Precision Neutron Diffraction Structure Determination of Protein and Nucleic Acid Components. VI. The Crystal and Molecular Structure of the Amino Acid L-Asparagine Monohydrate*

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A neutron diffraction study of L-asparagine monohydrate, $C_4H_8N_2O_3.H_2O$, has been carried out. The structure is orthorhombic, space group $P2_12_12_1$ with $Z = 4$. Cell parameters are $a = 5.593$ (5), $b = 9.827$ (10) and $c = 11.808$ (11) Å. Least-squares refinements based on 1787 reflections led to a final conventional R value of 0.026. The molecule is in the zwitterion form, and bond lengths involving hydrogen have been determined with an average precision of 0.002 Å. The molecules are linked together by seven distinct hydrogen bonds to form a three-dimensional network. Quantitative estimates are made of the effects of hydrogen bonding on the thermal motion of the NH_3^+ and NH_2 groups in the molecule. A comparison is made with an independent neutron diffraction study (preceding paper) and with the unpublished X-ray results of Kartha.

An X-ray diffraction structure determination of L-asparagine monohydrate, $C_4H_8N_2O_3.H_2O$, has been reported by Kartha & de Vries (1961). A neutron diffraction refinement has been carried out by Ramanadham, Sikka & Chidambaram (1972). Being unaware of the latter work until recently, we have carried out an independent neutron-diffraction refinement.

$a = 5.593$ (5) Å
$b = 9.827$ (10)
$c = 11.808$ (11)
M.W. = 150.14
$\varrho_c = 1.536$ g.cm⁻³, $\varrho_m = 1.543$ g.cm⁻³ (Kartha & de Vries, 1961)
$Z = 4$.

Experimental

Crystal data

Colorless crystals of L-asparagine monohydrate with well developed faces were easily obtained by slow cooling of an aqueous solution. A sample with a volume of 15.6 mm³ and with 14 bounding planes (see Fig. 1) was mounted with the crystallographic a axis near the principal axis of a goniometer head. X-ray diffraction patterns indicated the space group to be $P2_12_12_1$. The orthorhombic cell dimensions were refined by a least-squares technique, using the setting angles of 25 reflections automatically centered on a four-circle neutron diffractometer [$\lambda = 1.013$ (1) Å]:‖

Data collection

The intensities of the Bragg reflections were measured at the Brookhaven National Laboratory High 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 used with a scan range $\Delta 2\theta = 1.0°$ $(1 + 8 \tan \theta)$ and the step sizes were varied to obtain about 40 points in each scan. Data were collected for 2101 hkl and $hk\bar{l}$ reflections having $d^* \leq 1.4$ Å⁻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.

Background corrections were made by use of a method which divides peak and background in such a way that $\sigma(I)/I$ is minimized. I is the integrated intensity, and $\sigma(I)$ its standard deviation based on counting statistics (Lehmann, Hamilton & Larsen, 1972). Squared observed structure factors, obtained as $F_o^2 = I \sin 2\theta$, were then corrected for absorption using a 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 $\mu = 2.773$ cm⁻¹. Transmission coefficients ranged from 0.54 to 0.66. Finally, the two

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symmetry-related forms were averaged, giving 1003 unique reflections, with a correlation factor $R_c = \sum|F_o^2 - \bar{F}^2|/\sum F_o^2 = 0.039$, where $\bar{F}^2$ is the mean of the symmetry-related reflections.

Structure refinement

The starting parameters for the least-squares refinement were the final refined coordinates (Kartha, 1971) from the X-ray structure analysis including hydrogen atoms. Neutron-scattering lengths were taken to be: $b_O = 0.575$, $b_N = 0.940$, $b_C = 0.6626$, and $b_H = -0.3723$ (all $\times 10^{-12}$ cm). The function minimized was $\sum w|F_o^2 - |F_c|^2|^2$; weights were chosen as $w = 1/\sigma^2(F_o^2)$ with $\sigma^2(F_o^2) = \sigma_{count}^2(F_o^2) + (0.04\ F_o^2)^2$ (σ_{count}^2 is based on counting statistics). A total of 937 reflections with $F_o^2 > 3\sigma_{count}\ (F_o^2)$ were included in the refinement, the final cycles of which determined the positional and anisotropic thermal parameters for all atoms.

The crystal showed severe extinction, and extinction parameters were therefore refined, first isotropically, and in the last cycle anisotropically as Type I defined by Zachariasen (1967), and adapted by Coppens & Hamilton (1970)

$$E = \left[1 + \frac{2\bar{T}F_c^2 g\lambda^3 \times 10^4}{V^2 \sin 2\theta}\right]^{-1/4}.$$

E multiplies the calculated structure factor, g is the extinction parameter, $\bar{T}$ the average beam path length in the crystal for a reflection with Bragg angle θ, V the uni-tcell volume, F_c the calculated structure factor on an absolute scale, and λ the wavelength. For anisotropic extinction, $g = (D'ZD)^{1/2}$, where D is a unit vector which is perpendicular to the plane which contains the incident and diffracted beams, and Z is a symmetric tensor describing the mosaic spread in the crystal. The lowest value of E was 0.45 for the 020 reflection. (Agreement almost as good was achieved with an isotropic extinction parameter.) The refinement including anisotropic extinction used the full unaveraged data set of 1787 reflections with $F_o^2 > 3\sigma_{count}\ (F_o^2)$. The refined atomic parameters are given
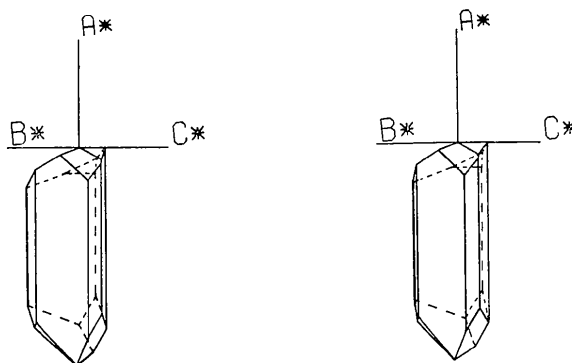

Fig. 1. Stereoscopic drawing of the crystal prepared by *ORTEP*. Reciprocal axes are given.

Table 1. *Fractional coordinates and temperature parameters*

The form of the anisotropic thermal ellipsoid is exp $-[\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl]$. The first column contains the atom names used in this paper, the second column contains the names used by Ramanadham *et al.* (1972). All parameters are multiplied by 10^5.

		x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C	C(1)	−12464 (17)	−5761 (9)	3565 (7)	1201 (25)	420 (8)	269 (5)	25 (13)	91 (9)	−17 (5)
C^α	C(2)	−30746 (16)	−2779 (8)	12969 (7)	1002 (23)	411 (8)	262 (5)	102 (11)	17 (10)	−35 (5)
C^β	C(3)	−20917 (18)	5669 (9)	22714 (7)	1218 (25)	411 (8)	325 (6)	94 (13)	2 (10)	−98 (5)
C^γ	C(4)	1639 (16)	−207 (9)	27919 (7)	1055 (23)	431 (8)	303 (5)	−26 (13)	2 (10)	−46 (5)
N	N(1)	−41654 (12)	−15658 (7)	17162 (5)	1161 (19)	490 (6)	322 (4)	−60 (9)	60 (7)	−46 (4)
$N^{\delta 2}$	N(2)	10503 (14)	6418 (8)	36819 (6)	1766 (23)	683 (8)	380 (4)	−46 (12)	−152 (7)	−128 (5)
O^2	O(1)	−548 (23)	4283 (11)	66 (9)	2058 (37)	526 (11)	456 (8)	−168 (18)	329 (14)	41 (7)
O^1	O(2)	−11881 (23)	−17472 (11)	−397 (8)	1878 (35)	492 (10)	379 (7)	47 (16)	213 (14)	−112 (7)
$O^{\delta 1}$	O(3)	11071 (20)	−10563 (12)	23908 (10)	1278 (32)	570 (11)	478 (8)	235 (18)	−98 (14)	−141 (8)
O^w	O(4)	30224 (30)	23018 (15)	11214 (11)	2771 (47)	811 (14)	478 (9)	−178 (25)	−121 (18)	29 (9)
H^{w1}	H(1)	18750 (54)	17421 (29)	7322 (21)	2993 (85)	1053 (27)	761 (18)	−225 (47)	−116 (36)	−59 (20)
H^{w2}	H(2)	35037 (52)	30200 (25)	6242 (21)	3387 (91)	872 (26)	706 (17)	−60 (45)	−8 (33)	100 (18)
H^2	H(3)	−31349 (43)	−20295 (22)	23230 (18)	2510 (66)	731 (21)	581 (14)	−79 (35)	−214 (28)	132 (14)
H^1	H(4)	−44533 (45)	−22465 (20)	10559 (17)	2893 (70)	615 (18)	515 (14)	−76 (34)	59 (25)	−125 (13)
H^3	H(5)	−58270 (40)	−13703 (23)	20737 (18)	1759 (60)	847 (23)	636 (14)	−166 (32)	329 (27)	−83 (15)
$H^{\delta 21}$	H(6)	25300 (43)	2680 (24)	40911 (17)	2229 (72)	995 (27)	519 (13)	−60 (37)	−232 (26)	−69 (14)
$H^{\delta 22}$	H(7)	2581 (52)	14962 (25)	39715 (17)	3282 (87)	935 (24)	564 (14)	201 (43)	−130 (28)	−258 (16)
H^α	H(8)	−45189 (40)	3151 (21)	9010 (17)	1832 (59)	783 (21)	545 (13)	373 (30)	−161 (23)	23 (13)
$H^{\beta 1}$	H(9)	−16955 (50)	15909 (21)	19777 (20)	3476 (89)	467 (18)	787 (17)	−73 (35)	−267 (33)	−14 (14)
$H^{\beta 2}$	H(10)	−34394 (40)	6827 (26)	29325 (17)	2034 (64)	1200 (27)	536 (14)	233 (38)	195 (25)	−299 (17)

in Table 1. The final values for the structure amplitudes are shown in Table 2 as $100 \times F_o^2$, $100 \times \sigma(F_o^2)$ and $100 \times |F_c|^2$ for those reflections used in the refinement.

The $\mathbf{Z}$ tensor used to calculate anisotropic extinction corrections has the following components: $z_{11} = 15 \cdot 3\,(7)$; $z_{22} = 11 \cdot 9\,(10)$; $z_{33} = 29 \cdot 7\,(15)$; $z_{12} = 4 \cdot 2\,(7)$; $z_{13} = -0 \cdot 8(6)$

$z_{23} = -15 \cdot 8\,(10)$. These extinction parameters correspond to a mosaic spread along the a, b, and c axes of $1 \cdot 49$, $1 \cdot 69$ and $1 \cdot 07$ seconds of arc respectively. Ramanadham et al. (1972) found for their crystal a value for g of $0 \cdot 23$ corresponding to a mosaic spread of 25 seconds of arc. This difference in mosaic spread can be

Table 2. *Observed and calculated structure amplitudes*

The quantities given are $100 \times F_o^2$, $100 \times \sigma(F_o^2)$, $100 \times |F_c^2|$. The extinction correction is applied to F_o^2.

attributed to the fact that they dipped their crystal in liquid nitrogen prior to the experiment whereas that was not the case in this study.

The agreement between observed and calculated squared structure amplitudes is: $R = [\sum |F_o^2 - |F_c|^2|]/\sum F_o^2 = 0.045$. The corresponding weighted value is $R_w = \{[\sum w |F_o^2 - |F_c|^2|^2]/\sum w F_o^4\}^{1/2} = 0.062$; while the conventional R index is $R = [\sum |F_o - |F_c||]/\sum F_o = 0.026$.

Description of the structure

The molecular structure

The molecular structure of L-asparagine is illustrated in Fig. 2. The thermal ellipsoids are shown on a 50% probability scale, and are typically small for an extensively hydrogen-bonded system containing only one hydrophobic CH_2 group. The molecule is in the zwitterion form.

The bond lengths and angles are listed in Tables 3 and 4. The hydrogen atom coordinates obtained from from the X-ray data (Kartha, 1971) by difference Fourier syntheses are qualitatively correct. The torsion angles, according to the IUPAC–IUB conventions (1970), are given in Table 5. The torsional angles ψ^1 and ψ^2 are within 10° of the values for a planar configuration of the group $N-C^\alpha-C-O^1-O^2$. The ammonium group is rotated in such a way as to place H^1 and O^1 near an eclipsed conformation. The distance between H^1 and O^1 is 2·291 (3) Å with the angle $N-H^1 \cdots O^1$ equal to 99·3°, so that we could characterize this contact as a close van der Waals contact between the negative O^1 and the positive H^1 atoms. Such contacts between the α-amino hydrogen atoms and the carboxyl group are not uncommon in amino acids, and, as we have discussed previously (Koetzle, Hamilton & Parthasarathy, 1972), the utility of calling such contacts hydrogen bonds is questionable. The group C^β, C^γ, $O^{\delta 1}$, $N^{\delta 2}$, $H^{\delta 12}$ and $H^{\delta 22}$ is nearly planar; the only significant deviations observed from the least-squares plane through the five atoms are 0·039 (2) Å

for $H^{\delta 21}$ and 0·022 (3) Å for $H^{\delta 22}$, corresponding mainly to a twisting of the NH_2 group about the $C^\gamma-N^{\delta 2}$ axis as shown by the torsion angles $\chi^{3,2,1}$ and $\chi^{3,2,2}$ (Table 5).

Table 3. *Molecular bond distances*

	Neutron	Corrected for thermal motion	X-ray
C—O¹	1·243 (1) Å	1·246 Å	1·243 Å
C—O²	1·261 (1)	1·264	1·258
C—Cᵅ	1·538 (1)	1·542	1·540
Cᵅ—Hᵅ	1·100 (2)		0·88
Cᵅ—N	1·490 (1)	1·494	1·493
Cᵅ—Cᵝ	1·522 (1)	1·525	1·517
N—H¹	1·040 (2)	1·053	0·88
N—H²	1·030 (2)	1·044	0·90
N—H³	1·039 (2)	1·053	0·87
Cᵝ—Hᵝ¹	1·087 (2)	1·099	0·97
Cᵝ—Hᵝ²	1·091 (2)	1·103	0·90
Cᵝ—Cᵞ	1·518 (1)	1·522	1·525
Cᵞ—Oᵟ¹	1·240 (1)	1·244	1·239
Cᵞ—Nᵟ²	1·332 (1)	1·334	1·329
Nᵟ²—Hᵟ²¹	1·026 (2)	1·033	0·78
Nᵟ²—Hᵟ²²	1·009 (3)	1·015	0·93
Oʷ—Hʷ¹	0·962 (3)	0·969	0·80
Oʷ—Hʷ²	0·957 (3)	0·963	0·78

Hydrogen bonding

There are seven hydrogen bonds, involving the seven H atoms bonded to nitrogen or oxygen, per asymmetric unit of the crystal. The complete hydrogen bonding network is drawn in Fig. 3, which displays the packing in one unit cell. Hydrogen-bond distances and angles are summarized in Table 6.

The NH_3^+ group is hydrogen bonded to O^1, $O^{\delta 1}$, and O^w. Only the second of these bonds is not bent, according to Baur's (1965) definition. The amide nitrogen donates hydrogen atoms to the carboxyl oxygen atoms O^1 and O^2; the second of these bonds is very bent (the $N-H \cdots O$ angle is 138·0°). The five $N-H \cdots O$ bonds are distributed over five different neighboring molecules, resulting in a complicated three-dimensional
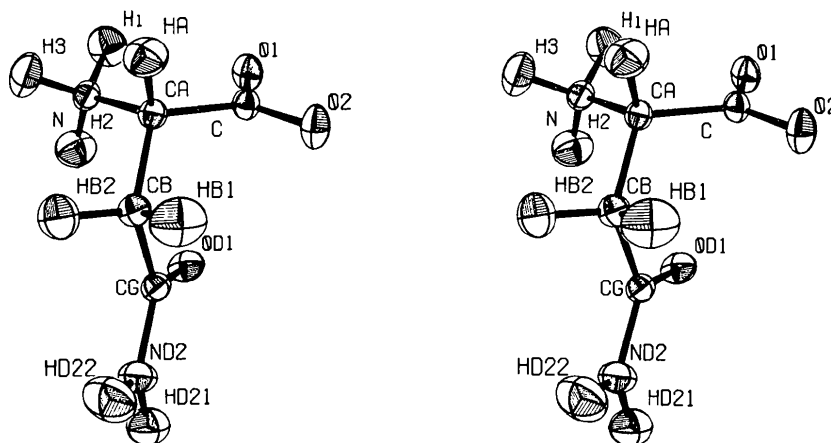

Fig. 2. Stereoscopic drawing of the molecule, with 50%-probability vibrational ellipsoids.

Table 4. *Molecular bond angles*

	Neutron	X-ray
O^1—C—C^2	126·0 (1)°	126·1°
O^1—C—C^α	117·8 (1)	117·8
O^2—C—C^α	116·0 (1)	115·9
H^1—N—C^α	111·2 (1)	113·1
H^2—N—C^α	112·3 (1)	109·8
H^3—N—C^α	110·1 (1)	109·3
H^1—N—H^2	109·1 (2)	110·2
H^1—N—H^3	106·6 (2)	104·0
H^2—N—H^3	107·3 (2)	110·1
C—C^α—C^β	114·2 (1)	114·5
N—C^α—C^β	111·1 (1)	110·9
H^α—C^α—C^β	107·3 (1)	110·3
C—C^α—N	110·5 (1)	110·2
C—C^α—H^α	106·4 (1)	103·5
N—C^α—H^α	106·9 (1)	106·9
C^α—C^β—C^γ	113·5 (1)	113·4
$H^{\beta 1}$—C^β—C^γ	108·2 (2)	104·4
$H^{\beta 2}$—C^β—C^γ	108·9 (2)	102·9
C^α—C^β—$H^{\beta 1}$	109·7 (2)	109·0
C^α—C^β—$H^{\beta 2}$	110·4 (2)	116·0
$H^{\beta 1}$—C^β—$H^{\beta 2}$	105·8 (2)	110·3
C^β—C^γ—$O^{\delta 1}$	120·7 (1)	120·7
C^β—C^γ—$N^{\delta 2}$	116·3 (1)	115·9
$O^{\delta 1}$—C^γ—$N^{\delta 2}$	123·0 (1)	123·3
C^β—$N^{\delta 2}$—$H^{\delta 21}$	119·8 (2)	120·8
C^γ—$N^{\delta 2}$—$H^{\delta 22}$	120·7 (2)	119·2
$H^{\delta 21}$—$N^{\delta 2}$—$H^{\delta 22}$	119·5 (2)	119·8
H^{w1}—O^w—H^{w2}	108·4 (2)	107·0

Table 5. *Torsion angles*

IUPAC–IUB Designation	Atoms involved	Angle
φ^1	C—C^α-N—H^1	−38·1 (2)°
φ^2	C—C^α-N—H^2	84·5 (2)
φ^3	C—C^α-N—H^3	−156·0 (2)
ψ^1	O^1—C –C^α—N	10·8 (1)
ψ^2	O^2—C –C^α—N	−174·0 (1)
χ^1	N—C^α-C^β—C^γ	72·2 (1)
$\chi^{2,3,1}$	C^α--C^β-C^γ—$O^{\delta 1}$	3·4 (1)
$\chi^{2,3,2}$	C^α--C^β-C^γ—$N^{\delta 2}$	−177·1 (1)
$\chi^{3,2,1}$	$O^{\delta 1}$-C^γ-$N^{\delta 2}$-$H^{\delta 21}$	−3·2 (2)
$\chi^{3,2,2}$	$O^{\delta 1}$-C^γ-$N^{\delta 2}$-$H^{\delta 22}$	178·3 (2)

eclipse of O^1 and H^1 mentioned above. At the same time, however, we observe a twisting of the N–C^α–C–O^1 group, so that the total effect of the hydrogen bonding on the molecule is a breakdown of the ideal configuration of the carboxyl and the ammonium group. The water hydrogen atoms contribute to the hydrogen bond network by linking O^2 atoms from two different L-asparagine molecules. These bonds are quite linear. The system of three hydrogen bonds around O^w is not planar; the H^{w1}–O^w···H^2 angle is 131·6 (2)°, and the angle H^{w2}–O^w···H^2 is 108·6 (2)°.

Thermal motion

In order to obtain the best geometrical parameters, the nonhydrogen atoms of the molecular framework were assumed to behave as a rigid body whose motion was described in terms of the T, L and S tensors (Schomaker & Trueblood, 1968). The r.m.s. difference between observed and calculated thermal parameters u_{ij} is 0·0019 Å², which compares well with the average

network of hydrogen bonds. There is no evidence for any *intra*molecular hydrogen bond, although O^1 is involved in a close contact with H^1, as mentioned above. O^1 accepts two hydrogen bonds, O^1···H^1 and O^1···$H^{\delta 22}$. The shortest of these bonds, O^1···H^1, is nearly parallel to C–O^1 and N–H^1, and favors the near

Table 6. *Hydrogen bond-distances and angles*

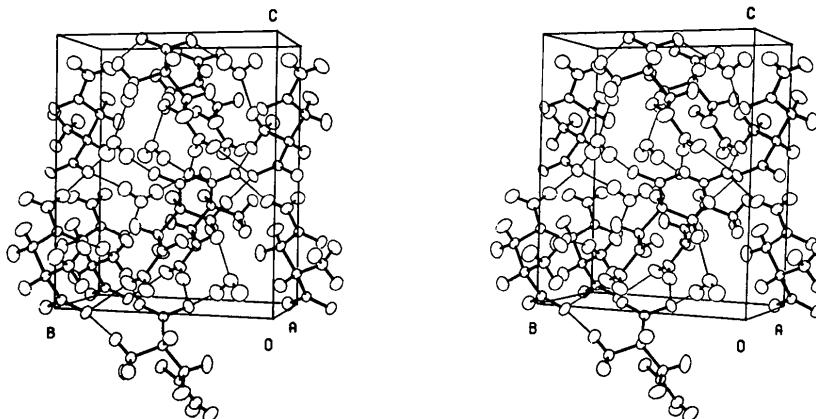| A–H···B–C	A···B	H···B	∠A–H···B	∠H···B–C
N–H^1···O^1–C	2·819 (1) Å	1·833 (2) Å	156·9 (2)°	139·5 (2)°
N–H^2···O^w	2·858 (2)	1·946 (3)	145·8 (2)	
N–H^3···$O^{\delta 1}$–C^γ	2·806 (1)	1·782 (3)	168·1 (2)	129·2 (2)
$N^{\delta 2}$–$H^{\delta 21}$···O^2–C	2·927 (1)	1·906 (3)	173·3 (2)	107·3 (2)
$N^{\delta 2}$–$H^{\delta 22}$···O^1–C	3·026 (1)	2·200 (3)	138·0 (2)	121·1 (2)
O^w–H^{w1}···O^2–C	2·843 (2)	1·888 (3)	171·5 (3)	133·5 (3)
O^w–H^{w2}···O^2–C	2·812 (2)	1·879 (3)	164·3 (3)	122·6 (3)

Fig. 3. Stereoscopic view of one unit cell. Molecular bonds, heavy lines; hydrogen bonds, thin lines.

standard deviation for u_{ij} of 0·0005 Å^2, when we take into consideration that the molecule is suspended in a strong hydrogen-bonded system. The effective screw translations were found to be negligible and the largest mean square translation and rotations are 0·023 (1) Å^2 and 0·0045 (7) rad^2 respectively. The corrected bond distances are given in Table 3.

Correction of the N–H and C–H distances for thermal motion was carried out by treating the appropriate groups as independent units in the following way. For the NH$_3$ group the contribution of the rigid body motion of the non-hydrogen framework was subtracted from the vibration tensors of the atoms H^1, H^2, H^3, and these three atoms were then assumed to behave as a rigid body with site symmetry $3m$ and principal axis along the C$^\alpha$–N axis. The r.m.s. goodness-of-fit for the residual u_{ij} is, in this case, 0·004 Å^2 and the only component of the T, L and S tensors which differs by more than two estimated standard deviations from zero is the rotation around the principal axis, with a mean square amplitude of 0·023 (4) rad^2. This value is somewhat larger than that for the ammonium group in alanine (Lehmann, Koetzle & Hamilton, 1972) which is 0·0106 (13) rad^2, indicating that the hydrogen bond system surrounding the ammonium group in asparagine is weaker than that in alanine. The H$\cdots$O distances in alanine range from 1·78 to 1·86 Å with an average of 1·82 Å, while in asparagine the average H$\cdots$O distance is 1·85 Å. The respective average N–H$\cdots$O angles in the two compounds are 164 and 157°, but there are still too few examples in the literature to decide whether the hydrogen bonding parameters offer a sufficient explanation of the differences in NH$_3^+$ libration between asparagine and alanine. If we assume the potential function for the ammonium group to be $V(\alpha)=\frac{1}{2}V_o(1-\cos 3\alpha)\approx\frac{1}{4}V_o3^2\alpha^2$, where α is the angle of rotation around the principal axis, and if we use the expressions for the harmonic oscillator, we find in asparagine a barrier to rotation, V_o, of 7(1) kcal.mole^{-1}. This is larger than the values of about 3·5 kcal.mole^{-1} found for compounds like ethane and propane from *ab initio* LCAO SCF MO calculations (Radom & Pople, 1970), thus indicating that the hydrogen bonding has a considerable effect on the barrier height.

For the CH$_2$ and the NH$_2$ groups we followed a modification of the procedure described by Kamb, Hamilton, La Placa & Prakash (1972) in their treatment of the rigid-body motions in Ice IX. The two groups were described in orthogonal coordinate systems with the first axis parallel to the H$\cdots$H vector and the second axis orthogonal to the plane of the group. We assumed the S tensors to be identically zero and the origins were chosen at the heavy atom. The components of the T tensor, L_{22}, L_{12}, L_{13}, L_{23} and a linear function of L_{11} and L_{33} can then be determined.

All the components of L were set to zero except for L_{22}, which is usually referred to as the rocking com-

ponent of the group. This description seems justified by the good agreement between observed and calculated u_{ij}; the goodness-of-fit for u_{ij} is 0·005 Å^2 for both the CH$_2$ and the NH$_2$ groups. The L_{22} component is 0·035 (6) and 0·017 (6) rad^2 for CH$_2$ and NH$_2$ respectively, showing that the rocking motion is less for hydrogen bonded groups. For the NH$_2$ group, most of the motion can be described by the translation. A calculation involving only the T tensor gave a goodness-of-fit of 0·006 Å^2, indicating that the correction for thermal motion will be small.

The water molecule was treated similarly to the CH$_2$ and NH$_2$ groups. The r.m.s. goodness-of-fit was 0·002 Å^2, and the translation was nearly isotropic with an average mean square value of 0·042 (5) Å^2. The L_{22} component was 0·021 (3) rad^2, in good agreement with the value given above for the hydrogen-bonded NH$_2$ group.

Corrected distances for the three groups are given in Table 3. The C$^\beta$–H$^{\beta 1}$ and C$^\beta$–H$^{\beta 2}$ distances compare well with the standard value of 1·096 Å. A calculation of the angles in the molecular framework after correction for thermal motion showed only small differences from the uncorrected case, so no special list is given for these quantities.

Inter-experimental comparison

The availability of the other neutron data set (Ramanadham, Sikka & Chidambaram, 1972) and the rather precise X-ray data set (Kartha & de Vries, 1961) has provided us with a splendid opportunity for inter-experimental comparison. We have analyzed the differences between the parameters for the three data sets, both by preparing half-normal probability plots (Abrahams & Keve, 1971) and by the use of χ^2 tests as we have previously discussed (Hamilton, 1969). For comparison with the X-ray data, we have considered only the heavy atom parameters, as there are known systematic differences between hydrogen atoms refined by neutrons and by the usual X-ray techniques. The three sets of parameters considered in the analysis were identified in the following way:

BNL   results of the present refinement;
BHA   the refinement of Ramanadham *et al.*;
XRAY   the X-ray parameters of Kartha & de Vries as further refined by Kartha.

In order to err on the conservative side in assessing the differences, we have used, with the BHA parameters, some slightly larger standard deviations which arose from our refinement of the Bhabha data (not reported here).

Half-normal probability plots of the parameter differences

$$|\Delta p_i/\sigma(p_i)|$$

were prepared for each pair of parameter sets. A few examples are shown in Fig. 4. In all but one case (the BNL–XRAY comparison for positional parameters)

the straight lines obtained indicate that the errors are indeed approximately normally distributed; the deviations of the slopes of these lines from unity however indicate that in most cases the pooled standard deviations are underestimated. The slopes of the lines are presented in Table 7. The underestimation of the
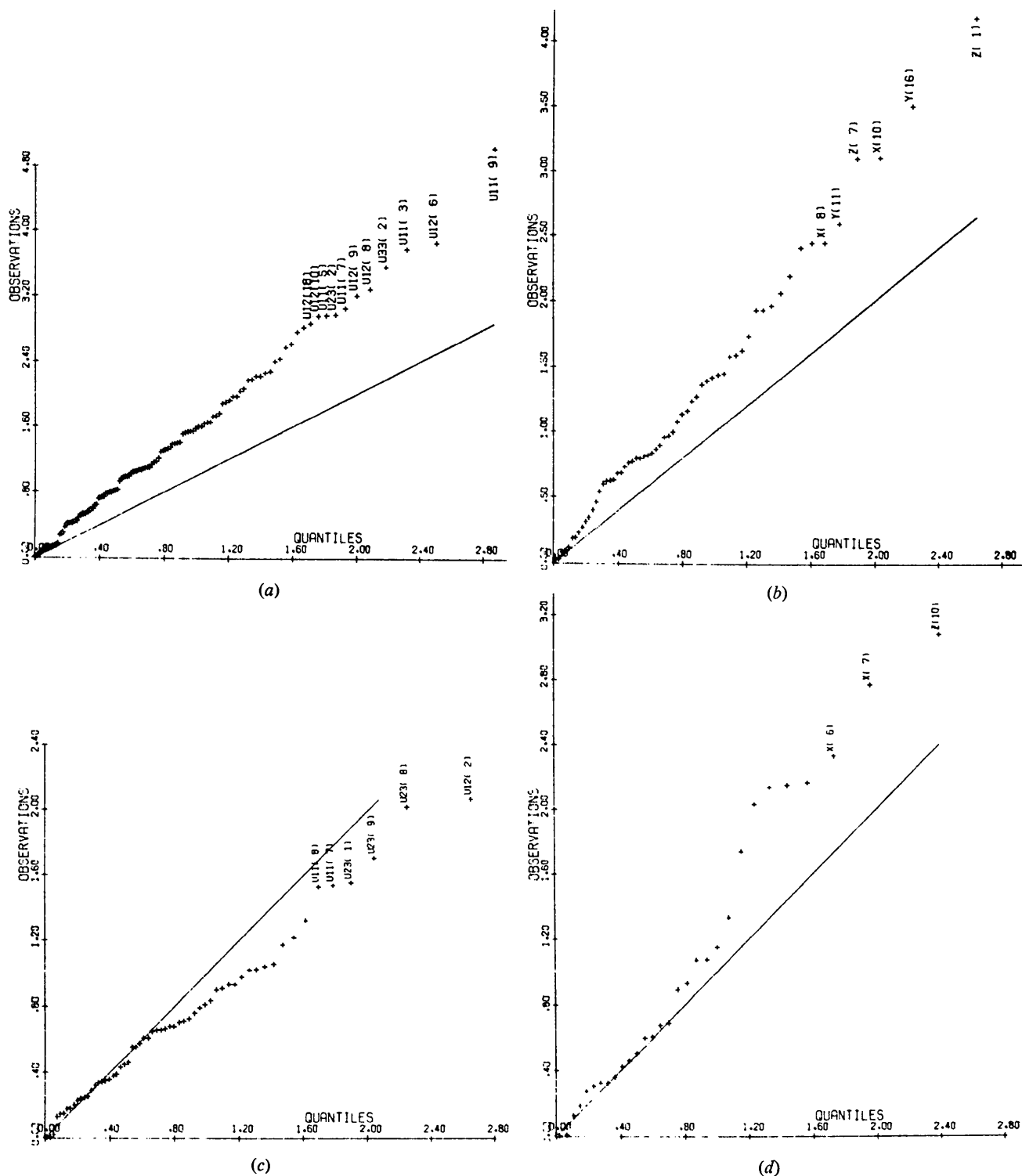

Fig. 4. Half-normal probability plots for parameter comparisons. The straight lines would be expected for normal populations with unit variance and zero mean. (a) BNL vs BHA thermal parameters. (b) BNL vs BHA position parameters. (c) BNL vs X-ray thermal parameters. (d) BNL vs X-ray position parameters. The other plots were all very similar to (a), (b), (c) with the slopes indicated in Table 7. Only plot (d) indicates significant deviations from non-normality.

pooled standard deviation for the difference defined by $\sigma=(\sigma_1^2+\sigma_2^2)^{1/2}$ cannot, of course, be divided *a priori* between the two experiments. However, since the BHA standard deviations are generally much larger than those for BNL and XRAY, for any comparisons involving these data sets the pooled standard deviation arises almost entirely from the BHA data set. Thus it seems fair to state that the underestimation of the σ's is about 1·3–1·6 for the parameters derived from the Bhabha data. For the BNL–XRAY comparisons, the standard deviations are of the same order of magnitude, and the best assumption seems to be that there are equal underestimates of error in both experiments, namely by a ratio of less than 1·3.

Table 7. *Summary of statistical comparisons among the three parameter sets*

	BNL–BHA	BNL–XRAY	BHA–XRAY
Normal probability plot slopes			
Position	1·6	1·0–1·3	1·6
Thermal	1·6	1·0	1·8

Parameter types for which there are significant values of χ_α^2 for $\alpha=0·01$

$x, z, \beta_{11}, \beta_{33}$	x	$z, \beta_{11}, \beta_{33},$
β_{12}, β_{13}		β_{12}, β_{13}

Largest values of mean Δ/σ

$-8·96\ (\beta_{11})$	$2·75\ (\beta_{33})$	$5·58\ (\beta_{11})$
$-6·70\ (\beta_{33})$		$8·39\ (\beta_{33})$

Although the normal probability plots give a good overall picture of the error distributions, other interesting facts are revealed only by a detailed examination of various parameter classes. The sum of the squares of the deviations for all the x parameters, for example, may be tested as χ^2, with the number of degrees of freedom being equal to the number of atoms. Parameters for which there are significant values of χ^2 at the 0·01 probability level are also indicated in Table 7. Two facts emerge from this comparison: the BNL and XRAY results agree well except for the x parameters. Secondly, the large parameter differences are for essentially the same classes of parameters for the BHA–XRAY and BHA–BNL comparisons. This strongly suggests that in addition to an underestimate of error in all data sets, there is some systematic difference between the BHA data set on the one hand and the BNL and XRAY data sets on the other hand.

More revealing yet is the examination of the mean values for the parameters. If we look at the mean values of the differences of the various parameters, *e.g.* $[\beta_{11}(1)-\beta_{11}(2)]$ averaged over all atoms, we can see, for example, whether the β_{11} for parameter set 1 are in general larger than those for parameter set 2. Again in Table 7 we have listed the parameter types for which the mean value divided by σ is unusually large. The principal offenders are β_{11} and β_{33} for comparisons between the BHA data and the XRAY or BNL data. The indication is that the values of β_{11} and β_{33} are systematically high in the Bhabha as compared with the BNL and XRAY sets. This suggests some system-

atic error in these parameters in the Bhabha data.

These comparisons are instructive in that they indicate the value of comparing two sets of data by normal probability plots, by χ^2 tests, and by tests on mean parameters to reveal systematic error.

Finally, we suggest that the considerably smaller standard deviations for the BNL parameters as compared to the Bhabha parameters and the good agreement between the BNL and X-ray parameters for the heavy atoms imply that the BNL structural parameters are the most definitive available for L-asparagine monohydrate, but that to be on the conservative side, the standard deviations recorded in our tables should be multiplied by 1·3.

Computer programs

All calculations were performed on a CDC 6600 computer using programs from the Brookhaven Crystallographic Computing Library. A brief description of these programs has been given by Schlemper, Hamilton & La Placa (1971).

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