Reduction in Crystal Symmetry of a Solid Solution: A Neutron Diffraction Study at 15 K of the Host/Guest System Asparagine/Aspartic Acid

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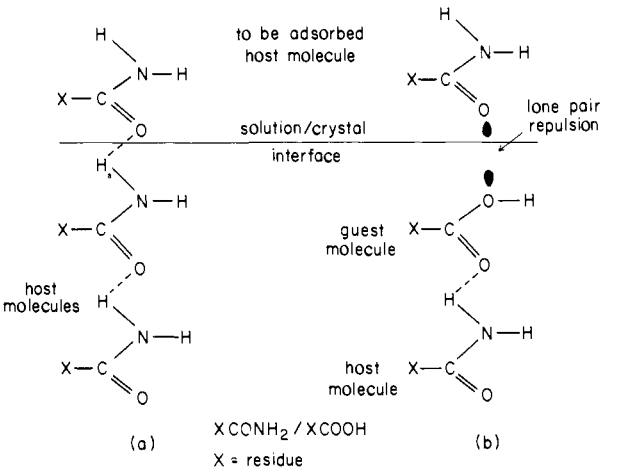
Abstract: We have demonstrated, for the first time by diffraction methods, that a solid solution composed of host and guest molecules can exhibit a crystal symmetry lower than that of the host. The study proves that the symmetry of a solid solution is dependent not only upon the host crystal structure and the guest molecular structure but also upon the surface structure and symmetry of the host crystal. The crystal structures of (*S*)-asparagine monohydrate [$D_2NCOCH_2CH(ND_3)CO_2 \cdot D_2O$] and of the solid solution (0.848:0.152) (*S*)-asparagine/(*S*)-aspartic acid [$DO_2CCD_2CD(ND_3)CO_2$] monohydrate were refined by using neutron diffraction data obtained at 15 K. The space group of the pure host crystal is $P2_12_12_1$ ($Z = 4$), whereas that of the host/guest crystal is monoclinic $P12_11$ with two molecular sites per asymmetric unit. The ratios of guest/host occupancies of the two independent sites are 0.173:0.827 and 0.132:0.868. The reduction in symmetry is in accordance with the preferred adsorption of guest aspartic acid on the (010) crystal face at half of the orthorhombic, symmetry-related surface sites. Aspartic acid mimics, at the preferred (010) surface sites, molecular asparagine, participating in all hydrogen bonds. At the less-favored (010) surface sites a "normal" N-H···O(host) hydrogen bond is replaced by O(hydroxyl)···O(host) repulsion between lone-pair electrons.

Recently we reported changes in crystal habit and composition during crystallization caused by the presence in solution of minor amounts (1-10%) of an additive with molecular structure similar to that of the host, but sufficiently different that only minute (up to 1%) amounts thereof were eventually occluded into the crystal.[1] We found also that those faces normal to directions in which crystal growth is inhibited are etched by the same additives on partial dissolution of the crystal.[2] A stereochemical correlation was established between the molecular structure of the additive, the crystal surface structure of the host, and the faces affected on growth and dissolution. We could infer that the additive may be adsorbed only at the surface sites on those faces such that the part of the absorbate that differs from the host emerges from the surface. Therefore, the additives will be anisotropically distributed within the crystal, preferentially occluded through different subsets at surface sites on the various faces leading to a mixed crystal of segments coherently compounded together. Furthermore, since the point symmetry of the crystal surface at which the additive is occluded is generally lower than that of the bulk, the occluded additive would occupy a subset of all the symmetry-related sites leading to a reduction in crystal symmetry.

The reduction in crystal symmetry has been inferred in crystalline host/additive systems such as (*R,S*)-serine/(*R,S*)-threonine[3] and in glycine/(*R,S*)-α-amino acids[4] by the change in crystal habit and from the enantiomeric segregation of additive occluded in the crystal. In these cases, reduction in symmetry could not be detected by X-ray diffraction measurements since the concentration of occluded additive was too low (maximally 0.1%).

The question then arose whether such a reduction in symmetry would occur in host/additive systems where the difference in molecular structure and shape is less pronounced than between serine and threonine or glycine and alanine. For this purpose we made use of the class of compounds $XCONH_2/XCO_2H$, in which the morphology of the host amide crystal undergoes a marked change when grown in the presence of the corresponding carboxylic acid.[1] In all cases examined there is a strong inhibition of growth along the direction in which the host amide molecules are interlinked by O=C—N—H_a···O=C hydrogen bonds, where H_a refers to the H atom that is in the antiplanar O=C—N—H conformation. The inhibition of crystal growth in these systems

Scheme I



may be explained, without exception, in terms of a replacement of an N-H_a···O(host amide) hydrogen bond at a normal surface site (see Scheme Ia) by a O(guest hydroxyl)···O(host amide) repulsion between the adjacent lone-pair electrons of the adsorbed carboxylic acid additive and of an oncoming amide molecule (Scheme Ib). The repulsive nature of this O···O interaction where the two oxygen atoms are separated by 2.9-3.0 Å (i.e., the N-H···O distance) has been experimentally demonstrated by the geometric deformations induced in the hydrogen-bonding arrangement of the 2:1 crystalline complex benzamide/succinic acid[5] in which the O(hydroxyl)···O(carbonyl) distance is forced to be as long as 3.6 Å. Furthermore, potential energy calculations[6] have

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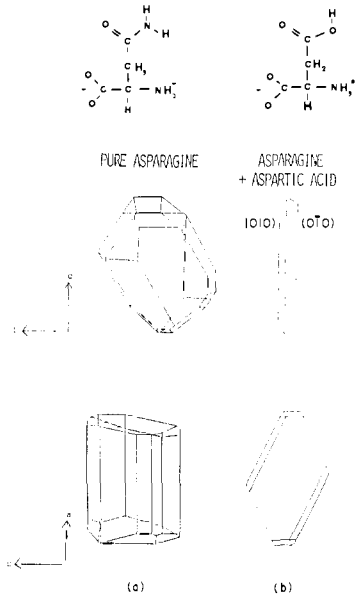

Figure 1. Crystal morphology obtained from aqueous solution. (a) Pure (*S*)-asparagine monohydrate; (b) (*S*)-asparagine monohydrate in presence of (*S*)-aspartic acid.

shown the O···O interaction energy to be about +2 kcal/mol for a separation distance of 3 Å.

In the preceding paper[7] we described the use of such an O···O repulsion to induce a reduction in crystal symmetry in the host/additive system cinnamamide/cinnamic acid. We demonstrated by solid-state photodimerization that the host/additive crystals were composed of two chiral enantiomorphous halves, the structure of the pure host crystal being monoclinic centrosymmetric (space group $P2_1/c$). The resemblance between host and guest was enough for the cinnamic guest to be occluded in all four "symmetry-related" sites of the unit cell, but with sufficiently different occupancies to lead to a reduction in crystal symmetry. However, the concentration of occluded cinnamic acid was too low (~1%) for detection of a lowering in crystal symmetry by X-ray or neutron diffraction.

A system that illustrates the above principles, and may be regarded as a true solid solution, is asparagine/aspartic acid,[8] in which as much as 16% of aspartic acid can be occluded. This increase in concentration of occluded guest, vis-á-vis cinnamic acid, is by virtue of the strong H bonding between asparagine (Asn) and aspartic acid (Asp). The racemic mixture (*R*,*S*)-asparagine resolves spontaneously upon crystallization from water as a conglomerate in space group $P2_12_12_1$, with the molecular structure and morphology[9] shown in Figure 1a. Crystallization of (*S*)-Asn in the presence of (*S*)-Asp yields {010} plates of Asn/Asp (Figure 1b). We may envisage preferental absorption of Asp at a subset of all possible (010) surface sites of a crystal growing from the (010) face as shown in Figure 2. The Asp molecules are easily absorbed at the A and C type surface sites on the (010) face since the lone-pair electron lobe of its hydroxyl oxygen atom emerges from the surface. Asp is less easily absorbed at B and D type surface sites on this face since the "normal" NH_a···O(carboxylate) hydrogen bond between Asn molecules at such a site would be replaced by an O(hydroxyl)···O(carboxylate) repulsion between Asp and Asn. Under such conditions Asp should, on eventual occlusion, preferentially occupy two of the four symmetry-related sites (A and C type sites) so that the symmetry of Asn/Asp would

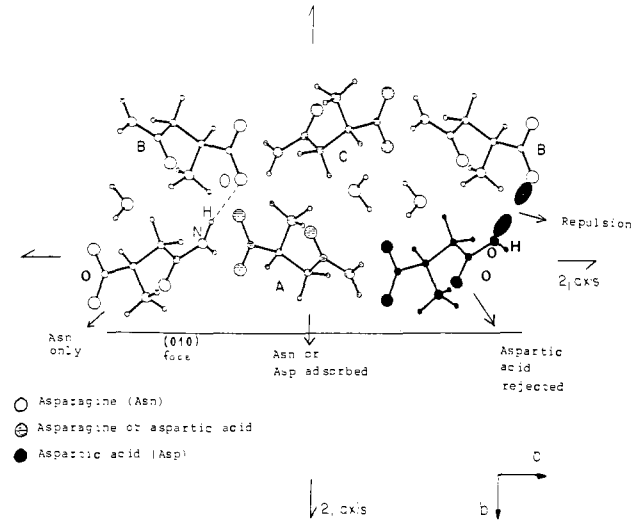

Figure 2. Proposed structure for the preferential adsorption of aspartic acid on the (010) surface of asparagine.

be reduced to $P12_11$, as opposed to $P2_12_12_1$ in the pure crystal. Naturally the roles of the (A,C) and (B,D) surface sites would be reversed were the crystal to grow only from the (0$\bar{1}$0) face. This reduction from orthorhombic to monoclinic symmetry should be easily discernible from the Laue diffraction symmetry of the crystal, provided the Asp molecules are occluded through only one of the two opposite {010} faces. Otherwise the crystal will contain two halves, each of symmetry $P12_11$, related to each other by overall 2-fold symmetry about the *a* and *c* axes passing through the center of the crystal. The true symmetry will thus be masked in a manner analogous to that of a twinned crystal; the overall crystal symmetry will appear to be orthorhombic, certainly in terms of the Laue diffraction pattern. This factor may be responsible for the fact that no other diffraction experiments appear to have been reported describing such a reduction in symmetry of mixed crystals.

A conventional X-ray diffraction study[10] of a single crystal of Asn/Asp cooled to approximately 100 K did not allow detection of the drop in crystal symmetry, presumably because the X-ray scattering powers of the host and guest molecules are almost equal. Thus we embarked on a neutron diffraction study at 15 K with asparagine C–H (protonated) and aspartic acid C–D (deuteriated), so as to take advantage of the radically different neutron scattering amplitudes of H and D. Neutron data of pure Asn were also measured at 15 K in order to obtain precise molecular parameters in the host crystal for use in the structure refinement of the Asn/Asp solid solution. Furthermore, to facilitate detection of the hydroxyl hydrogen atom of guest Asp and to lower the background level of the incoherent neutron scattering, the labile H atoms attached to N and O atoms of Asn and Asp were replaced by D atoms.

The principle we shall demonstrate in this paper is at variance with the following symmetry constraint outlined by Kitaigorodsky in his analysis on solid solutions.[11] He wrote that *guest molecules will occupy all symmetry-related sites in the crystal with equal probability*. This requirement is completely consistent with bulk structural properties, but breaks down when surface structure is to be taken into account.

Results and Discussion

The details of the neutron intensity data measurements on a single crystal of pure Asn (C–H protonated) and Asn (C–H protonated)/Asp (C–D deuteriated) are given in the Experimental

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Table I. Crystallographic Data and Neutron Diffraction Measurements[a]

	pure asparagine	asparagine/ aspartic acid
principal faces	{011}, {101}, {012} {111}	{101}, {001}, {010} {111}
dimensions mm	2.6 × 2.8 × 1.4	3.2 × 5.8 × 3.0
V, mm^3	3.68	9.06
absorp coeff, μ·cm^{-1})[b]	0.799	0.799
	Crystal Data	
space group	$P2_12_12_1$	$P12_11$
lattice constants		
a, Å	5.581 (2)	5.570 (2)
b, Å	9.730 (2)	9.765 (2)
c, Å	11.683 (3)	11.674 (3)
a, deg	90.00	89.99 (2)
β, deg	90.00	90.05 (2)
γ, deg	90.00	89.96 (2)
cell vol, Å^3	634.4 (5)	635.0 (7)
d, g·cm^{-3}	1.436	1.439
	Diffraction Measurements	
temp, K	15.0 (5)	15.0 (5)
wavelength, Å	1.0505 (1)	1.0505 (1)
scan method	$\omega/2\theta$	$\omega/2\theta$
scan lengths ($0° < 2\theta < 55°$)	3.0	4.2
in 2θ ($55° < 2\theta < 106°$)	1.39 + 2.67 tan θ	3.53 + 2.40 tan θ
reciprocal lattice sector	$(+h,+k,+l)$	$(\pm h,\pm k,+l)$
sin θ/λ limit, Å^{-1}	0.765	0.765
no. of total observns	1424	4871
no. of independ observns (N)	1424	4871 (P1)
internal agreement[c] assumed space group		
$P2_12_12_1$		0.0282
$P12_11$		0.0185
$P112_1$		0.0249
$P2_11$		0.0267

[a] Numbers in parentheses are estimated standard deviations in the least significant digit given, here and throughout this paper. [b] Evaluated with the mass absorption coefficient of hydrogen (μ/ρ = 24.88 cm^2·g^{-1}), taken from ref 16c. [c] $R(F^2) = \sum_i|\bar{F}_o^2 - F_{o,i}^2|/\sum_i F_{o,i}^2$.

Section. These measurements are summarized in Table I. The crystal structure of pure Asn has been precisely determined in the straightforward refinement, yielding fit indexes $R(F) = 0.020$, and $R_w(F) = 0.021$. The fractional atomic coordinates and displacement parameters are given in Tables III and IV, respectively. The bond distances and angles are given in Table V. Figure 3 shows the asparagine and water molecules with atomic notation; Figure 4 illustrates the packing of molecules with space group symmetry $P2_12_12_1$.

Table I lists the internal agreement factors of the Asn/Asp data involving observed symmetry-related $F^2(hkl)$ for the four point groups 222, 121, 112, and 211 corresponding to space groups $P2_12_12_1$, $P12_11$, $P112_1$, and $P2_11$, respectively. The $R(F^2)$ factor of 0.0185 for point group 121 (i.e., space group $P12_11$) is significantly lower than those of the other three point groups. We note that 121, 112, and 211 correspond to the same point group but with the 2-fold symmetry axis assumed to be parallel to b, c, and a, respectively. Least-squares refinements for the four models were carried out (see Table II). Many constraints on interatomic distances, "atomic displacement parameters", and molecular occupancy factors were imposed in the refinement because of host/guest molecular overlap and the symmetry requirement that the monoclinic structures each contain two independent sites, whereas the orthorhombic structure contains only one site. Refinement assuming $P2_12_12_1$ symmetry yielded an occupancy of 0.150 (2) for guest Asp, and $R(F)$ and $R_w(F)$ of 0.0349 and 0.0260, respectively. The two "monoclinic" structures $P112_1$ and $P2_11$ are no different from the orthorhombic $P2_12_12_1$

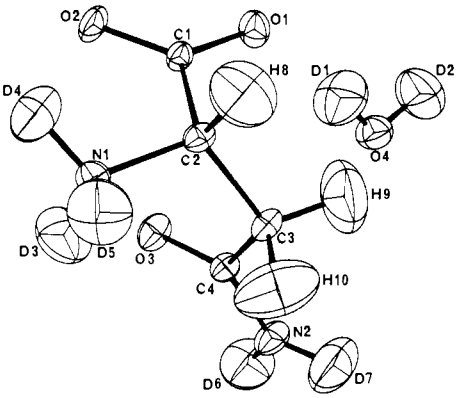

Figure 3. Individual asparagine and water molecules with key to atomic nomenclature. They are oriented as in sites C (Figure 4). The thermal ellipsoids, which enclose 98% probability surfaces,[12] represent the U_{ij} parameters in Table IV.

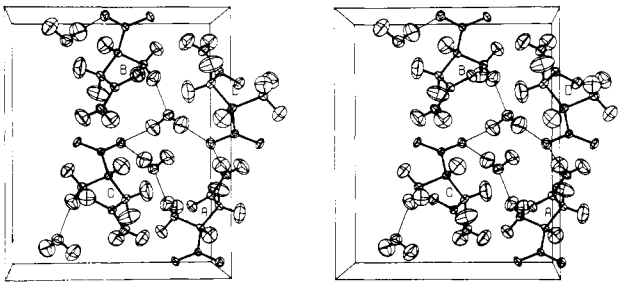

Figure 4. Stereoscopic diagram of the asparagine structure at 15 K viewed down $+a$ in a right-handed system with b horizontal. The equivalent molecules (A, B, C, D) are designated as in the text and in Table II. The thermal ellipsoids enclose 98% probability surfaces. Hydrogen bonds are indicated by thin lines.

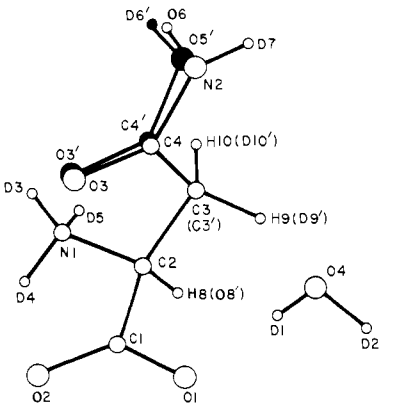

Figure 5. The overlap between asparagine and aspartic acid molecules in one of the two sites in space group $P12_11$.

Table II. Refinement of Asn/Asp in Assumed Space Groups

	space gp	no. of reflcns	no. of param	$R(F)$	$R_w(F)$	refined occupancy of aspartic acid at sites[a] A	B	C	D
a	$P2_12_12_1$	4837	169	0.0349	0.0260	0.150 (2)	0.150 (2)	0.150 (2)	0.150 (2)
b	$P12_11$	4835	237	0.0330	0.0243	0.173 (2)	0.132 (2)	0.173 (2)	0.132 (2)
c	$P112_1$	4836	237	0.0352	0.0263	0.148 (3)	0.155 (3)	0.155 (3)	0.148 (3)
d	$P2_11$	4837	237	0.0353	0.0264	0.145 (3)	0.145 (3)	0.158 (3)	0.158 (3)
e	$P1$	4837	293	0.0333	0.0245	0.161 (3)	0.127 (3)	0.185 (3)	0.135 (2)

[a] (A) x, y, z; (B) $^1/_2 + x, ^1/_2 - y, -z$; (C) $-x, ^1/_2 + y, ^1/_2 - z$; (D) $^1/_2 - x, -y, ^1/_2 + z$.

Table III. Fractional Coordinates of Asparagine at 15 K: Orthorhombic $P2_12_12_1$

atom[a]	x	y	z	OCC
C1	−0.1287 (1)	−0.0568 (1)	0.0345 (1)	1.000
C2	−0.3101 (1)	−0.0261 (1)	0.1303 (1)	1.000
C3	−0.2099 (1)	0.0601 (1)	0.2277 (1)	1.000
C4	0.0164 (1)	−0.0006 (1)	0.2796 (1)	1.000
N1	−0.4184 (1)	−0.1560 (1)	0.1737 (0)	1.000
N2	0.1075 (1)	0.0653 (1)	0.3698 (0)	1.000
O1	−0.0108 (2)	0.0450 (1)	−0.0023 (1)	1.000
O2	−0.1214 (2)	−0.1760 (1)	−0.0047 (1)	1.000
O3	0.1087 (2)	−0.1053 (1)	0.2377 (1)	1.000
O4	0.2970 (2)	0.2264 (1)	0.1127 (1)	1.000
D1	0.1793 (2)	0.1717 (1)	0.0724 (1)	0.808 (5)
D2	0.3449 (3)	0.3010 (1)	0.0627 (1)	0.808 (5)
D3	−0.3146 (2)	−0.2030 (1)	0.2353 (1)	0.839 (3)
D4	−0.4473 (2)	−0.2249 (1)	0.1071 (1)	0.839 (3)
D5	−0.5848 (2)	−0.1363 (1)	0.2101 (1)	0.839 (3)
D6	0.2573 (2)	0.0272 (1)	0.4098 (1)	0.839 (3)
D7	0.0333 (2)	0.1529 (1)	0.3997 (1)	0.839 (3)
H8	−0.4557 (3)	0.0329 (2)	0.0905 (1)	1.000
H9	−0.1692 (4)	0.1639 (2)	0.1969 (2)	1.000
H10	−0.3451 (3)	0.0717 (2)	0.2957 (1)	1.000

[a] The hydrogen atoms corresponding to D1 through D7 have occupancy factors (1 − OCC).

Table IV. Atomic Displacement Parameters[a] (×10⁴) of Asparagine at 15 K: Orthorhombic $P2_12_12_1$

atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C1	38 (3)	33 (3)	37 (3)	−5 (2)	6 (2)	−1 (2)
C2	36 (3)	43 (3)	36 (3)	−7 (2)	−5 (2)	2 (3)
C3	42 (3)	42 (3)	45 (3)	−15 (2)	−3 (2)	3 (2)
C4	35 (3)	44 (3)	41 (3)	−6 (2)	−3 (2)	1 (2)
N1	40 (2)	48 (2)	43 (2)	−0 (2)	6 (2)	−8 (2)
N2	60 (2)	66 (2)	56 (2)	−19 (2)	−19 (2)	5 (2)
O1	61 (3)	47 (3)	63 (3)	2 (3)	15 (3)	−7 (3)
O2	71 (3)	39 (3)	56 (3)	−21 (3)	8 (3)	1 (3)
O3	49 (3)	57 (3)	74 (3)	−16 (3)	−2 (3)	18 (3)
O4	80 (4)	79 (3)	65 (3)	−5 (3)	−3 (3)	−9 (3)
D1	155 (5)	156 (5)	162 (5)	−27 (4)	−30 (4)	−52 (4)
D2	207 (6)	134 (5)	145 (5)	38 (4)	8 (4)	−34 (4)
D3	131 (4)	125 (4)	131 (4)	35 (3)	−39 (4)	3 (4)
D4	181 (5)	103 (4)	111 (4)	−36 (3)	5 (4)	−24 (4)
D5	94 (4)	145 (4)	151 (4)	2 (4)	44 (4)	−4 (4)
D6	123 (4)	167 (5)	137 (4)	−3 (4)	−47 (4)	18 (4)
D7	183 (5)	127 (4)	150 (4)	−52 (4)	−9 (4)	37 (4)
H8	134 (7)	163 (6)	162 (7)	19 (6)	−42 (6)	39 (6)
H9	259 (9)	93 (6)	245 (8)	24 (6)	−36 (7)	−35 (7)
H10	143 (7)	289 (9)	140 (6)	−58 (6)	34 (6)	25 (7)

[a] Displacement parameters are of the following form $\exp(-2\pi^2\sum\sum h_i h_j a_i^* a_j^* U_{ij})$.

structure, since the occupancies of Asp at the two independent sites for each structure do not differ significantly: 0.148 (3) vs 0.155 (3) for $P112_1$ and 0.145 (3) vs 0.158 (3) for $P2_111$ (see Table II). Moreover, the $R(F)$ and $R_w(F)$ values for $P2_12_12_1$, (0.0349, 0.0260) are as low as those for $P112_1$ and $P2_111$ (0.0352, 0.0263; and 0.0353, 0.0264). On the other hand, the $R(F)$ and $R_w(F)$ values for $P12_11$ (0.0330, 0.0243) are significantly lower than those of the other three orthorhombic and monoclinic space groups. According to the Hamilton test[13] (see Experimental Section), $P12_11$ is the preferred space group with a significance level greater than 99.5% (Table II). The occupancies of Asp at the two independent sites of space group $P12_11$ are 0.132 (3) and 0.173 (2), decidedly different from one another. The crystal structure has also been refined in space group $P1$ with four independent sites, but with constraints applied to the molecules. This refined triclinic structure does not differ significantly from the $P12_11$ structure (see Table II). The final atomic parameters from refinement in space group $P12_11$ are given in Table VI. Figure 5 shows the extent of overlap between the asparagine and aspartic acid molecules.

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Table V. Bond Distances (Å) and Bond Angles (deg) in Asparagine at 15 K

Bond Distances			
C1–O2	1.212 (1)	C1–O1	1.247 (1)
C1–C2	1.539 (1)	C2–N1	1.490 (1)
C2–C3	1.520 (2)	C3–C4	1.520 (1)
C4–O3	1.242 (1)	C4–N2	1.334 (1)
C2–H8	1.098 (2)	C3–H9	1.096 (2)
C3–H10	1.102 (2)	N1–D3	1.031 (1)
N1–D5	1.039 (1)	N1–D4	1.040 (1)
N2–D6	1.027 (1)	N2–D7	1.010 (1)
O4–D1	0.968 (2)	O4–D2	0.969 (2)
Bond Angles			
O1–C1–C2	114.36 (9)	O2–C1–C2	114.65 (9)
O2–C1–O1	130.98 (11)	C3–C2–C1	114.18 (6)
N1–C2–C1	110.48 (8)	N1–C2–C3	111.25 (8)
H8–C2–C1	106.28 (12)	H8–C2–C3	107.50 (12)
H8–C2–N1	106.70 (11)	C4–C3–C2	112.94 (8)
H9–C3–C2	109.81 (15)	H9–C3–C4	108.47 (13)
H10–C3–C2	110.15 (10)	H10–C3–C4	108.74 (12)
H10–C3–H9	106.52 (17)	N2–C4–C3	116.41 (8)
O3–C4–C3	120.39 (10)	O3–C4–N2	123.19 (9)
D3–N1–C2	112.70 (9)	D4–N1–C2	110.81 (8)
D4–N1–D3	108.88 (12)	D5–N1–C2	110.20 (10)
D5–N1–D3	107.36 (9)	D5–N1–D4	106.65 (10)
D6–N2–C4	119.74 (10)	D7–N2–C4	121.50 (9)
D7–N2–D6	118.75 (10)	D2–O4–D1	107.81 (15)
C4–O3–D5	127.85 (9)		

Hydrogen Bond Distances and Angles

A–H···B–C	A···B	H···B	⟨A–H···B	⟨H···B–C
N1–D4···O2–C1	2.881 (1)	1.892 (2)	156.6 (1)	138.8 (1)
N1–D3···O4–C1	2.828 (1)	1.907 (2)	147.0 (1)	
N1–D5···O3–C2	2.787 (2)	1.767 (2)	166.3 (1)	
N2–D6···O1–C1	2.935 (1)	1.914 (2)	173.7 (1)	106.5 (1)
N2–D7···O2–C1	2.914 (1)	2.064 (2)	140.0 (1)	121.6 (1)
O4–D1···O1–C1	2.780 (2)	1.821 (2)	171.0 (1)	131.4 (1)
O4–D2···O1–C1	2.811 (2)	1.863 (2)	163.9 (1)	122.7 (1)

The relative occupancies of Asp of the two independent sites [0.173 (2) at the A type site vs 0.132 (2) at the B type site] are consistent with our proposed mechanism of preferred absorption of aspartic acid at site A rather than B at the (010) surface of the crystal. The crystal grew in solution resting on its (0$\bar{1}$0) face so that of the two {010} faces Asp was absorbed primarily through the exposed (010) face. In terms of our proposed mechanism of preferred absorption of Asp at those (010) surface sites, which requires the lone-pair electron of the hydroxyl oxygen atom to emerge from the (010) surface, Asp should be preferentially absorbed at sites of type A and C in Figures 2 and 4. These sites are unambiguously defined in our crystal of Asn/Asp since we know which {010} face was exposed to solution, and moreover, the absolute configuration of the constituent molecules fixes the absolute structure of the crystal. In the crystal structure of cinnamamide/cinnamic acid,[7] the guest molecules are also preferentially occluded through those surface sites that require the lone-pair electrons of the hydroxyl oxygen atom to emerge from the surface. The percentage differences in occupancy values at sites A and C vs B and D here in Asn/Asp are not nearly as pronounced as those in cinnamamide/cinnamic acid.[7] This result is due to the much more extensive hydrogen bonding between host and guest in Asn/Asp than in cinnamamide/cinnamic acid. (In each case, one hydrogen bond is broken per molecular pair.) The relatively small difference in occupancy of Asp at the A and C vs B and D type sites requires further explanation in terms of the marked difference in morphologies of pure Asn and Asn/Asp. Asp adsorbed on a B or D type site does not induce a pronounced further inhibition of crystal growth, but Asp adsorbed on an A or C type site inhibits the further deposition of the neighboring solute molecule above its site (see Figure 2).

The reduced symmetry of the crystal structure of asparagine/aspartic acid implies a general revision of our description of symmetries of solid solutions. The tendency toward reduced symmetry has already been inferred in several systems where the

Table VI. Fractional Coordinates and U_{iso} or U_{equiv} ($Å^2 \times 10^4$) Displacement Parameters of Asparagine/Aspartic Acid at 15 K: Monoclinic b Unique $P12_11$[a]

atom	x	y	z	OCC	U_{iso} or U_{equiv}[b]
		Molecule A			
C1	−0.1225 (2)	−0.0510 (1)	0.0336 (1)	1.0000	
C2	−0.3047 (1)	−0.0209 (1)	0.1291 (1)	1.0000	
C3	−0.2075 (2)	0.0652 (1)	0.2264 (1)	0.827 (2)	
C4	0.0190 (2)	0.0051 (1)	0.2783 (1)	0.827 (2)	
N1	−0.4117 (1)	−0.1512 (1)	0.1715 (1)	1.0000	
N2	0.1077 (2)	0.0703 (1)	0.3694 (1)	0.827 (2)	
O1	−0.0034 (2)	0.0507	−0.0026 (1)	1.0000	
O2	−0.1156 (2)	−0.1690 (1)	−0.0062 (1)	1.0000	
O3	0.1141 (2)	−0.0985 (1)	0.2357 (1)	0.827 (2)	
O4	0.2987 (2)	0.2323 (2)	0.1144 (1)	1.0000	
D1	0.1832 (2)	0.1771 (2)	0.0725 (1)	0.967 (2)	
D2	0.3501 (2)	0.3051 (2)	0.0640 (1)	0.967 (2)	
D3	−0.3075 (2)	−0.1980 (2)	0.2336 (1)	0.967 (2)	
D4	−0.4391 (2)	−0.2194 (2)	0.1046 (1)	0.967 (2)	
D5	−0.5784 (2)	−0.1323 (2)	0.2077 (1)	0.967 (2)	
D6	0.2574 (2)	0.0325 (1)	0.4097 (1)	0.827 (2)	
D7	0.0316 (2)	0.1570 (1)	0.3998 (1)	0.827 (2)	
D8	−0.4501 (7)	0.0375 (4)	0.0875 (4)	0.827 (2)	
D8	−0.435 (2)	0.053 (1)	0.095 (1)	0.173 (2)	
H9	−0.1674 (6)	0.1681 (2)	0.1948 (3)	0.827 (2)	
H10	−0.3453 (5)	0.0760 (4)	0.2926 (2)	0.827 (2)	
C3′	−0.1966 (8)	0.0551 (5)	0.2301 (3)	0.173 (2)	
C4′	0.0249 (9)	−0.0064 (6)	0.2867 (4)	0.173 (2)	
O3′	0.120 (1)	−0.1076 (7)	0.2498 (6)	0.173 (2)	
O5′	0.100 (1)	0.0511 (7)	0.3839 (6)	0.173 (2)	
D6′	0.244 (2)	0.012 (1)	0.4149 (7)	0.173 (2)	
D9′	−0.137 (2)	0.1580 (7)	0.206 (1)	0.173 (2)	
D10′	−0.312 (2)	0.067 (1)	0.3054 (7)	0.173 (2)	
		Molecule B			
C1	0.3777 (2)	0.5600 (1)	−0.0335 (1)	1.0000	65 (1)
C2	0.1946 (1)	0.5294 (1)	−0.1288 (1)	1.0000	62 (1)
C3	0.2922 (1)	0.4435 (1)	−0.2261 (1)	0.868 (2)	71 (1)
C4	0.5184 (1)	0.5035 (1)	−0.2786 (1)	0.868 (2)	64 (1)
N1	0.0870 (1)	0.6595 (1)	−0.1713 (1)	1.0000	80 (1)
N2	0.6072 (1)	0.4384 (1)	−0.3692 (1)	0.868 (2)	71 (1)
O1	0.4966 (2)	0.4583 (1)	0.0029 (1)	1.0000	87 (1)
O2	0.3854 (2)	0.6783 (1)	0.0057 (1)	1.0000	93 (1)
O3	0.6116 (1)	0.6072 (1)	−0.2361 (1)	0.868 (2)	73 (2)
O4	0.7895 (2)	0.2766 (2)	−0.1145 (1)	1.0000	102 (1)
D1	0.6838 (2)	0.3313 (2)	−0.0727 (1)	0.967 (2)	189 (2)
D2	0.8503 (2)	0.2035 (2)	−0.0641 (1)	0.967 (2)	192 (2)
D3	0.1907 (2)	0.7065 (2)	−0.2334 (1)	0.967 (2)	181 (1)
D4	0.0595 (2)	0.7278 (2)	−0.1044 (1)	0.967 (2)	173 (2)
D5	−0.0799 (2)	0.6403 (2)	−0.2075 (1)	0.967 (2)	175 (1)
D6	0.7588 (1)	0.4771 (1)	−0.4094 (1)	0.868 (2)	155 (2)
D7	0.5303 (1)	0.3516 (1)	−0.3995 (1)	0.868 (2)	179 (2)
H8	0.0495 (7)	0.4706 (4)	−0.0875 (4)	0.868 (2)	189 (6)
D8	0.066 (3)	0.454 (2)	−0.095 (2)	0.132 (2)	189 (6)
H9	0.3324 (6)	0.3405 (2)	−0.1945 (3)	0.868 (2)	223 (6)
H10	0.1548 (4)	0.4324 (4)	−0.2926 (2)	0.868 (2)	231 (6)
C3′	0.302 (1)	0.4535 (6)	−0.2297 (4)	0.132 (2)	71 (1)
C4′	0.525 (1)	0.5136 (7)	−0.2862 (5)	0.132 (2)	64 (1)
O3′	0.622 (2)	0.6140 (9)	−0.2491 (8)	0.132 (2)	73 (2)
O5′	0.595 (1)	0.4574 (9)	−0.3847 (7)	0.132 (2)	71 (1)
D6′	0.738 (2)	0.497 (1)	−0.4168 (9)	0.132 (2)	155 (2)
D9′	0.363 (3)	0.351 (1)	−0.206 (1)	0.132 (2)	223 (6)
D10′	0.192 (2)	0.0443 (2)	−0.3067 (9)	0.132 (2)	231 (6)

Separation Distances (in Å) between Disordered Atomic Sites (See Figure 5)

C3–C3′···0.124 (5)	C3–C3′···0.121 (9)
C4–C4′···0.153 (6)	C4–C4′···0.138 (2)
N2–O5′···0.256 (7)	N2–O5′···0.267 (9)
O3–O3′···0.190 (7)	O3–O3′···0.176 (6)
D6–D6′···0.221 (10)	D6–D6′···0.240 (7)
H9–D9′···0.238 (11)	H9–D9′···0.242 (11)
H10–D10′···0.254 (10)	H10–H10′···0.281 (15)

[a] For carbon, nitrogen, and oxygen atoms, displacement parameters are listed and have the form $\exp[-8\pi^2 U_{iso} (\sin^2\theta/\lambda)]$; for deuterium and hydrogen atoms, the parameters are equivalent isotropic displacements defined as $^1/_3$ of the trace of the orthogonalized U_{ij} matrix. [b] U_{iso} or U_{equiv} are given for atoms of both molecule A and molecule B.

guest occupies only a minor fraction of the crystallographic sites[1,7,14] and now demonstrated conclusively in three systems by diffraction methods[15] (the present study included) where the concentration of occluded guest is greater than 7%.

Experimental Section

Crystals of Asn·D_2O were obtained by slow evaporation from aqueous D_2O solutions of enantiomerically pure (*S*)-Asn [$^+H_3NCH(CH_2CONH_2)CO_2^-$] and recrystallized about five times to ensure pronounced deuteriation of the labile (water) amide and amino groups. A large crystal (see Table I) was eventually obtained and proved suitable for the neutron diffraction measurements.

Crystals of the solid solution were obtained from D_2O solution of (*S*)-asparagine [$^+H_3NCH(CH_2CONH_2)CO_2^-$] grown in the presence of 25% (*S*)-aspartic acid [$^+H_3NCD(CD_2COOH)CO_2^-$]. The resulting precipitate was recrystallized 20 times in D_2O to ensure almost complete deuteriation of the labile amino, amide, and hydroxyl groups. Finally, an isolated large {010} platelike crystal (Table I) of Asn (C–H protonated)/Asp (C–D deuteriated) was grown (from its observed inception) with one of its two opposite {010} faces resting on the flat glass bottom of the beaker. This face was fixed for the purpose of X-ray measurements and structure analysis to be (0$\bar{1}$0). Thus we were assured that, for the most part, Asp additive was not occluded into the crystal through the (0$\bar{1}$0) face in contact with the glass. The pure Asn crystal was completely transparent, whereas the crystal of Asn/Asp was opaque. X-ray Weissenberg photographs of the latter confirmed that it was a single crystal.

Neutron Data Collection. The neutron diffraction measurements were carried out at the Brookhaven high-flux beam reactor. The two crystals were each cooled to 15 K. Details of the neutron data measurements on the single crystals of Asn and Asn/Asp are given in Table I. A full hemisphere of reflections in the 2θ range 0–106° (and a full sphere between 25 and 35°) was measured on the crystal of Asn/Asp in order to make certain of the possible reduction in space group symmetry.

Table I lists the internal agreement factors for Asn/Asp involving the match between equivalent absorption-corrected[16] $F^2(hkl)$ data, assuming the orthorhombic and the three monoclinic point groups (i.e., with the unique axis pointing along *b*, *c*, and *a*, respectively). Here we note that the Asp/Asn crystal with morphologic symmetry close to 222 had been mounted with the *c* axis aligned approximately 8° from the diffractometer ϕ axis so that no obvious bias was made in favor of point groups in terms of the intensity measurement procedure due to multiple reflection effects. Point symmetry 121 corresponding to space group $P12_11$ (i.e., monoclinic with *b* the unique axis) yields an internal agreement factor significantly lower than orthorhombic 222 (corresponding to space group $P2_12_12_1$) or monoclinic 211 and 112 (corresponding to space groups $P2_111$ and $P112_1$).

General Remarks on the Structure Refinement. The overall refinement of the two crystal structures using the observed structure factors, but corrected for extinction,[17] was carried out with the program SHELX.[18] Constraints on many interatomic distances, "atomic displacement parameters", and molecular occupancy factors were imposed in the refinement because of host/guest molecular overlap and the symmetry requirement that the monoclinic structures each contain two independent sites whereas the orthorhombic structure contains only one site. The refined molecular structure of pure Asn was used in the structural model in order to minimize the number of parameters for the refinement of the Asn/Asp structure.

Crystal Structure Refinement of Pure Asn. The starting model was taken from the low-temperature X-ray crystal structure refinement of pure Asn·H_2O.[10] The initial refinement was carried out with the program LINEX.[19] All the atoms were refined with anisotropic atomic displace-

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ment parameters. The anisotropic extinction parameters[17] for a type I crystal with Lorentzian mosaicity were refined and used to correct each observed F_o value. The extinction effects were pronounced, the largest correction to F_o being 3.70. The relative deuterium/hydrogen concentration of the amine, amide, and water groups was determined by refining the crystal structure for different inserted values of the scattering length of the "deuterium" atoms. An $R(F)$ factor of 0.021 was obtained with an overall D to H concentration of 0.82:0.18.

The structure was also refined with SHELX by making use of the observed structure factors corrected for extinction. Intially, four occupancies of the deuterium atoms of the amino, water, and amide groups were refined, yielding values of 0.834 (4) for ND_3, 0.808 (7) for D_2O, 0.836 (7) for ND(6), and 0.850 (7) for ND (7). The refinement was continued with two occupancy parameters, one for D_2O and the other for the remaining labile deuterium atoms (Table III). The final cycle of refinement using 1398 reflections with 188 parameters yielded $R(F)$ = 0.0203 and $R_w(F)$ = 0.021. This study thus provided the precise molecular parameters of Asn at 15 K (Table V) that were needed in a constrained refinement of the solid solution Asn/Asp against 15 K neutron data.

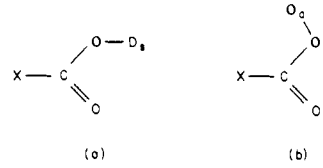
Structure Refinement of Asparagine/Aspartic Acid. The crystal structure of Asn/Asp was refined in each of the five space groups $P2_12_12_1$, $P12_11$, $P112_1$, $P2_111$, and $P\bar{1}$. In order to reduce the number of parameters to a minimum, the following strategy was adopted: The N–D bonds were restrained to have the same length, compatible with the fact that the five bonds are approximately equal in length in pure Asn. The C, N, and O atoms (see Figure 3) were treated isotropically in Asn/Asp. In addition, the overlapping atoms of Asn and Asp were constrained to have the same displacement parameters.

For the monoclinic space groups $P12_11$, $P112_1$, and $P2_111$, which contain two independent molecular sites, the 1···2 and 1···3 intramolecular distances (1···2 specifics bonded atoms, and 1···3 two atoms bonded to the same atom) of chemically equivalent groups of the two sites were restrained to have the same value. Moreover, the corresponding atoms belonging to these two independent molecular sites were constrained to have the same isotropic displacement and equivalent U_{ij} displacements, i.e., compatible with orthorhombic symmetry. For the $P\bar{1}$ unit cell, which contains four independent molecular sites, an analogous refinement procedure was adopted. In order to resolve the atoms of the overlapping asparagine and aspartic acid molecules, it was first assumed that their glycyl moieties overlapped completely; the molecules were assumed to differ only in terms of the geometry of their $COND_2$ and CO_2D moieties and the rotational conformation about the two C–C bonds $C–CH_2–CO–ND_2$ and $C–CD_2–CO_2D$ (Figure 5). Thus, the CH_2COND_2 and CD_2CO_2D moieties were initially refined as rigid groups. At a later stage, the CH_2COND_2 moiety was refined with 1···2 and 1···3 distances taken from the crystal structure of pure Asn; for CD_2CO_2D the 1···2 and 1···3 distances involving the carboxyl group were taken from the low-temperature neutron diffraction structure analysis of *N*-acetyl-L-cysteine.[20]

The sum of occupancies of Asn and Asp at a given molecular site was constrained to have a value equal to 1. The labile D atoms belonging to the amino acid carboxyl and water groups were all refined with the same occupancy, compatible with the fact that in the crystal of pure Asn all the labile D atoms were found to have approximately the same occupancy.

The structure was first refined assuming $P2_12_12_1$ symmetry. The asymmetric unit comprised one molecular site containing overlapping Asn and Asp molecules. In order to resolve the Asn and Asp molecules, the CH_2CONH_2 and CD_2CO_2D moieties were first treated as rigid bodies as described above. Deuteriation of the amine, amide, carboxyl, and water groups was almost complete, with the relative D to H concentration of 0.96:0.04. Comparison of the magnitudes of the observed and calculated structure factors indicated that the extinction was negligible. Indeed, modeling of isotropic extinction correction with the program LINEX had a negligible effect on the $R(F)$ factor. Continued refinement of the $P2_12_12_1$ crystal structure in which the rigid treatment of the CH_2COND_2 and CD_2CO_2D groups was replaced by 1···2 and 1···3 distance restraints yielded $R(F)$ = 0.0349, $R(F^2)$ = 0.0448, and $R_w(F)$ = 0.0261 for all 4837 reflections (see Table IIa). The relative occupancies of the Asp and Asn molecules were 0.150 (2) and 0.850 (2).

The hydroxyl group O–D of Asp may, in principle, adopt either the synplanar (1a) or antiplanar (1b) conformation. We had assumed, in



the refinement, the synplanar (1a) which is the commonly observed and more stable conformation.[21] The low-temperature X-ray structure refinement of Asn/Asp also demonstrated the presence of 1a, but not of 1b. To establish conclusively the orientation of the hydroxyl deuterium atom D6′, shown in Figure 5, the atom was omitted from the refinement. The occupancies of the amide atoms D6 and D7 were refined to compensate the lack of D6′. Refinement yielded an occupancy of 0.545 (2) for D6 and 0.455 (2) for D7, the sum of 0.545 + 0.455 being constrained to equal 1. The fraction 0.455/0.545 = 0.834 is in excellent agreement with the Asn occupancy of 0.85 as determined by the overall refinement. This result shows that the carboxyl group adopts the O=C—O—D synplanar conformation exclusively.

Assumed Monoclinic Space Groups $P12_11$, $P112_1$, and $P2_111$. The three monoclinic structure models each contain two independent molecular sites. The molecular structures and atomic displacement tensors of the two independent sites were almost identical, by virtue of the refinement restraints, but not the occupancies of their constituent Asn and Asp molecules. The results of these refinements are summarized in Table II. According to the Hamilton test,[13] $P12_11$ is the preferred space group with a significance level >99.5%. The $R_w(F)$ values for monoclinic space groups $P112_1$ and $P2_111$ are not significantly different from that of $P2_12_12_1$. Indeed, the values of the occupancies of Asp at the two independent sites in these two space groups (Table IIc,d) are compatible with $P2_12_12_1$ symmetry; the average occupancy of Asp for space group $P112_1$ is 0.152 (4) and 0.152 (7) for space group $P2_111$. These two estimated standard deviations of 0.004 and 0.007 are in the same range as the individual deviations in occupancy of 0.003 (Table II) as derived from the structure-factor least-squares refinement. On the other hand, the two occupancies of Asp for space group $P12_11$, 0.173 (2) and 0.132 (3) (Table IIb), are significantly different from one another.

Assumed Triclinic Space Group $P\bar{1}$. For triclinic space group $P\bar{1}$, the refined occupancies of sites A, B, C, and D closely mimic those of the monoclinic $P12_11$ structure. The Hamilton test[13] is in accord with this observation: the $R_w(F)$ value for $P\bar{1}$ does not show significant improvement over that of $P12_11$.

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Registry No. $D_2NCOCH_2CH(ND_2)CO_2D·D_2O$, 117711-63-4; $D_2NCOCH_2CH(ND_2)CO_2D·^1/_2D_2O$, 117711-64-5; $DO_2CCD_2CD(ND_2)CO_2D·^1/_2D_2O$, 117773-67-8; H-Asn-OH, 70-47-3; H-Asp-OH, 56-84-8; neutron, 12586-31-1.

Supplementary Material Available: Tables of observed and calculated structure factors for Asn/Asp and for Asn at 15 K (38 pages). Ordering information is given on any current masthead page.

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