°) *for azaserine and related molecules*

IUPAC designation	Atoms involved in bond or plane	L-Azaserine *A*(a)	L-Azaserine *B*(a)	L-Glutamine(b)	L-Serine(c)	L-*O*-Serine phosphate(d)
	C(1)–O(1)	1·241	1·231	1·238	1·248	1·206
	C(1)–O(2)	1·250	1·262	1·260	1·251	1·299
	C(1)–C(2)	1·543	1·551	1·537	1·527	1·532
	C(2)–N(1)	1·510	1·504	1·496	1·488	1·477
	C(2)–C(3)	1·499	1·511	1·525	1·519	1·517
	C(3)–O(3)	1·435	1·443	–	1·413	1·433
	C(5)–N(2)	1·321	1·302	–	–	–
	N(2)–N(3)	1·137	1·120	–	–	–
ψ^1	N(1)–C(2)–C(1)–O(1)	−13·6	−4·1	−15·6	+2·1	−2·6
ψ^2	N(1)–C(2)–C(1)–O(2)	+166·9	+174·8	+167·0	−179·3	+178·7
χ^1	N(1)–C(2)–C(3)–O(3)	+75·0	+58·3	+66·1	+70·2	+61·3
χ^2	C(2)–C(3)–O(3)–C(4)	−105·5	+178·4	+175·5	–	+153·2
$\chi^{3,1}$	C(3)–O(3)–C(4)–C(5)	−178·9	+170·9	+167·2	–	–
$\chi^{3,2}$	C(3)–O(3)–C(4)–O(4)	−1·0	−9·2	−13·3	–	–

(*a*) This work: atom labels for distances and angles are for azaserine and appropriate substitutions must be made for other compounds in the table. Torsion angles were calculated according to the IUPAC convention (IUPAC–IUB Commission on Biochemical Nomenclature, 1970): estimated standard deviations: σ(C–O) ~ 0·007 Å, σ(C–N) ~ 06007 Å, σ(C–C) ~ 0·006 Å and σ(dihedral angles) ~ 0·5°.

(*b*) Work of Koetzle *et al.* (1973); estimated standard deviations: σ(C–O) ~ 0·003 Å, σ(C–N) ~ 0·002 Å, σ(C–C) ~ 0·003 Å and σ(dihedral angles) ~ 0·3°.

(*c*) Work of Frey, Lehmann, Koetzle & Hamilton (1973); estimated standard deviations: σ(C–O) ~ 0·003 Å, σ(C–N) ~ 0·002 Å, σ(C–C) ~ 0·003 Å and σ(dihedral angles) ~ 0·3°.

(*d*) Work of Sundaralingam & Putkey (1970); estimated standard deviations: σ(C–O) ~ 0·005 Å, σ(C–N) ~ 0·005 Å, σ(C–C) ~ 0·005 Å and σ(dihedral angles) ~ 0·4°.

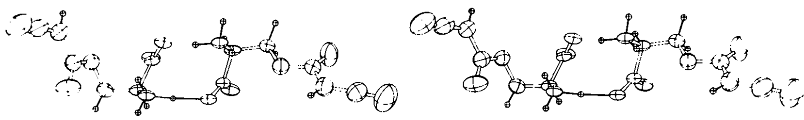


Fig. 3. Stereoscopic view of the asymmetric unit. Hydrogen atoms have been assigned isotropic B values of 0·5 Å^2 for clarity.

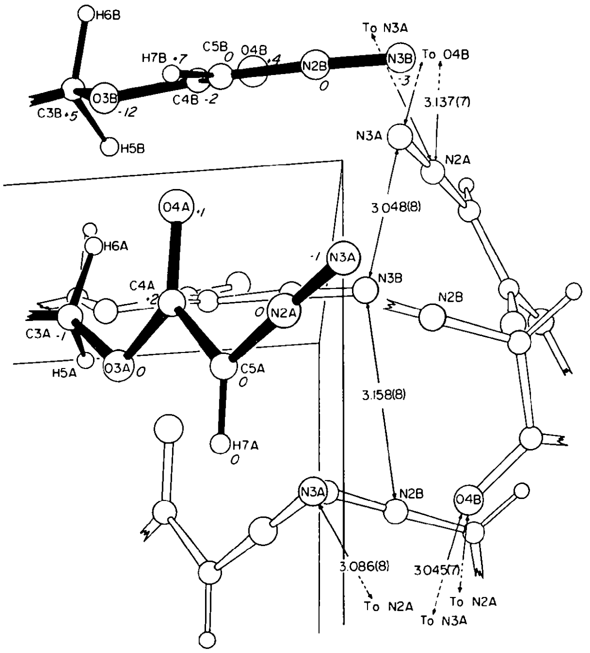


Fig. 4. Section illustrating details of molecular geometry in the region of the van der Waals interactions. Deviations of atoms from the least-squares planes through H(7), C(3), C(4), C(5), O(3), O(4), N(2), and N(3) are indicated (italicized numerals) in Å × 10^2.

derived from the LTAS data. Discussion of deterioration and errors in the model will utilize the results of both the LTAS and RTAS data sets.*

Molecular structure

The bond distances and angles and the numbering of the atoms are shown in Fig. 2. Atom positions and thermal parameters are listed in Table 2. Fig. 3 is an *ORTEP* plot (Johnson, 1965) of the asymmetric unit illustrating the thermal ellipsoids. Both molecules in the asymmetric unit (*A* and *B*) exist as zwitterions and are compared to several related molecules in Table 3. Glutamine, serine and *O*-serine phosphate adopt conformations very similar to that of molecule *B*, but molecule *A* is substantially different. The major conformational difference between *A* and *B* (and *A* and glutamine, serine, and *O*-serine phosphate) is due to the 76·1° difference in the dihedral angle C(2)—C(3)—O(3)—C(4).

The conformation of molecule *B* is such that O(4*B*) occupies a semistaggered position between H(5*B*) and H(6*B*) with O···H distances of 2·63 and 2·68 Å respectively. Molecule *A*, on the other hand, exists in a less favorable conformation such that O(4*A*) has a nearly eclipsed relationship with H(6*A*), and the O···H distance of 2·33 Å is somewhat less than the sum of the van der Waals radii (estimated to be > 2·6 Å, Bondi, 1964). These conformational details are illustrated in Fig. 4.

Bond lengths and angles in the carboxylate portion of the two azaserine molecules compare quite well with the other entries in Table 3 except for L-*O*-serine phosphate in which one of the carboxyl O atoms is protonated. Bond lengths involving C(2) compare well with similar bonds in amino acids and the C(3)—O(3) distances are in good agreement with those in L-serine and L-*O*-serine phosphate (Frey, Lehmann, Koetzle & Hamilton, 1973; Sundaralingam & Putkey, 1970). The C(4)—O(3) distance of 1·330 Å in molecule *B* agrees well with similar bonds observed in recent structures, for example, vitamin A acetate (1·327 Å) and dimethyl terephthalate (1·331 Å) as reported by Oberhänsli, Wagner & Isler (1974) and Brisse & Pérez (1976) respectively. The C(4)—O(3) bond length of 1·367 Å observed in molecule *A* differs substantially from the 1·330 Å value in molecule *B*, but it is unclear if this deviation can be attributed to deterioration (~10%) which occurred during the LTAS data collection or is associated with the semi-eclipsed conformation. In addition, the C(2)—C(3)—O(3) angles differ significantly between molecules *A* and *B*, the angle in molecule *A* being 4·9° larger than that observed for molecule *B*. The carbonyl distances [C(4)—O(4)] and angles are reasonable but the C(4)—C(5) bond lengths (1·432 and 1·429 Å for molecules *A* and *B* respectively) are substantially shorter than the 1·486 Å observed for the corresponding sp^2—sp^2 bond in dimethyl terephthalate. This is only slightly less than the 1·495 Å sp^2—sp^3 bond length observed in the acetate group of vitamin A acetate. The extensive resonance system involved in the diazoacetyl group is responsible for the short C(4)—C(5) bond length.

---

* A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 33013 (15 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

The C(5)—N(2) distance [1·321 (9), 1·302 (7) Å respectively] is in agreement with the 1·34 (5) Å observed in diazomethane by electron diffraction methods (Boersch, 1935). The N(2)—N(3) distance [1·137 (9), 1·120 (7) Å respectively] is essentially identical to the 1·13 (4) Å distance found for diazomethane. Values of 1·32 and 1·12 Å have been determined for diazomethane by microwave spectroscopy (Cox, Thomas & Sheridan, 1958). We are unaware of any other crystal structure study of an N—N bond of this type.

The C(4)—C(5)—N(2) angles differ significantly between molecules *A* and *B*; the difference is 3·8°. Molecule *B* assumes a conformation such that the angle is very close to the theoretical value of 120°. The C(5)—N(2)—N(3) group is essentially linear with angles of 179·3 (6) and 178·0 (7)° in molecules *A* and *B* respectively.

Deviations from the least-squares planes through atoms C(1), C(2), O(1) and O(2) are shown in Fig. 5. The molecular fragment consisting of atoms H(7), C(3), C(4), C(5), N(2), N(3), O(3) and O(4) is planar. Deviations from the least-squares planes are shown in Fig. 4.

Intramolecular distances and angles for C—H bonds are reasonable. The N—H distances and angles (Table 4) are also reasonable, except for the N(1*A*)—H(1*A*) distance which is sensitive to deterioration [*cf.* 1·31 (5) Å for the RTAS data and 1·21 (4) Å for the LTAS data]. The mean value for five N—H distances [excluding N(1*A*)—H(1*A*)] is 0·91 Å with a standard deviation of the mean of 0·04 Å, in good agreement with N—H bond lengths as determined in other X-ray structure analyses (Hamilton & Ibers, 1968).

Table 4. N—H *bond lengths* (Å) *and* H—N—H *and* H—N—C *bond angles* (°)

	A(21°C)	*A*(0°C)	*B*(21°C)	*B*(0°C)
H(1)—N(1)	1·31 (5)	1·21 (4)	1·14 (5)	1·04 (3)
H(2)—N(1)	0·63 (5)	0·82 (3)	0·73 (4)	0·88 (3)
H(3)—N(1)	1·11 (12)	0·90 (4)	0·86 (6)	0·91 (3)
H(1)—N(1)—C(2)	98 (3)	103 (2)	112 (3)	116 (3)
H(1)—N(1)—H(2)	112 (5)	112 (3)	103 (4)	104 (3)
H(1)—N(1)—H(3)	121 (6)	118 (3)	128 (5)	118 (3)
H(2)—N(1)—C(2)	100 (5)	102 (3)	109 (4)	108 (2)
H(2)—N(1)—H(3)	113 (8)	112 (3)	107 (6)	110 (3)
H(3)—N(1)—C(2)	109 (7)	108 (3)	98 (5)	101 (2)

Although azaserine is considerably less active as an alkylating agent and more stable than diazomethane, these differences in reactivity are not reflected as substantial differences in the bond lengths observed in the two compounds. It appears that within the series diazomethane, DON, and azaserine, compound stability is increased on extending the π-overlap system and activity as an alkylating agent is decreased.

Crystal structure and hydrogen bonding

The azaserine crystal structure contains alternating sheets of hydrogen-bonding and van der Waals interactions. Details of the hydrogen-bonding scheme are summarized in Table 5 and a graphic representation is given in Fig. 5. H(1*B*) is involved in a bifurcated hydrogen bond with O(1*A*) of one molecule and O(2*A*) of the molecule in the next unit cell along **b**, although the H(1*B*)···O(2*A*) interaction must be regarded as very weak. The N—H—O angles vary from 130 to 175°, within the ranges observed in amino acid and peptide structures. It is interesting that H(1*A*) is involved in the only hydrogen bond that O(1*B*) participates in and that the N(1*A*)—H(1*A*) distance is substantially longer than expected, as noted above. Presumably, the N(1*A*)—H(1*A*) bond is weaker than a normal N—H covalent bond and the H(1*A*)···O(1*B*) distance of 1·54 (4) Å indicates substantial H···O interaction.

There are several intermolecular distances in the *bc* plane that are less than or approximately equal to the sums of the van der Waals radii proposed by Bondi (1964) who suggested that the radius of N≡N normal to the bond may be as much as 1·7 Å. The van der Waals interactions are illustrated in Fig. 4.

Table 5. *Hydrogen-bonding distances and angles*

A—H···*B*	*A*···*B*	∠*A*—H···*B*	H···*B*	Symmetry† operation
N(1*A*)—H(1*A*)···O(1*B*)*	2·736 (4) Å	169 (4)°	1·54 (4) Å	(i)
N(1*A*)—H(2*A*)···O(2*B*)	2·890 (4)	166 (3)	2·09 (3)	(ii)
N(1*A*)—H(3*A*)···O(2*A*)	2·882 (6)	140 (4)	2·14 (4)	(ii)
N(1*B*)—H(1*B*)···O(1*A*)	2·935 (4)	128 (3)	2·18 (4)	(iii)
N(1*B*)—H(1*B*)···O(2*A*)	3·183 (5)	140 (3)	2·31 (4)	(i)
N(1*B*)—H(2*B*)···O(1*A*)	2·717 (4)	173 (3)	1·84 (3)	(iv)
N(1*B*)—H(3*B*)···O(2*B*)	2·976 (6)	158 (3)	2·12 (4)	(ii)

* O(1*B*) takes part in only one hydrogen bond while O(1*A*), O(2*A*) and O(2*B*) all participate in two.

† Symmetry operations relating atom *B* to its original position in the unit cell are (i) $1-x, y+\frac{1}{2}, 1-z$; (ii) $x, y+1, z$; (iii) $1-x, y-\frac{1}{2}, 1-z$; (iv) $x, y, z+1$.

A stereoscopic view illustrating the hydrogen bonding and packing in the crystal is shown in Fig. 6.

Effects of deterioration on molecular parameters

As noted above for the RTAS data set, the decrease in the intensities of the six monitor reflections was ~40% relative to the initial values, and the rate of decrease of the individual reflections varies widely (see Fig. 1). Different deterioration rates could result from disordering of the structure (Blake & Phillips, 1962) and from structural change. Whatever the situation for azaserine, it is clear that correcting for deterioration on the basis of an average curve for the monitor reflections is but a rough approximation and is likely to lead to an unreliable model. Deterioration being systematic with time presumably introduces systematic error, and, although the error may have a random component which will increase the estimated standard deviations, the systematic component will not be included in the standard deviations. Thus, they will be underestimates of the true error.

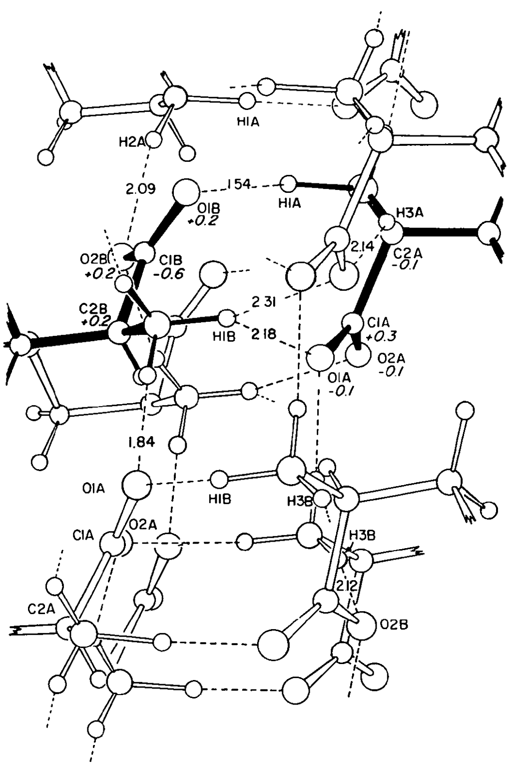


Fig. 5. Section illustrating details of the hydrogen-bonding scheme. Deviations of atoms from the least-squares planes through O(1), O(2), C(1) and C(2) are indicated (italicized numerals) in Å × 10^2.

The r.m.s. difference $[= (\Sigma \Delta_i^2/n)^{1/2}]$ in bond length between molecules *A* and *B* for the RTAS determination is 0·043 Å, considerably exceeding the r.m.s. standard deviation of 0·018 Å. Three bonds [C(1)–O(2), C(4)–O(3) and C(5)–N(2)] account for most of the discrepancy between the two sets of bond lengths (see Fig. 2), but, if one were to apply strictly statistical criteria, these bonds would be said to differ significantly (Cruickshank, 1949).

The precision of the LTAS analysis is much improved, and the agreement in bond lengths between molecules *A* and *B* is much better, the r.m.s. difference being 0·016 Å compared with a value of 0·010 Å for the r.m.s. standard deviation. Two of the three bonds noted above do not now differ significantly in length, but the C(4)–O(3) lengths still differ by 3·7 times the standard deviation of the difference. Although the difference would be considered significant on the basis of statistical criteria, one should note that this bond is sensitive to deterioration and that the monitor reflections decreased ~10% while the LTAS data were collected. Thus the difference may be attributed to deterioration, but one should be aware that both the

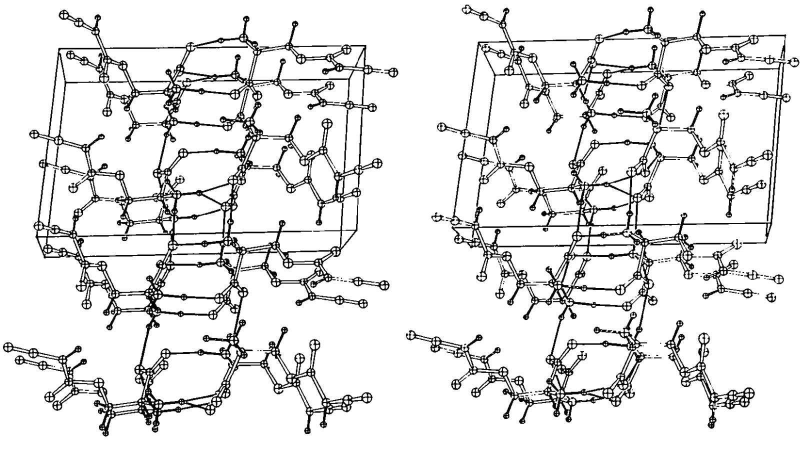


Fig. 6. Stereoscopic view of the contents of four unit cells illustrating packing and hydrogen bonding in the crystal. All atoms are shown as spheres of different isotropic *B* values according to atom type; oxygen (1·5), nitrogen (1·25), carbon (1·0) and hydrogen (0·5 Å^2).

C(4)–O(3) bond and the C(3)–O(3)–C(4) bond angle are in the region where the two molecules are conformationally very different.

Table 4 illustrates an apparent effect of deterioration on the N–H distances and angles. Individually, the changes may not be significant but, in general, the derived parameters are improved in the LTAS data in the sense that agreement with expected values is much better.

It is clear from the results for azaserine at two temperatures that the molecular parameters are sensitive to deterioration; in particular in the RTAS results the C(5)–N(2) bond lengths differ by 0·099 Å, exceeding the estimated standard deviation of the difference by a factor of 4·5. This suggests that in macromolecular crystallography, where deterioration is a general problem, the effects may be serious and much greater than indicated by the estimated standard deviations.

It should also be recognized that, at least for the case of azaserine, the relatively small decrease in the temperature of ~20°C reduced deterioration by a factor of ~4, improving the overall quality of the data. For RTAS, 82% of the data were $>2\sigma_I$ but, for LTAS, 90% of the data exceeded $2\sigma_I$ even though the crystals used were essentially identical in volume. The precision in the derived parameters improved by a factor of ~2 and the accuracy, as indicated by improved agreement between the two molecules, was much improved, the average difference in equivalent bond distances in the two molecules decreasing from 0·032 to 0·013 Å. The improvement in the quality of the LTAS data is reflected in the various residuals and the goodness of fit.

We used the XRAY system of programs (Stewart, 1976) and the *ORTEP* program (Johnson, 1965).

This work has been supported by USPHS Grant GM-10828 from the National Institutes of Health and the University of Washington Institutional Cancer Grant IN-26 from the American Cancer Society.

References

Blake, C. C. F. & Phillips, D. C. (1962). *Biological Effects of Ionizing Radiations at the Molecular Level.* Vienna: IAEA.

Boersch, H. (1935). *Monatsh. Chem.* **65**, 311–337.

Bondi, A. (1964). *J. Phys. Chem.* **68**, 441–451.

Brisse, R. & Pérez, S. (1976). *Acta Cryst.* B**32**, 2110–2115.

Cochran, W. & Penfold, B. R. (1952). *Acta Cryst.* **5**, 644–653.

Cox, A. P., Thomas, L. F. & Sheridan, J. (1958). *Nature (London)*, **181**, 1000–1001.

Cruickshank, D. W. J. (1949). *Acta Cryst.* **2**, 65–82.

Dawid, I. B., French, T. C. & Buchanan, J. M. (1963). *J. Biol. Chem.* **238**, 2178–2185.

French, T. C., Dawid, I. B. & Buchanan, J. M. (1963). *J. Biol. Chem.* **238**, 2186–2193.

French, T. C., Dawid, I. B., Day, R. A. & Buchanan, J. M. (1963). *J. Biol. Chem.* **238**, 2171–2177.

Frey, M. N., Lehmann, M. S., Koetzle, T. F. & Hamilton, W. C. (1973). *Acta Cryst.* B**29**, 876–884.

Hamilton, W. C. & Ibers, J. A. (1968). *Hydrogen Bonding in Solids.* New York: Benjamin.

Hartman, S. C. & Buchanan, J. M. (1959). *Annu. Rev. Biochem.* **28**, 365–410.

Hill, D. L. & Bennett, L. L. Jr (1969). *Biochemistry*, **8**, 122–130.

International Tables for X-ray Crystallography (1974). Vol. IV, Tables 2.2a and 2.3.1, p. 3. Birmingham: Kynoch Press.

IUPAC–IUB Commission on Biochemical Nomenclature (1970). *J. Mol. Biol.* **52**, 1–17.

Johnson, C. K. (1965). *ORTEP.* Report ORNL-3794. Oak Ridge National Laboratory, Tennessee.

Koetzle, T. F., Frey, M. N., Lehmann, M. S. & Hamilton, W. C. (1973). *Acta Cryst.* B**29**, 2571–2575.

Levenberg, B., Melnick, I. & Buchanan, J. M. (1957). *J. Biol. Chem.* **225**, 163–176.

Main, P., Woolfson, M. M. & Germain, G. (1971). *MULTAN. A Computer Program for the Automatic Solution of Crystal Structures.* Univ. of York Printing Unit, York, England.

Oberhänsli, W. E., Wagner, H. P. & Isler, O. (1974). *Acta Cryst.* B**30**, 161–166.

Stewart, J. M. (1976). The XRAY 76 system. Tech. Rep. TR-446. Computer Science Center, Univ. of Maryland, College Park, Maryland.

Stewart, R. F., Davidson, E. R. & Simpson, W. T. (1965). *J. Chem. Phys.* **42**, 3175–3187.

Sundaralingam, M. & Putkey, E. F. (1970). *Acta Cryst.* B**26**, 790–800.