GM-22548 from the National Institutes of Health, and CHE-77-09649 from the National Science Foundation.

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Acta Cryst. (1981). **B37**, 1591–1596

Structure of *N*-Acetyl-L-cysteine: X-ray ($T = 295$ K)* and Neutron ($T = 16$ K)† Diffraction Studies

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(Received 27 October 1980; accepted 10 February 1981)

Abstract

N-Acetyl-L-cysteine, $C_5H_9NO_3S$, $M_r = 163.20$, crystallizes in the space group PI , with $a = 5.766$ (1), $b = 6.433$ (1), $c = 5.014$ (1) Å, $\alpha = 102.80$ (2), $\beta = 102.77$ (1), $\gamma = 95.81$ (1)°, $Z = 1$ and final $R(F^2) = 0.024$ for a total of 1232 independent reflections, at $T = 16$ K. The structure was first deduced from

X-ray data obtained at 295 K. A subsequent neutron diffraction study carried out at 16 K has provided a more precise description of the hydrogen bonds than was possible from the X-ray experiment. The thiol group acts as both donor and acceptor, being involved in S–H...O and N–H...S interactions.

Introduction

The thiol or sulfhydryl group in cysteine residues has attracted a great deal of attention because of its ability to take part in a variety of biochemical reactions (Friedman, 1973; Jocelyn, 1972). The role of the thiol group as a possible hydrogen-bond donor and acceptor has been explored in a number of studies (Gordy &

* Structure and Conformation of Amino Acids Containing Sulfur. IV. Work supported by NCI, CA 23704.

† Precision Neutron Diffraction Structure Determination of Protein and Nucleic Acid Components. XIX. Part XVIII: Kvick, Canning, Koetzle & Williams (1980). Research carried out under contract with the US Department of Energy, and supported by its Office of Basic Energy Sciences.

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high-angle data ($2\theta \geq 60^\circ$) and fixed at $\Delta 2\theta = 3.0^\circ$ for the low-angle data. The step size was adjusted to give approximately 75 steps in each scan. As a general check on experimental stability, the intensities of two reflections were monitored every 100 measurements. These did not vary to any significant degree during the entire period of data collection.

Integrated intensities of reflections were obtained from the scan profile data by a method described by Takusagawa & Koetzle (1978). The intensities were corrected for absorption by a modification of the analytical method of de Meulenaer & Tompa (1965) (Alcock, 1970; Templeton & Templeton, 1973). Squared structure factors were obtained as $F_o^2 = I \sin 2\theta$ and averaged for Friedel-related reflections. The variance of each reflection was estimated as follows: $\sigma^2(F_o^2) = \sigma_{\text{count}}^2 + AF_o^8 + BF_o^4 + C$. Constants A , B and C were determined by a least-squares procedure minimizing the quantity $\sum [\langle \Delta F_o^2 \rangle - \sigma(F_o^2)]^2$, where

$$\langle \Delta F_o^2 \rangle = \sum_{i=1}^{n'} |\langle F_o^2 \rangle - F_{oi}^2|/n'$$

and n' is the number of observations for a given reflection hkl and its Friedel equivalent. The resulting values on an absolute scale are $A = 3.21 \times 10^{-10}$, $B = 2.08 \times 10^{-4}$ and $C = 6.23 \times 10^{-3}$ (with F^2 expressed in barn: 1 barn $\equiv 10^{-28} \text{ m}^2$).

Refinement

The structure was first deduced from the X-ray data. Chemical and stereochemical criteria were used to select a plausible molecular model, starting from an F_o

synthesis with all positive coefficients (the S atom was positioned at the origin) and removing peaks caused by the false center of inversion. The structure was refined by block-diagonal least-squares techniques, with anisotropic thermal parameters for non-H atoms and isotropic thermal parameters for H atoms, to yield an overall agreement factor $R = \sum |k|F_o| - |F_c|/ \sum k|F_o| = 0.057$ for 806 independent X-ray reflections. The function minimized was $\sum w[|F_o| - (1/k)|F_c|]^2$; the differential synthesis weighting of $w = 1/f_c$ was used, where f_c is the scattering factor of the C atoms.

X-ray coordinates of non-H atoms were used as initial values for the refinement based on the neutron data. After three cycles of full-matrix refinement of positional and thermal parameters, a ΔF synthesis showed all H-atom positions. Positional parameters for all atoms except S, which remained fixed at the origin, and anisotropic thermal parameters were varied together with the coherent neutron scattering lengths of S and N atoms and a type I isotropic extinction correction parameter (Becker & Coppens, 1975). The least-squares procedure minimized $\sum w(F_o^2 - k^2 F_c^2)^2$ and was carried out with a modified version of program ORFLS by Busing, Martin & Levy (1962). Weights were chosen as $w = 1/\sigma^2(F_o^2)$. The most significant extinction correction value (on F^2) is 0.35 for the reflection 111. Final positional and equivalent isotropic thermal parameters for both the X-ray and neutron refinements are listed in Table 3.* Neutron

* Lists of anisotropic thermal parameters and structure factors and a stereoscopic view of the molecule have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 36053 (17 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 3. Fractional atomic coordinates and equivalent isotropic thermal parameters with *e.s.d.*'s in parentheses

	Neutron values (16 K)				X-ray values (room temperature)			
	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i> (Å ²)	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i> (Å ²)
S	0.0000	0.0000	0.0000	0.57 (2)	0.000000	0.000000	0.000000	2.74 (9)
O(1)	0.5255 (2)	-0.3050 (2)	0.0809 (2)	0.64 (1)	0.5127 (8)	-0.3032 (6)	0.079 (1)	3.02 (11)
O(2)	0.2321 (2)	-0.4600 (2)	0.2343 (2)	0.76 (1)	0.224 (1)	-0.4546 (8)	0.225 (2)	4.32 (15)
O(3)	0.5082 (2)	0.3107 (2)	0.7944 (2)	0.63 (1)	0.5034 (9)	0.3122 (7)	0.788 (1)	3.09 (11)
N	0.5400 (2)	0.0844 (2)	0.3979 (2)	0.50 (1)	0.5285 (8)	0.0850 (7)	0.3935 (9)	2.06 (11)
C(1)	0.3669 (2)	-0.3027 (2)	0.2334 (2)	0.47 (1)	0.3575 (9)	-0.2996 (8)	0.226 (1)	2.13 (11)
C(2)	0.3617 (2)	-0.0827 (2)	0.4208 (2)	0.44 (1)	0.3514 (9)	-0.0802 (8)	0.412 (1)	1.99 (11)
C(3)	0.1060 (2)	-0.0283 (2)	0.3556 (2)	0.52 (1)	0.103 (1)	-0.0271 (9)	0.348 (1)	2.34 (12)
C(4)	0.6030 (2)	0.2736 (2)	0.5915 (2)	0.45 (1)	0.594 (1)	0.2747 (8)	0.586 (1)	2.22 (11)
C(5)	0.7962 (2)	0.4335 (2)	0.5597 (2)	0.67 (1)	0.783 (1)	0.430 (1)	0.555 (2)	3.00 (15)
H(1)	0.5216 (3)	-0.4609 (2)	-0.0344 (3)	1.48 (2)	0.43 (2)	-0.41 (2)	-0.08 (3)	6.00 (100)
H(2)	0.4039 (3)	-0.0983 (2)	0.6397 (3)	1.37 (2)	0.39 (2)	-0.09 (2)	0.63 (3)	6.00 (100)
H(3A)	0.0993 (3)	0.1203 (2)	0.5059 (3)	1.67 (2)	0.10 (1)	0.11 (2)	0.50 (2)	3.00 (100)
H(3B)	-0.0184 (3)	-0.1597 (2)	0.3772 (3)	1.68 (2)	-0.03 (1)	-0.12 (1)	0.38 (1)	1.00 (100)
H(S)	0.1386 (4)	0.1888 (3)	0.0352 (4)	2.46 (3)	0.15 (3)	0.14 (3)	0.00 (3)	1.00 (100)
H(N)	0.6143 (3)	0.0584 (2)	0.2311 (3)	1.82 (2)	0.60 (1)	0.06 (1)	0.26 (2)	2.00 (100)
H(5A)	0.7460 (3)	0.5938 (2)	0.6026 (4)	2.27 (3)	0.77 (3)	0.60 (3)	0.66 (3)	7.00 (100)
H(5B)	0.8330 (3)	0.3944 (3)	0.3528 (3)	2.27 (3)	0.78 (3)	0.40 (3)	0.35 (3)	7.00 (100)
H(5C)	0.9606 (3)	0.4395 (3)	0.7200 (4)	2.41 (3)	0.94 (2)	0.40 (2)	0.65 (2)	3.00 (100)

scattering lengths used for C, H and O atoms are $b_C = 6.648$, $b_H = -3.740$ and $b_O = 5.803$ fm (Shull, 1972). The refined scattering lengths for S and N were found to be 2.90 (3) and 9.29 (3) fm respectively, which are in good agreement with values of $b_S = 2.847$ (1) and $b_N = 9.36$ (2) fm presented by Koester (1977).

Discussion

The molecular structure of *N*-acetyl-L-cysteine in the crystalline state and the atomic nomenclature used in this paper are illustrated in Fig. 1 (Johnson, 1976). Bond distances and angles from the neutron diffraction study shown in Fig. 2 are consistent with corresponding dimensions determined by neutron diffraction in amino acids (Koetzle & Lehmann, 1976) and peptides (Freeman, Paul & Sabine, 1970; Griffin & Coppens, 1975; Koetzle, Hamilton & Parthasarathy, 1972; Kvik, Al-Karaghoulis & Koetzle, 1977). For *N*-acetyl-L-cysteine, the differences in bond distances and angles between the neutron and X-ray studies are given in Fig. 3. The neutron bonding parameters agree quite well with those determined in the X-ray study, except for the C(1)—O(1), C(1)—O(2) and C(4)—C(5) distances and the expected discrepancies in parameters involving H atoms. In the following discussion, reference is made mainly to the more precise neutron parameters. The reported bond distances are not corrected for thermal motion, and the apparent foreshortening of the C(1)—O(1), C(1)—O(2) and C(4)—C(5) bonds in the X-ray study is an effect of the large rigid-body librations of the carboxyl and methyl groups at room temperature.

Torsion angles and the molecular conformation are shown in Fig. 4. The amide group shows small deviations from planarity: displacements of individual atoms from the least-squares plane are listed in Table 4. C(2) is essentially coplanar with the carboxyl group C(1), O(1) and O(2); the bond angles around C(1) accordingly sum to 360.0° .

Fig. 1. Molecular view of *N*-acetyl-L-cysteine at 16 K. Thermal ellipsoids are drawn at the 97% probability level. A full stereoscopic drawing has been deposited.

Fig. 2. (a) Bond distances (Å) at 16 K (neutron values). E.s.d.'s are 0.002 Å or less. (b) Bond angles (°) at 16 K (neutron). E.s.d.'s are 0.2° or less.

Fig. 3. Differences between the neutron and X-ray results for bond distances [$D = D(N) - D(X)$] and angles [$A = A(N) - A(X)$]. Units for distances and angles are 0.001 Å and 0.1° respectively. Estimated pooled standard deviations are 0.006–0.008 Å and 0.4–0.6°.

The neutron thermal parameters have been fit to general rigid-body motions described by T, L, and S tensors (Schomaker & Trueblood, 1968). When the H atoms are excluded from the rigid body, the r.m.s. error of fit, $\langle \Delta U_{ij}^2 \rangle^{1/2}$, is 0.0005 Å². On the other hand, when the H atoms are included in the calculation, $\langle \Delta U_{ij}^2 \rangle^{1/2}$ is increased to 0.0061 Å². This fact suggests that the backbone of the molecule can be considered to behave

Fig. 4. Torsion angles ($^{\circ}$) around bonds: (a) C(1)–C(2), (b) C(2)–C(3), (c) C(3)–S, (d) C(2)–N, (e) C(4)–C(5) (neutron values). E.s.d.'s are 0.2° or less.

Table 4. Deviations ($\text{\AA}$) from the least-squares plane through the amide group [N, H(N), C(4), O(3)] and C(2), C(5) (neutron results)

The equation of the plane is $3.7865x - 3.8429y + 2.4503z - 2.6934 = 0$.

O(3)	–0.017 (2)	O(1)	0.667 (3)
N	0.002 (1)	O(2)	0.527 (4)
C(2)	0.025 (2)	C(1)	0.431 (3)
C(4)	–0.012 (2)	H(1)	0.969 (4)
C(5)	0.027 (2)	H(2)	0.781 (3)
H(N)	–0.026 (3)	H(5A)	–0.673 (3)
S	–2.693 (3)	H(5B)	–0.190 (3)

as a rigid body, but as might be expected the H atoms are moving somewhat independently from the backbone. When the methyl group C(4)–C(5)–H(5A,5B,5C) is taken as a rigid body, the largest principal axis of L corresponds to a r.m.s. libration of $L_1 = 6.8^{\circ}$ about the C–C bond, and $\langle \Delta U_{ij}^2 \rangle^{1/2}$ is $0.0020 \text{ \AA}^2$. This calculation indicates that the

C(5)–H(5A,5B,5C) distances should be increased by $0.017 \text{ \AA}$ to allow for effects of thermal motion, while the corresponding corrections calculated for bond distances in the non-H backbone are less than $0.002 \text{ \AA}$.

The crystal structure is illustrated in Fig. 5, and Fig. 6 outlines the hydrogen-bonding scheme. The hydrogen atom H(1) of the carboxyl group forms a hydrogen bond to the acetyl oxygen O(3) of a neighboring molecule [H(1)···O(3) $1.513 (2) \text{ \AA}$]; this hydrogen bonding is a common specific feature for *N*-acyl amino acids (Chen & Parthasarathy, 1978). These moderately strong hydrogen bonds link molecules head-to-tail to form infinite chains along the [011] direction. These chains are joined by weak S–H(S)···O(2) [$2.216 (2) \text{ \AA}$] and N–H(N)···S [$2.747 (2) \text{ \AA}$] hydrogen bonds to form a three-dimensional network. The H(S)···O distance of $2.216 (2) \text{ \AA}$ observed here is considerably shorter than the analogous contact of $2.40 (2) \text{ \AA}$ found in orthorhombic L-cysteine (Kerr *et al.*, 1975). In addition to the interaction with S, the amide hydrogen H(N) possesses a shorter intra-

Fig. 5. Stereoscopic illustration of the molecular packing, viewed approximately along [001]. Thin lines denote hydrogen bonds.

Fig. 6. Outline of the hydrogen-bonding scheme. E.s.d.'s for distances and angles are $0.002 \text{ \AA}$ and 0.2° respectively (neutron values).

molecular contact with O(1); this may be regarded as a bifurcated interaction (Koetzle *et al.*, 1972). Intramolecular N—H...O contacts similar to that found here are observed in many amino acids, *e.g.* α -glycine (Jönsson & Kvick, 1972).

The H(N)...S distance of 2.747 (2) Å is substantially shorter than 3.0 Å, the sum of normal van der Waals radii (Bondi, 1964),* and is similar to the H...S

distances for a bifurcated O—D... $\overset{\text{S}}{\underset{\text{S}}{\text{S}}}$ interaction in

Schlippe's salt where Raman spectroscopic data indicate that all D and H atoms are involved in hydrogen bonding (Mereiter, Preisinger & Guth, 1979). Thus, this contact may be considered to be a weak hydrogen bond. The orientation of the S...H(N) vector with respect to the thiol group ($\theta = 24.2^\circ$, $\phi = 154.7^\circ$) falls in the normal range for electrophilic contacts, as described by Rosenfield, Parthasarathy & Dunitz (1977).

FT and TFK wish to thank Joseph Henriques for technical assistance in operating the neutron diffractometer, and our colleagues in the Brookhaven crystallography group for their critical review of the manuscript.

* The X-ray value of the H...S distance is 2.92 (8) Å.

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