# Crystal and Molecular Structure of Aspartame · HCl · $2H_2O$

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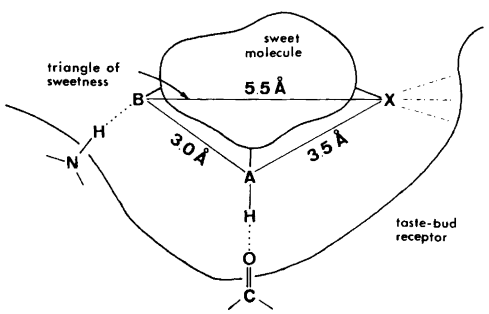
The crystal and molecular structure of the hydrochloride salt of the peptide sweetener aspartame (α-L-Asp-L-Phe methyl ester) has been determined at 120 K using 3877 reflections with $I > 2.5\sigma I$. Space group $P2_12_12_1$, cell dimensions $a = 6.768(1)$, $b = 9.796(1)$ and $c = 26.520(3)$ Å; final $R$ factor 0.033. While the $N$-terminal L-Asp group in the structure of aspartame itself forms a six-membered ring with an intramolecular hydrogen bond between the carboxylate and the protonated amino terminus, the corresponding group in the hydrochloride adopts a completely different conformation with a weak intramolecular hydrogen bond between the carboxyl group and the N atom of the L-Phe residue. The L-Phe methyl ester moiety is rather similar in the two structures. Of the many possible conformations of aspartame, only one may be expected to function as a substrate at the receptor site for sweet taste, and a proposal is made for this active conformation.

The artificial sweetener α-L-aspartyl-L-phenylalanine methyl ester (aspartame) is about 200 times sweeter than sucrose, and great interest has been shown in investigating the structural and conformational characteristics of the molecule. The crystal structure of aspartame (AM) has recently been reported by Hatada *et al.*[1] to be zwitterionic with a hydrophilic aspartyl side and a highly hydrophobic phenylalanine side. The present paper deals with the crystal structure of the acidic form of the molecule, i.e. aspartame hydrochloride (ACL). ACL is also sweet and is used commercially as a sweetener, but suffers from an even greater lack of stability in aqueous solution than the neutral compound.[2,3]

The carbonyl groups of proteins are hydrogen bond acceptors and the amide groups are hydrogen bond donors. A molecule containing two complementary sites, denoted AH and B respectively, about 3 Å apart can bind to the protein. If the protein forms part of the appropriate taste receptors at the tip of the tongue, the sensation of sweet taste is triggered. Studies of sweet molecules by Kier[4] led to the recognition of another common factor for sweetness, a hydrophobic group denoted X. Thus, A–B–X form a molecular arrangement called the "triangle of sweetness" (Scheme 1). A review on sweet compounds is given in Ref. 5.

## Experimental

Long rod-shaped crystals of aspartame · HCl · $2H_2O$ were prepared by repeated evaporation of a 0.1 M HCl solution of aspartame. The data collection procedure is summarized in Table




*Scheme 1.*

*Table 1.* Data collection.

| | |
|---|---|
| Instrument | Nicolet P3 |
| Radiation | Graphite Crystal Monochromated Mo*K*α |
| Scanning mode | θ/2θ |
| Scan speed/° $min^{-1}$ | 3.0 |
| Scan range/° | $2\theta_{\alpha 1}-1.0$ to $2\theta_{\alpha 2}+1.0$ |
| Background count | For 35 % of scan time at scan limits |
| Temperature/K | 120 |
| 2θ range/° | 5.0–70.0 |
| Crystal dimensions/mm | 0.50×0.50×0.35 |
| No. of refl. measured | 4389 |
| No. of unique refl. $I > 2.5\sigma I$ | 3877 |

1. Cell parameters were determined by least-squares fit to the diffractometer settings for 25 general reflections. Standard deviations in the measured intensities were calculated as $\sigma I = [C_T+(0.02C_N)^2]^{1/2}$, where $C_T$ is the total number of counts and $C_N$ is the scan count minus the background count. The intensities were corrected for Lorentz and polarization effects, but not for absorption. The chlorine atom and a large part of the molecule were recognized in the *E* map produced by MITHRIL,[6] and subsequent Fourier synthesis revealed the positions of all the non-hydrogen atoms, including two water molecules, in the asymmetric unit. Hydrogen atoms were introduced in theoretical positions, except those connected to oxygen atoms which were obtained from a difference Fourier synthesis. All positional parameters, anisotropic temperature factors for the non-hydrogen atoms and isotropic temperature factors for hydrogen atoms were refined by least-squares methods, giving $R = 0.033$ and $R_w = 0.037$ with goodness of fit $S = [\Sigma w\Delta^2/(m-n)]^{1/2} = 1.95$. The final parameters are given in Table 2. Atomic scattering factors for free heavy atoms and spherically bonded hydrogen atoms were taken from Ref. 7.

Lists of structure factors and anisotropic thermal parameters are available from the author on request.

## Crystal data

α-L-Aspartyl-L-phenylalanine methyl ester·HCl·$2H_2O$, $C_{14}H_{23}ClO_7N_2$: orthorhombic, $a = 6.768(1)$, $b = 9.796(1)$, $c = 26.520(3)$ Å, $V =$

*Table 2.* Fractional coordinates for aspartame·HCl·$2H_2O$ with standard deviations and equivalent isotropic temperature factors, $B_{eq}$, for non-hydrogen atoms.

| Atom | *x* | *y* | *z* | $B_{eq}$/Å$^2$ |
|---|---|---|---|---|
| C1 | 0.7609(1) | 0.8045(1) | 0.9129(1) | 1.6 |
| OD1 | 0.4573(2) | 0.2268(1) | 0.9457(1) | 1.6 |
| OD2 | 0.4016(2) | 0.1529(1) | 1.0241(1) | 2.2 |
| O1 | 0.2552(2) | 0.6649(1) | 0.9537(1) | 2.0 |
| O21 | 0.0721(2) | 0.2567(1) | 0.8648(1) | 1.9 |
| O22 | −0.2072(2) | 0.3777(1) | 0.8530(1) | 1.9 |
| OW1 | 0.3830(2) | −0.0233(1) | 0.9251(1) | 1.7 |
| OW2 | 0.0998(2) | −0.0309(1) | 0.8558(1) | 2.1 |
| N1 | 0.5939(2) | 0.6173(1) | 0.9999(1) | 1.4 |
| N2 | 0.2534(2) | 0.4686(1) | 0.9089(1) | 1.3 |
| CA1 | 0.5050(2) | 0.4991(1) | 0.9732(1) | 1.1 |
| CB1 | 0.4481(2) | 0.3916(1) | 1.0124(1) | 1.3 |
| CG1 | 0.4332(2) | 0.2451(1) | 0.9948(1) | 1.3 |
| C1 | 0.3261(2) | 0.5528(1) | 0.9440(1) | 1.2 |
| CA2 | 0.0671(2) | 0.4978(1) | 0.8836(1) | 1.3 |
| CB2 | 0.0926(3) | 0.5979(1) | 0.8388(1) | 1.5 |
| CG21 | 0.2321(2) | 0.5487(1) | 0.7984(1) | 1.5 |
| CG22 | 0.4284(3) | 0.5915(2) | 0.7979(1) | 1.9 |
| CG23 | 0.5574(3) | 0.5482(2) | 0.7603(1) | 2.4 |
| CG24 | 0.4928(3) | 0.4584(2) | 0.7235(1) | 2.4 |
| CG25 | 0.2980(3) | 0.4141(2) | 0.7237(1) | 2.2 |
| CG26 | 0.1684(3) | 0.4592(2) | 0.7605(1) | 1.8 |
| C2 | −0.0193(2) | 0.3628(1) | 0.8667(1) | 1.4 |
| CM2 | −0.3042(3) | 0.2562(2) | 0.8334(1) | 2.4 |
| HOD1 | 0.444(4) | 0.141(3) | 0.939(1) | |
| HW11 | 0.299(4) | −0.027(3) | 0.903(1) | |
| HW12 | 0.478(5) | −0.068(3) | 0.921(1) | |
| HW21 | 0.005(5) | −0.073(3) | 0.869(1) | |
| HW22 | 0.071(4) | 0.051(3) | 0.854(1) | |
| HN11 | 0.635(3) | 0.682(2) | 0.978(1) | |
| HN12 | 0.702(3) | 0.594(2) | 1.017(1) | |
| HN13 | 0.505(4) | 0.665((2) | 1.022(1) | |
| HN2 | 0.295(3) | 0.396(2) | 0.906(1) | |
| HCA1 | 0.602(3) | 0.465(2) | 0.951(1) | |
| HB11 | 0.549(3) | 0.397(2) | 1.038(1) | |
| HB12 | 0.322(3) | 0.410(2) | 1.028(1) | |
| HCA2 | −0.027(3) | 0.537(2) | 0.908(1) | |
| HB21 | −0.137(3) | 0.681(2) | 0.852(1) | |
| HB22 | −0.049(3) | 0.613(2) | 0.825(1) | |
| HG22 | 0.473(4) | 0.653(2) | 0.821(1) | |
| HG23 | 0.707(4) | 0.579(2) | 0.759(1) | |
| HG24 | 0.585(4) | 0.429(2) | 0.700(1) | |
| HG25 | 0.249(4) | 0.353(2) | 0.696(1) | |
| HG26 | 0.034(3) | 0.436(2) | 0.759(1) | |
| HM21 | −0.446(4) | 0.285(3) | 0.827(1) | |
| HM22 | −0.302(3) | 0.183(2) | 0.858(1) | |
| HM23 | −0.233(4) | 0.225(2) | 0.805(1) | |

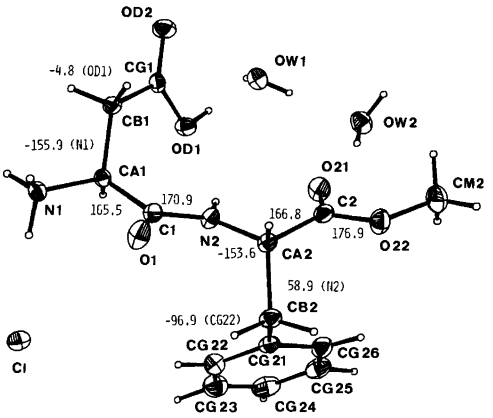



*Fig. 1.* The asymmetric unit with the protonated Aspartame molecule, the two water molecules W1 and W2 and the chlorine atom. Torsion angles along the peptide chain are given (e.s.d.'s 0.1–0.2°).

1758.1(4) Å$^3$, $Fw = 366.8$, $Z = 4$, $F_{000} = 776$, space group $P2_12_12_1$, $D_C = 1.386$ g cm$^{-3}$.

## Description and discussion

A drawing of the molecule with the chlorine atom and two water molecules in the asymmetric unit is shown in Fig. 1, together with torsion angles along the peptide backbone. The atomic numbering is identical to that used in Ref. 1. Bond lengths and bond angles for heavy atoms are shown in Fig. 2. There are no deviations from accepted values for peptides, but there are some significant differences when compared with the corresponding parameters in AM (values for AM in parentheses): O22–CM2 1.456 Å (1.416 Å), CA2–C2 1.514 Å (1.471 Å) and N1–CA1–CB1 108.3° (111.3°). Whereas the phenyl ring bond lengths in ACL are quite uniform (1.384–1.401 Å), the differences are greater in AM (1.363–1.418 Å). Some changes in bond angles are also observed at CA2 and, of course, in the carboxyl group which is ionized in AM. O–H and N–H distances are given in Table 2 (e.s.d.'s 0.02–0.03 Å). C–H distances range from 0.92 to 1.06 Å (mean 0.97 Å) with e.s.d.'s 0.02 Å.

The geometry of the aspartyl side chain resembles that observed in other short linear peptides.[8–11] The intramolecular hydrogen bond OD1···N1 observed in AM is not present (distance 4.2 Å), but instead, a weak intramolecular hydrogen bond to N2 is observed. In this connection it is interesting to note that this N–H moiety is essential, since replacement by O or N–$CH_3$ is accompanied by a loss of sweet taste.[12] The aromatic ring of the L-Phe residue is planar, with all

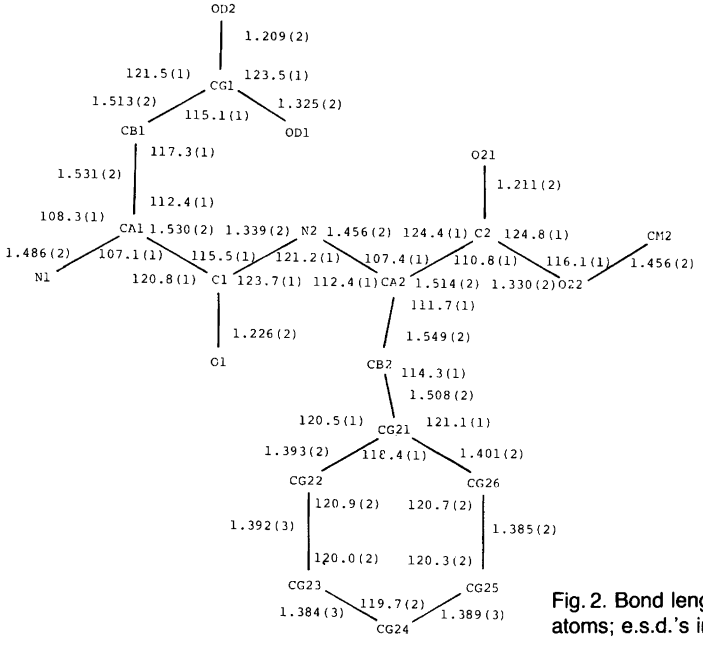



Fig. 2. Bond lengths and bond angles between heavy atoms; e.s.d.'s in parentheses.

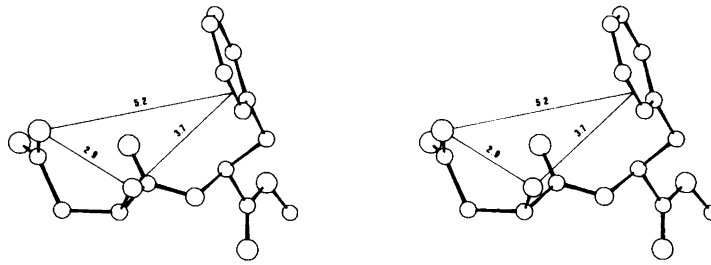


*Fig. 3.* Stereoscopic drawing of the aspartame molecule in the AspIII–PheII conformation. The possible "triangle of sweetness" has been indicated. Distances shown are between N1, OD1 and the centre of the C25–C26 bond.

atoms within 0.009 Å of a least-squares plane through the ring.

The main peptide chain backbone is in a rather extended conformation, as reflected by the torsion angles (°): Asp: $\psi = 165.5(1)$, $\omega = 170.9(1)$, $\chi^1 = -155.9(1)$; Phe: $\varphi = -153.6(1)$, $\psi = 166.8(1)$ (O22), $\chi^1 = 58.9(2)$, $\chi^{21} = -96.9(2)$ (CG22). The peptide bond is significantly nonplanar, and $\chi^{21}_2$ is close to a right angle, as is observed for aromatic residues in peptides and proteins.[13]

An α-amino acid can be assumed to exist in solution in three staggered rotameric forms with respect to the Cα–Cβ bond, with $\chi^1 = 180$ (I), $-60$ (II) and 60° (III). A dipeptide would thus give rise to nine essentially different conformations. In the case of aspartame, only one of these may be expected to be active at the receptor site and thereby be responsible for the sweet taste. Investigations on aspartame by Lelj *et al.*,[14] using NMR methods, indicate that all conformations are populated and that the most stable form is AspII–PheI. The geometry of AM in the crystal is best described as AspII–PheIII and of ACL as AspI–PheIII. Since the A–B part of the A–B–X triangle in Aspartame must be the Aspartyl moiety with $\chi^1$ either $-60$ (AspII) or 60° (AspIII), the crystal conformation of ACL, which according to Lelj *et al.* is the least populated in solution, cannot be the active one.

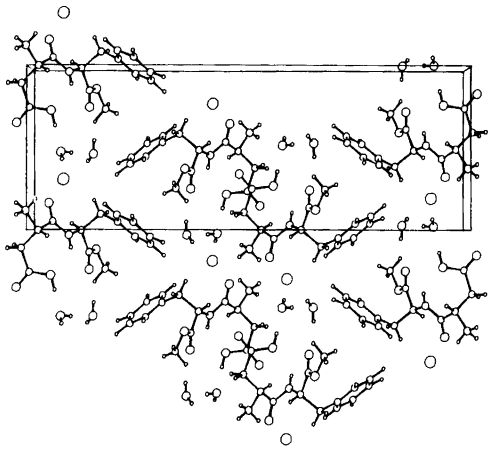


Fig. 4. The crystal packing with the unit cell viewed along the *a* axis.

An analogue of Aspartame, α-L-Asp-L-Met methyl ester,[15] which also tastes sweet, has been found by NMR to be present predominantly as AspII–MetII at high pH and predominantly (with an almost equal population) as AspIII–MetII at low pH. The same paper claims that the II conformation is normally preferred for L-Phe residues in solution, as it is for aromatic residues (57%) in the solid phase according to a survey made by Benedetti *et al.*[13] Fig. 3 shows that with $\chi^1_2 \approx -60°$ (PheII) the Aspartame molecule can satisfy the A–B–X triangle requirements, with the aromatic ring as the hydrophobic group. The torsion angles for this conformation are as follows (°): $\psi_1 = 82.0$, $\omega_1 = -172.0$, $\chi^1_1 = 60.0$ (AspIII); $\chi^{21}_1 = -65.0$ (OD1), $\varphi_2 = -128.0$, $\psi_2 = 166.8$, $\chi^1_2 = -60.0$ (PheII); $\chi^{21}_2 = -85.0$ (CG22). Bond lengths and bond angles are as in Fig. 2, except for the carboxyl group for which the values of Hatada *et al.*[1] have been used. No other conformation can give as good a fit to Kier's[4] triangle, although AspII–PheII is fairly close with a minimum OD1···*ring* distance of about 6.4 Å. The conclusion is that if the theory of the triangle of sweetness is correct, the confor-

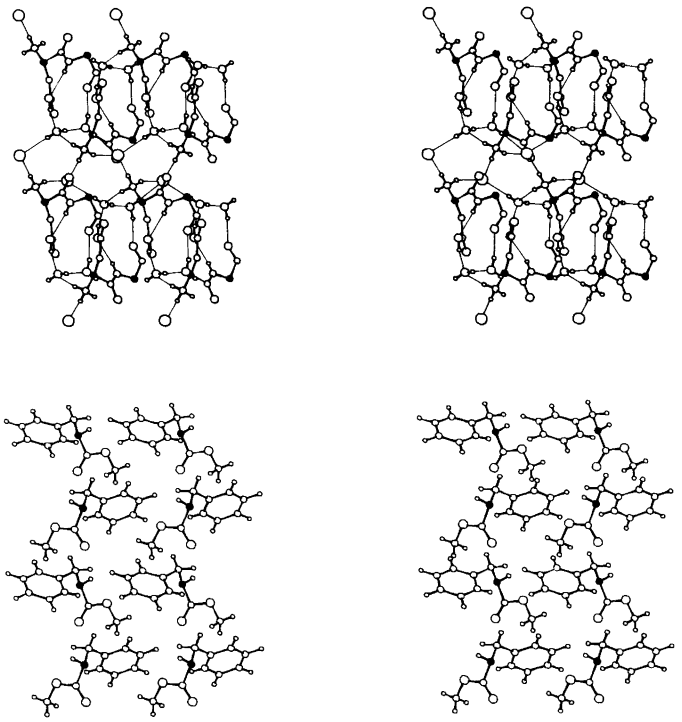


*Fig. 5.* Stereoscopic drawing of partial molecules, illustrating the separate layers in the crystal. Top: A hydrophilic layer with all but the four last hydrogen bonds in Table 3 lined; HCA1, HB11 and HB12 are omitted. Bottom: A hydrophopic layer; CA2, C2 and O22 are shown in both layers and Cα-atoms are in black for clarity.

mation triggering response at the receptor site of the taste bud for sweetness is very likely to be AspIII–PheII, although the possibility that it is AspII–PheII cannot be entirely excluded.

The unit cell content is shown in Fig. 4. It can be seen that the crystal is divided into hydrophobic and hydrophilic layers perpendicular to the *z*-axis. These layers are shown in Fig. 5. The hydrophilic layers exhibit an extensive hydrogen bond network. Data for the hydrogen bonds are given

*Table 3.* Hydrogen bond and hydrogen bond-like distances/Å and angles/°.

| D | H | A | D–H | D···A | H···A | D–H···A |
|---|---|---|---|---|---|---|
| OW1 | HW12 | Cl | 0.78 | 3.081 | 2.30 | 177 |
| OW2 | HW21 | Cl | 0.84 | 3.186 | 2.35 | 174 |
| N1 | HN11 | Cl | 0.91 | 3.157 | 2.26 | 168 |
| N1 | HN13 | Cl | 0.96 | 3.318 | 2.41 | 158 |
| OD1 | HOD1 | OW1 | 0.87 | 2.560 | 1.70 | 172 |
| N1 | HN12 | OW1 | 0.89 | 2.938 | 2.08 | 161 |
| OW1 | HW11 | OW2 | 0.82 | 2.656 | 1.84 | 177 |
| OW2 | HW22 | O21 | 0.83 | 2.834 | 2.03 | 163 |
| N2 | HN2 | OD1[a] | 0.77 | 2.911 | 2.25 | 145 |
| CA1 | HCA1 | OD2 | 0.94 | 3.071 | 2.43 | 159 |
| N1 | HN11 | O1 | 0.91 | 2.693 | 2.50 | 93 |
| N1 | HN12 | O1 | 0.89 | 2.693 | 2.52 | 92 |
| N1 | HN13 | O1[b] | 0.96 | 2.693 | 2.47 | 93 |

[a]Intramolecular bond. [b]Angle CA1–N1···O1 = 178.1°.

in Table 3. It is interesting to note that the hydrogen atoms of N1, in addition to forming three regular hydrogen bonds of intermediate strength to two chlorine atoms and one water molecule, are also involved in three almost identical van der Waals contacts to O1. In an analysis of 56 amino acid crystal structures made by Jeffrey and Mitra,[16] no such arrangement was found, but an N atom acts as a donor for six hydrogen bonds in the AM structure. O22 accepts a weak hydrogen bond from CA1 and in addition it is close to CA2 (also 3.071 Å), although no hydrogen bond-like interaction occurs here (O22···HCA2 2.63 Å). There are no other intermolecular contacts of less than 3.2 Å. The water molecules W1 and W2 are involved in four and three hydrogen bonds, respectively. The H–O–H bond angle in W1 is very large, 116(3)°, while the corresponding angle in W2 is 108(3)°.

In the hydrophobic layer, the methyl group is placed about midway between two Phenyl rings with closest contacts CG23···CM2 3.380(2) and CG24···CM2 3.524(2) Å, respectively. Neighbouring aromatic rings stack at an angle of 102° and the shortest intermolecular distance between them is CG22···CG24, 3.678(2) Å.

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