A Neutron Diffraction Study of Glycyl-L-threonine Dihydrate

By A. Sequeira, M. Ramanadham, H. Rajagopal and V. M. Padmanabhan

Neutron Physics Division, Bhabha Atomic Research Centre, Trombay, Bombay 400 085, India

Abstract

Glycyl-L-threonine dihydrate [$C_6H_{12}N_2O_4 . 2H_2O$, $NH_3^+ . CH_2 . CONH . CH(CHOHCH_3) . COO^- . 2H_2O$] crystallizes in space group $P2_12_12_1$ with $Z = 4$ and $a = 9.592$ (16), $b = 10.002$ (10) and $c = 10.632$ (11) Å. Three-dimensional (3D) neutron intensity data with $\sin \theta/\lambda \le 0.5$ Å^{-1} have been collected on a computer-controlled four-circle neutron diffractometer. All 16 H atoms of the asymmetric unit have been located in a 3D neutron Fourier map. Full-matrix least-squares refinements, including anisotropic temperature factors for all atoms and an extinction factor, led to a weighted $R(F^2)$ value of 0.085 for 754 observations. Structural parameters of this dipeptide compare favourably with similar values observed in the neutron diffraction studies of amino acids and dipeptides. The peptide group is non-planar due to small but significant deviations of $\Delta\omega$ and θ_C [2.9 (5) and −2.9 (12)° respectively] from zero. The two C—O bond distances of the terminal α-COO$^-$ group are consistent with the hydrogen bonding involving this group. The crystal structure is stabilized by a 3D network of hydrogen bonds, nine such distinct hydrogen bonds being contributed by each asymmetric unit.

Introduction

A series of neutron diffraction studies of amino acids (for references, see Ramanadham & Chidambaram, 1978) were carried out in our laboratory in order to obtain precise information regarding their molecular structure, conformation and hydrogen-bonding interactions. The present work on glycyl-L-threonine dihydrate ($GT . 2H_2O$) is part of this series and, to the best of our knowledge, the first neutron diffraction study of a dipeptide with one residue other than glycine. The crystal structure of $GT . 2H_2O$ was earlier determined by Yadava & Padmanabhan (1973) using X-ray diffraction techniques.

Experimental

Crystals of $GT . 2H_2O$ were grown by slow evaporation from a saturated aqueous solution containing 2% alcohol at room temperature (298 K). The crystals were generally prismatic with their principal bounding planes of the form {011}. The length of the crystal used in the experiment was 4.6 mm and the widths of the pairs of faces (011), (01$\bar{1}$) and (0$\bar{1}$1), (01$\bar{1}$) were 1.2 and 0.95 mm respectively. The crystal was mounted with its prism axis (crystallographic a axis) along the φ axis of the on-line TDC-312 computer-controlled four-circle neutron diffractometer (Sequeira *et al.*, 1978) situated at the CIRUS reactor in Trombay. The wavelength [1.026 (1) Å] of the incident neutron beam was determined by using the diffraction pattern from a KCl crystal. Cell parameters and the crystal orientation were refined using the program *REFINE* (Srikanta & Sequeira, 1968*b*) from the 2θ peak values of 25 reflections obtained by $\theta : 2\theta$ scans. The space-group extinctions for $P2_12_12_1$ were confirmed. Integrated intensities of 766 independent observations with $\sin \theta/\lambda \le 0.5$ Å^{-1} were recorded in the symmetrical setting using the $\theta : 2\theta$ step-scan mode. The scan step was 0.1° and the scan length was varied to include at least 10 background steps on either side of the peak. Two reflections, 002 and 400, were measured at regular intervals to monitor the stability of the crystal and the reproducibility of the measurements. Intensity fluctuations in these reflections were within 3% from the mean values during the entire experiment. The integrated intensities were reduced to F_o^2 values by applying the standard Lorentz and absorption corrections using the program *DATARED* (Srikanta & Sequeira, 1968*a*), which had *ORABS* (Wehe, Busing & Levy, 1962) as a subroutine. The linear absorption coefficient ($\mu = 0.22$ mm^{-1}) was calculated using 35×10^2 fm^2 as the incoherent scattering cross section for H atoms. The computed values of the transmission coefficients were in the range 0.790 to 0.831.

Refinement

All 16 H atoms of the asymmetric unit were located in a 3D neutron Fourier map computed using the program *FORDAP* (Zalkin, 1962) on the basis of observed neutron F_o values and phases calculated from the X-ray non-hydrogen atomic positions. The initial model, consisting of positional and isotropic tempera-

ture factors of all the atoms along with a scale factor and an extinction factor, was then subjected to a series of least-squares refinements using the program *TRXFLS* (H. Rajagopal & A. Sequeira, 1977, unpublished), a modified version of *ORFLS* (Busing, Martin & Levy, 1962). The quantity minimized was $\sum w(F_o^2 - |F_c|^2)^2$, where the weights were given by $w = [\sigma^2(F_o^2)_{count} + (0.03F_o^2)^2]^{-1}$. The neutron scattering lengths used in the calculation of F_c were: $b_H = -3.72$, $b_C = 6.626$, $b_N = 9.4$, $b_O = 5.75$ fm. Individual anisotropic temperature factors were introduced into the refinement during the final stages. The refinement was terminated when no parameter shift was more than one tenth of its estimated standard deviation. The extinction correction was of the form $y = (1 + 2x)^{-1/2}$ (Zachariasen, 1967) and the parameter refined was G ($=x/QT$).

The presence of a significant number of weak reflections, some of which had negative F_o^2 values owing to poor signal to noise ratio, prompted us to carry out independent series of refinements with different modes of treatment for the weak observations. In the first series, all the observations with $F_o^2 < \sigma(F_o^2)$ were rejected. In the second series, negative values of F_o^2 were replaced with zero. In the third series, all the observations were used without any alteration. Differences among the three sets of refined parameters thus obtained were not statistically significant. However, the structural model chosen for further discussion was that obtained from the second series of refinement ($F_o^2 = 0$ if $F_o^2 < 0$). The final agreement factors for this refinement are:

	For 754 observations actually used	For 581 observations with $F_o^2 > \sigma(F_o^2)$
$R(F)$	0.119	0.063
$R(F^2)$	0.101	0.081
$wR(F^2) = [\sum w(F_o^2 - |F_c|^2)^2/\sum wF_o^4]^{1/2}$	0.085	0.079.

12 out of 766 observations were found to have recording errors. They were rejected during the final cycles of refinement. However, they are listed in the final structure factor table (deposited). The final values of σ $\{=[\sum w(\Delta F^2)^2/(n - m)]^{1/2}\}$ and the extinction parameter, $r^*\lambda^{-1}$, are 1.17 and 0.07502 × 10^4 respectively.

An attempt was made to treat the negative and weak observations by Bayesian statistics as proposed by French & Wilson (1978). A computer program was accordingly written (*TNEABS*, M. Ramanadham, 1979, unpublished), which was used to recalculate F_o^2 and $\sigma(F_o^2)$ values of 341 ($F_o^2 < 3\sigma$ initially) out of 766 observations. Refinement based on this data set resulted in a model closer to that obtained in the refinement where all the observations, including the negative ones, were used without any alterations. This method is being explored further and the results will be published separately.

Results and discussion

Fractional coordinates and U_{eq} (Å^2) for all the atoms of GT.2H₂O are listed in Table 1.* The nomenclature used here and elsewhere in this paper is in accordance with the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature (1970). Derived parameters (bond distances, bond angles, hydrogen-bond parameters, torsion angles, *etc.*) have been computed using the programs *ORFFE* (Busing, Martin & Levy, 1964) and *TORANG* (M. Ramanadham, 1970, unpublished). Corrections for thermal motion were not applied to any of the derived parameters. The program *PLANE* (M. Ramanadham & S. K. Sikka, 1970, unpublished) has been used to compute the weighted least-squares planes. All the figures have been generated using the program *ORTEP* II (Johnson, 1976). Fig. 1 is a view of the GT molecule.

Results of this neutron study confirm many of the features of the earlier X-ray structure of GT.2H₂O (Yadava & Padmanabhan, 1973). However, the two

---

* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 36094 (7 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 1. *Fractional atomic coordinates* (×10^4) *and equivalent mean-square thermal-vibration amplitudes, with e.s.d.'s in parentheses*

	x	y	z	U_{eq} ($\text{Å}^2 \times 10^3$)
N(1)	7089 (6)	503 (5)	6271 (5)	29 (2)
H(11)	7339 (15)	1079 (14)	6908 (18)	59 (6)
H(12)	6572 (14)	−257 (12)	6698 (12)	43 (5)
H(13)	7958 (16)	177 (16)	5814 (18)	75 (7)
CA(1)	6146 (7)	1196 (6)	5396 (7)	34 (3)
HA(11)	5222 (14)	1392 (14)	5870 (18)	65 (7)
HA(12)	5994 (16)	571 (15)	4574 (12)	60 (6)
C(1)	6792 (7)	2490 (7)	4971 (7)	24 (2)
O(1)	8022 (8)	2766 (7)	5201 (9)	38 (3)
N(2)	5913 (5)	3309 (4)	4389 (4)	30 (2)
H(2)	4917 (13)	3033 (11)	4327 (12)	29 (4)
CA(2)	6334 (7)	4580 (6)	3855 (6)	25 (2)
HA(2)	7401 (16)	4763 (12)	4087 (12)	43 (5)
C(2)	5476 (6)	5739 (6)	4395 (6)	25 (2)
O(T1)	4419 (8)	5470 (8)	5048 (7)	35 (3)
O(T2)	5874 (8)	6878 (7)	4082 (8)	40 (3)
CB	6150 (8)	4550 (8)	2401 (6)	39 (3)
HB	6362 (15)	5510 (14)	2059 (13)	52 (5)
OG(1)	4738 (8)	4234 (9)	2141 (7)	38 (3)
HG(1)	4677 (13)	3449 (13)	1727 (13)	39 (5)
CG(2)	7133 (9)	3558 (10)	1823 (9)	59 (4)
HG(21)	8176 (21)	3789 (22)	2073 (26)	118 (11)
HG(22)	7012 (23)	2548 (18)	2160 (21)	104 (9)
HG(23)	6964 (20)	3508 (21)	820 (17)	99 (9)
O(W1)	5359 (13)	8961 (11)	7621 (10)	75 (5)
H(W11)	4641 (21)	8384 (14)	7399 (13)	67 (7)
H(W12)	5303 (16)	9261 (16)	8483 (13)	58 (6)
O(W2)	6655 (9)	2292 (8)	8286 (8)	36 (3)
H(W21)	6254 (15)	1774 (14)	8916 (13)	49 (5)
H(W22)	7492 (17)	2653 (14)	8611 (13)	53 (6)
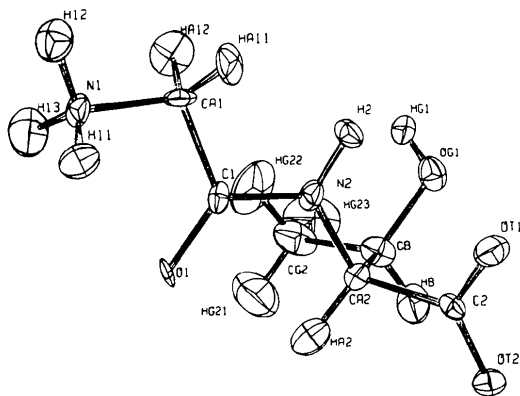
Fig. 1. The GT molecule.

Table 2. *Covalent bond distances* (Å) *with their e.s.d.'s*

N(1)—H(11)	0·921 (18)	CA(2)—CB	1·556 (9)
N(1)—H(12)	1·015 (14)	CB—HB	1·047 (16)
N(1)—H(13)	1·018 (17)	CB—OG(1)	1·418 (11)
N(1)—CA(1)	1·471 (9)	CB—CG(2)	1·501 (12)
CA(1)—HA(11)	1·038 (17)	OG(1)—HG(1)	0·901 (16)
CA(1)—HA(12)	1·084 (15)	CG(2)—HG(21)	1·060 (23)
CA(1)—C(1)	1·504 (9)	CG(2)—HG(22)	1·078 (21)
C(1)—O(1)	1·236 (10)	CG(2)—HG(23)	1·080 (20)
C(1)—N(2)	1·328 (8)	O(W1)—H(W11)	0·929 (21)
N(2)—H(2)	0·997 (13)	O(W1)—H(W12)	0·966 (18)
N(2)—CA(2)	1·449 (8)	O(W2)—H(W21)	0·930 (17)
CA(2)—HA(2)	1·069 (17)	O(W2)—H(W22)	0·946 (18)
CA(2)—C(2)	1·534 (9)		
C(2)—O(T1)	1·258 (10)		
C(2)—O(T2)	1·246 (9)		

Table 3. *Bond angles* (°) *with their e.s.d.'s*

CA(1)—N(1)—H(11)	109·3 (11)	N(2)—CA(2)—HA(2)	109·1 (8)
CA(1)—N(1)—H(12)	109·6 (9)	C(2)—CA(2)—HA(2)	107·3 (8)
CA(1)—N(1)—H(13)	110·7 (11)	CB—CA(2)—HA(2)	110·0 (9)
H(11)—N(1)—H(12)	105·4 (14)	CA(2)—C(2)—O(T1)	118·5 (6)
H(11)- N(1)—H(13)	109·8 (14)	CA(2)—C(2)—O(T2)	115·3 (6)
H(12)—N(1)—H(13)	111·9 (13)	O(T1)—C(2)—O(T2)	126·1 (7)
N(1)—CA(1)—C(1)	110·0 (6)	CA(2)—CB—OG(1)	107·8 (6)
N(1)—CA(1)—HA(11)	107·8 (11)	CA(2)—CB—CG(2)	110·3 (6)
N(1)—CA(1)—HA(12)	108·7 (9)	OG(1)—CB—CG(2)	111·9 (7)
C(1)—CA(1)—HA(11)	109·6 (9)	CA(2)—CB—HB	107·8 (9)
C(1)—CA(1)—HA(12)	108·0 (10)	OG(1)—CB—HB	108·8 (10)
HA(11)—CA(1)—HA(12)	112·7 (14)	CG(2)—CB—HB	110·0 (10)
CA(1)—C(1)—O(1)	121·7 (7)	CB—OG(1)—HG(1)	110·5 (11)
CA(1)—C(1)—N(2)	114·1 (6)	CB—CG(2)—HG(21)	110·3 (15)
O(1)—C(1)—N(2)	124·1 (7)	CB—CG(2)—HG(22)	114·6 (14)
C(1)—N(2)—CA(2)	123·1 (5)	CB—CG(2)—HG(23)	109·9 (13)
C(1)—N(2)—H(2)	117·9 (8)	HG(21)—CG(2)—HG(22)	102·9 (19)
CA(2)—N(2)—H(2)	119·0 (8)	HG(21)—CG(2)—HG(23)	113·5 (20)
N(2)–CA(2)– C(2)	111·5 (5)	HG(22)—CG(2)—HG(23)	105·6 (18)
N(2)—CA(2)—CB	109·9 (5)	H(W11)—O(W1)—H(W12)	113·1 (17)
C(2)—CA(2)—CB	109·0 (5)	H(W21)—O(W2)—H(W22)	107·5 (15)

Table 4. *Torsion angles* (°) *and their e.s.d.'s*

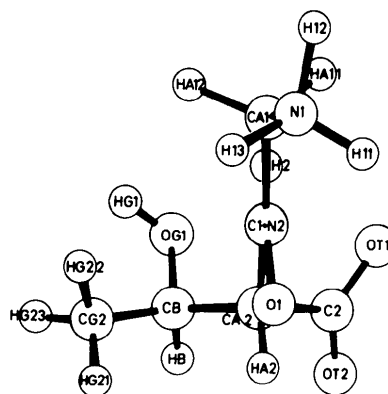|		
$\varphi(1,1)$	C(1)—CA(1)—N(1)—H(11)	52·7 (13)
$\varphi(1,2)$	C(1)—CA(1)—N(1)—H(12)	167·8 (9)
$\varphi(1,3)$	C(1)—CA(1)—N(1)—H(13)	−68·3 (12)
$\psi(1,1)$	N(1)—CA(1)—C(1)—N(2)	−167·0 (5)
$\psi(1,2)$	N(1)—CA(1)—C(1)—O(1)	10·2 (10)
ω	CA(1)—C(1)—N(2)—CA(2)	−177·1 (5)
$\varphi(2,1)$	C(1)—N(2)—CA(2)—C(2)	−123·7 (6)
$\varphi(2,2)$	C(1)—N(2)—CA(2)—CB	115·3 (7)
$\varphi(2,3)$	C(1)—N(2)—CA(2)—HA(2)	−5·4 (11)
$\psi(2,1)$	N(2)—CA(2)—C(2)—O(T1)	−10·6 (8)
$\psi(2,2)$	N(2)—CA(2)—C(2)—O(T2)	172·7 (6)
$\chi(1,1)$	N(2)—CA(2)—CB—OG(1)	56·8 (8)
$\chi(1,2)$	N(2)—CA(2)—CB—CG(2)	−65·7 (8)
$\chi(1,3)$	N(2)—CA(2)—CB—HB	174·2 (10)
$\chi(2,1)$	CA(2)—CB—OG(1)—HG(1)	−116·8 (12)
$\chi(2,2,1)$	CA(2)—CB—CG(2)—HG(21)	−56·9 (17)
$\chi(2,2,2)$	CA(2)—CB—CG(2)—HG(22)	58·6 (16)
$\chi(2,2,3)$	CA(2)—CB—CG(2)—HG(23)	177·3 (13)

Fig. 2. A view of the GT molecule down the C(1)—N(2) bond.

C—O distances of the α-carboxyl group in the X-ray study indicated that this group might be un-ionized, but the present work clearly establishes that the GT molecule is in the zwitterion form in the crystal structure of GT.2H₂O.

Bond distances (Table 2) and bond angles (Table 3) obtained in the present study compare well with

the corresponding parameters obtained from the neutron diffraction studies of perdeutero-α-glycylglycine (Freeman, Paul & Sabine, 1970), glycylglycine.HCl.H₂O (Koetzle, Hamilton & Parthasarathy, 1972) and L-threonine (Ramanadham, Sikka & Chidambaram, 1973). However, some of the bond distances in GT involving the H atoms are shorter than the corresponding values in other structures. In particular the N(1)—H(11) distance of 0·921 (18) Å is very short. However, most of the H atoms involved in shorter-than-expected bond distances also have larger and more anisotropic thermal motion associated with them. We attribute these shorter bond distances to the limited accuracy of the experimental data and, to some extent, to the thermal motion, rather than to any special features of the GT molecule. Torsion angles, listed in Table 4, indicate that there are differences in the main-chain conformation of the three dipeptides. Also, the conformation of the threonyl residue in GT is different from that of the L-threonine molecule. Fig. 2 presents a view of the GT molecule down the

C(1)–N(2) bond. The bulk of the molecule seems to be made up of two approximately planar groups of atoms, namely, [CA(1), C(1), O(1), N(2), H(2), CA(2), HA(2)] and [CA(2), C(2), CB, CG(2), HG(23)] which are mutually perpendicular. The value of the angle between the normals of the two weighted least-squares planes is 88·9°.

Table 5. *Comparison of torsion angles* (°) *in* GT.2H$_2$O, *perdeutero-α-glycylglycine, and glycylglycine*. HCl.H$_2$O *studied by neutron diffraction*

	GT.2H$_2$O	Perdeutero-α-GG	GG.HCl. H$_2$O
ω_1 CA(1)–C(1)–N(2)–CA(2)	−177·1 (5)	−176·5	176·8
ω_2 O(1)–C(1)–N(2)–H(2)	−173·7 (11)	171·6	175·8
ω_3 O(1)–C(1)–N(2)–CA(2)	5·8 (11)	0·3	− 0·9
ω_4 CA(1)–C(1)–N(2)–H(2)	3·4 (12)	11·8	−6·6
$\Delta\omega$ $\frac{\omega_1 + \pi}{}$	2·9 (5)	3·6	· 3·2
θ_N $\begin{cases} \omega_1 - \omega_1 + \pi \\ \text{or} \\ \omega_2 - \omega_3 + \pi \end{cases}$	0·5 (13)	8·1	− 3·4
θ_C $\begin{cases} \omega_1 - \omega_3 + \pi \\ \text{or} \\ \cdot \omega_2 + \omega_4 + \pi \end{cases}$ (mod 2π)	−2·9 (12)	3·4	2·4

Table 6. *Coefficients of weighted least-squares planes and atomic deviations from them* (Å ×10^3)

L, M, N, D are the least-squares coefficients in the planes with equations $LX + MY + NZ - D = 0$, where X, Y, Z are in Å.

	Plane 1	Plane 2	Plane 3	Plane 4	Plane 5
L	−0·2412	−0·2126	−0·2582	−0·1958	0·5770
M	0·4330	0·4353	0·4138	0·4528	0·0226
N	0·8685	0·8748	0·8730	0·8699	0·8165
D	4·107	4·306	3·978	4·449	6·960
CA(1)	−28 (7)	−20 (7)	4 (7)		
C(1)	−9 (7)	17 (7)	−15 (7)	1 (7)	
O(1)	39 (9)		9 (9)		
N(2)	12 (5)	12 (5)	2 (5)	−1 (5)	
H(2)	66 (12)			4 (12)	
CA(2)	−29 (6)	−18 (6)		1 (6)	−5 (6)
C(2)					15 (6)
O(T1)					−9 (7)
O(T2)					−10 (8)

Dihedral angles associated with the peptide group are listed in Table 5 for the three structures; GT, perdeutero-α-glycylglycine and glycylglycine.HCl.-H$_2$O. Values of $\Delta\omega$, θ_C and θ_N, which measure the non-planar distortions in the peptide group due to pure ω rotation and pyramidal distortions of bonds at atoms C and N respectively (Ramachandran, Lakshminarayanan & Kolaskar, 1973), show that the peptide group is non-planar in all three structures. Magnitudes of $\Delta\omega$ and θ_C are comparable in these structures, but θ_N has a larger variation. It is almost zero in GT. Therefore, unlike the other two dipeptides, the non-planar distortions in the peptide group of GT are mainly due to $\Delta\omega$ and θ_C. Similar conclusions have been arrived at on the basis of the weighted least-squares planes (Table 6) and χ^2 tests (Hamilton, 1964) carried out on the weighted sums of the squares of the atomic deviations from the corresponding least-squares planes. The terminal carboxyl group is only approximately planar (see Table 6). The angle between the normals of the least-squares planes associated with the peptide and carboxyl groups is 54·6°.

Fig. 3 shows the hydrogen-bonding network in the crystal structure of GT.2H$_2$O. There are nine distinct hydrogen bonds (Table 7) per asymmetric unit. Parameters associated with these hydrogen bonds, with the exception of the N(1)–H(11) distance, are comparable with similar values obtained from the neutron diffraction studies of amino acids and other structures (Ramanadham & Chidambaram, 1978). The three hydrogen bonds N(1)–H(11)··· O(W2), O(W2)···H(W11)–O(W1) and O(W1)–H(W12)···O(T1) join the two water molecules and a GT molecule to form a closed loop-like structure. These structures, related to one another by a screw axis and joined by hydrogen bonds N(1)–H(13)···O(T1) and N(2)–H(2)···O(1), form chains parallel to the *a* axis. The rest of the hydrogen bonds interconnect these chains. The hydrogen bond N(2)–H(2)···O(1) is similar to that observed in the
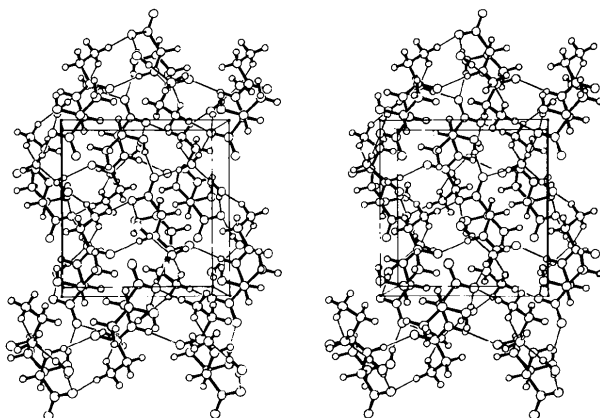
Table 7. *Hydrogen-bond parameters with e.s.d.'s in parentheses*

X–H···Y	$D_{(X-H)}$ (Å)	$D_{(H···Y)}$ (Å)	$D_{(X···Y)}$ (Å)	∠(HXY) (°)
N(1)–H(11)···O(W2^i)	0·921 (18)	2·012 (19)	2·823 (10)	23·6 (9)
N(1)–H(12)···O(W1^{ii})	1·015 (14)	1·711 (17)	2·682 (13)	13·5 (8)
N(1)–H(13)···O(T1^{iii})	1·018 (17)	1·796 (18)	2·812 (10)	2·4 (10)
N(2)–H(2)···O(1^{iv})	0·997 (13)	2·048 (15)	3·006 (10)	13·1 (8)
OG(1)–HG(1)···O(T2^v)	0·901 (16)	1·868 (15)	2·754 (11)	8·6 (9)
O(W1)–H(W11)···O(W2^{vi})	0·929 (21)	1·807 (19)	2·729 (15)	6·0 (10)
O(W1)–H(W12)···O(T1^{vi})	0·966 (18)	1·993 (16)	2·910 (16)	15·1 (10)
O(W2)–H(W21)···O(T1^{vii})	0·930 (17)	1·825 (16)	2·742 (11)	7·8 (10)
O(W2)–H(W22)···O(T2^{viii})	0·946 (18)	1·711 (18)	2·650 (12)	5·4 (10)

Equivalent positions of acceptor atoms

(i) x,y,z	(iv) −0·5 + x, 0·5 − y, 1·0 − z	(vii) 1·0 − x, −0·5 + y, 1·5 − z
(ii) x, − 1·0 + y,z	(v) 1·0 − x, −0·5 + y, 0·5 − z	(viii) 1·5 − x, 1·0 − y, 0·5 + z
(iii) 0·5 + x, 0·5 − y, 1·0 − z	(vi) 1·0 − x, 0·5 + y, 1·5 − z	

Fig. 3. A stereoscopic view of the hydrogen-bonding network in the crystal structure of GT.$2H_2O$.

antiparallel β sheets of some protein structures. There are no intramolecular or multifurcated hydrogen bonds in the crystal structure of GT.$2H_2O$. The H—O—H angles of the two water molecules are consistent with the configuration of hydrogen bonds around them (Chidambaram, Sequeira & Sikka, 1964). The two C—O bond lengths in α-COO$^-$, though indistinguishable within the level of experimental uncertainty, nevertheless seem to agree with the results of the analysis, made by Ramanadham & Chidambaram (1978), of the influence of hydrogen bonding on C—O distances using the bond-length–bond-strength correlations due to Brown & Shannon (1973).

References

BROWN, I. D. & SHANNON, R. D. (1973). *Acta Cryst.* A29, 266–282.

BUSING, W. R., MARTIN, K. O. & LEVY, H. A. (1962). *ORFLS.* Report ORNL-TM-305. Oak Ridge National Laboratory, Tennessee.

BUSING, W. R., MARTIN, K. O. & LEVY, H. A. (1964). *ORFFE.* Report ORNL-TM-306. Oak Ridge National Laboratory, Tennessee. Adapted to the BESMA-6 computer by H. RAJAGOPAL.

CHIDAMBARAM, R., SEQUEIRA, A. & SIKKA, S. K. (1964). *J. Chem. Phys.* 41, 3616–3622.

FREEMAN, H. C., PAUL, G. L. & SABINE, T. M. (1970). *Acta Cryst.* B26, 925–932.

FRENCH, S. & WILSON, K. (1978). *Acta Cryst.* A34, 517–525.

HAMILTON, W. C. (1964). *Statistics in Physical Science*, pp. 174–183. New York: Ronald Press.

IUPAC–IUB COMMISSION ON BIOCHEMICAL NOMENCLATURE (1970). *J. Mol. Biol.* 52, 1–17.

JOHNSON, C. K. (1976). *ORTEP* II. Report ORNL-5138. Oak Ridge National Laboratory, Tennessee. Implemented on the DEC-1077 computer by H. RAJAGOPAL.

KOETZLE, T. F., HAMILTON, W. C. & PARTHASARATHY, R. (1972). *Acta Cryst.* B28, 2083–2090.

RAMACHANDRAN, G. N., LAKSHMINARAYANAN, A. V. & KOLASKAR, A. S. (1973). *Biochim. Biophys. Acta*, 303, 8–13.

RAMANADHAM, M. & CHIDAMBARAM, R. (1978). *Advances in Crystallography*, edited by R. SRINIVASAN, pp. 81–103. New Delhi: Oxford & IBH Publishing Co.

RAMANADHAM, M., SIKKA, S. K. & CHIDAMBARAM, R. (1973). *Pramana*, 1, 247–259.

SEQUEIRA, A., MOMIN, S. N., RAJAGOPAL, H., SONI, J. N., CHIDAMBARAM, R., DILIP KUMAR, RAMANA RAO, A. & GOPU, V. M. (1978). *Pramana*, 10, 289–302.

SRIKANTA, S. & SEQUEIRA, A. (1968a). Program *DATARED* (unpublished). Adapted to the BESM-6 computer by H. RAJAGOPAL.

SRIKANTA, S. & SEQUEIRA, A. (1968b). Program *REFINE* (unpublished). Adapted to the BESM-6 computer by H. RAJAGOPAL.

WEHE, D. J., BUSING, W. R. & LEVY, H. A. (1962). *ORABS.* Report ORNL-TM-229. Oak Ridge National Laboratory, Tennessee.

YADAVA, V. S. & PADMANABHAN, V. M. (1973). *Acta Cryst.* B29, 854–858.

ZACHARIASEN, W. H. (1967). *Acta Cryst.* 23, 558–564.

ZALKIN, A. (1962). Program *FORDAP* (unpublished). Adapted to the BESM-6 computer by H. RAJAGOPAL.