

## Further conformational aspects of the amino-acid histidine: crystal and molecular structure of histidine dihydrochloride\*

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*(Received 20 June 1974)*

### Abstract

Histidine dihydrochloride crystallizes in the orthorhombic system, space group  $P2_12_12_1$ , with unit-cell data  $a = 6.970(4)$ ,  $b = 16.165(7)$ ,  $c = 8.964(6)\text{\AA}$ ,  $V_c = 1010.0\text{\AA}^3$  and  $Z = 4$ . Intensities for 1360 independent reflections were collected on an automated diffractometer. The structure was solved by standard heavy-atom methods and refined by full-matrix least squares, based on  $F$ , to an  $R$ -value of 0.027. The final weighted  $R$ -value and goodness-of-fit are 0.029 and 1.9, respectively. The histidine residue exists in the crystal as the carboxylic acid with protonation of the primary amine nitrogen and the imidazole ring. The overall conformation of the molecule is extended, similar to that observed in *L*-histidine, *DL*-histidine hydrochloride dihydrate, and one of the two independent molecules in the structure of *L*-*N*-acetylhistidine. There is an extensive array of hydrogen bonds formed in the crystal, which, presumably, together with the observed electrostatic interactions, account for the adopted molecular conformation.

\* This investigation was supported by the donors of the Petroleum Research Fund, administered by the American Chemical Society, and by a National Institutes of Health Biomedical Science Support Grant. The diffraction equipment was purchased through a grant from the National Science Foundation.

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### Introduction

The molecular stereochemistry of the amino-acid histidine has been the subject of several recent crystal structure analyses: *L*-histidine hydrochloride monohydrate (Donohue, Lavine & Rollett, 1956; Donohue & Caron, 1964; Oda & Koyama, 1972), *DL*-histidine hydrochloride dihydrate (Bennett, Davidson, Harding & Morelle, 1970), orthorhombic *L*-histidine (Madden, McGandy & Seeman, 1972), monoclinic *L*-histidine (Madden, McGandy, Seeman, Harding & Hoy, 1972) and *L*-*N*-acetylhistidine monohydrate (Kistenmacher & Marsh, 1971; Kistenmacher, Hunt & Marsh, 1972).

It would appear that the discriminating feature that determines the molecular topology of the histidine fragment is the torsion angle about the C(2)–C(3)[C<sup>α</sup>–C<sup>β</sup>] bond (Kistenmacher & Marsh, 1971). In the two forms of *L*-histidine, in *DL*-histidine hydrochloride dihydrate and in one of the two independent molecules in *L*-*N*-acetylhistidine monohydrate, this torsion angle has a value of about 180° and the molecular conformation is open and extended. However, in *L*-histidine hydrochloride monohydrate, in the metal-histidine coordination complexes (see Freeman, 1967, for a review) and in the second independent molecule in *L*-*N*-acetylhistidine monohydrate, the histidine molecular fragment is observed in a compact and closed conformation (torsion angle about C(2)–C(3) equal to ± 50°). The occurrence of both the open and the closed molecular conformations in *L*-*N*-acetylhistidine clearly indicates that the adopted molecular conformation is very much dependent on the forces operating in the crystal–inter- and intramolecular hydrogen bonds, electrostatic forces, van der Waals forces, and the like.

Westhof (1972) has recently studied the structure and formation of radiation-induced radicals in histidine by electron spin resonance methods. In particular he has studied free radical formation in crystals of *L*-histidine dihydrochloride. Analysis of the esr spectrum is best explained (Westhof, 1972) if it is assumed that the histidine moiety is in an open and extended conformation.

Our interest in the structure of histidine dihydrochloride is then twofold: (1) as an aid in interpreting and understanding the above mentioned electron spin resonance results for X-ray induced radicals; (2) a further extension of our understanding of the crystal forces operative on the molecular conformation of the histidine molecule. A brief report on the structure of histidine dihydrochloride has been published (Kistenmacher & Sorrell, 1973).

### Experimental

Crystals of histidine dihydrochloride were kindly supplied to us by Professor Eric Westhof, Department of Biology, University of Regensburg, West Germany. The crystals decompose on exposure to humid air, and we have found it necessary to coat the crystals with a thin film of petroleum grease and seal them in thin-walled Lindemann glass capillaries. Oscillation and Weissenberg

photographs indicated an orthorhombic lattice. The observed systematic absences ( $h00$ ,  $h = 2n + 1$ ;  $0k0$ ,  $k = 2n + 1$ ;  $00l$ ,  $l = 2n + 1$ ) are consistent with the space group  $P2_12_12_1$ . Unit-cell dimensions were obtained from the complete angular settings of 14 reflections measured on a diffractometer. The density of the crystals was measured by flotation methods, and it indicated that there were four molecules of histidine dihydrochloride per unit cell — one molecule per asymmetric unit. Complete crystal data are given in Table 1.

Table 1. *Crystal data for histidine dihydrochloride*

|   |  |
|---|--|
|   | C <sub>6</sub> H <sub>11</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>2</sub> |
| $a = 6.970(4)\text{\AA}$                                | $MW = 228.04$  |
| $b = 16.165(7)$   | Space group: $P2_12_12_1$  |
| $c = 8.964(6)$  | $Z = 4$  |
| $V_c = 1010.0\text{\AA}^3$                              | $D_m = 1.50(1)\text{ g cm}^{-3}$   |
| $\mu = 6.2\text{ cm}^{-1}$                              | $D_c = 1.50$   |
| $\bar{\lambda}(\text{Mo } K\alpha) = 0.71069\text{\AA}$ | $F(000) = 472e$  |

Intensity measurements were made on a Syntex P $\bar{1}$  computer-controlled diffractometer equipped with a highly-oriented graphite monochromator crystal. The crystal used in data collection was cleaved from a larger crystal; the maximum and minimum dimensions of the crystal were 0.4mm and 0.3mm, respectively. Intensity data were collected with Mo  $K\alpha$  radiation by the  $\theta-2\theta$  scan technique. Individual scan speeds were determined by a rapid scan at each Bragg peak, and the rate of scanning varied from a minimum of  $2^\circ/\text{min}$  to a maximum of  $24^\circ/\text{min}$ . Three standards were measured after every 100 reflections during the course of the experiment, and their intensities showed no unusual fluctuations or decay with time. All reflections in the  $+h$ -hemisphere to  $2\theta = 55^\circ$  were surveyed; these totalled some 5843 reflections including standards and systematic absences. We have made no attempt to determine the absolute configuration of the crystal under study, but have simply assumed that the absolute molecular configuration is  $L$ . The four octants of data were combined to yield a set of 1360 independent intensities of which two had intensities less than zero (assigned an  $F$  and  $w(F)$  equal to zero). The reflections with intensities greater than zero were assigned observational variances based on the following equation:

$$\sigma^2(I) = S + (B_1 + B_2)(T_S/2T_B)^2 + (pI)^2,$$

where  $S$ ,  $B_1$  and  $B_2$  are the scan and background counts,  $T_S$  and  $T_B$  are the scan and individual background counting times ( $T_B = 1/4T_S$  for all reflections) and  $p$  was taken to be 0.03 and represents the error proportional to the diffracted beam intensity (Busing and Levy, 1957). The intensities and their standard deviations were corrected for Lorentz and polarization effects, but not for absorption ( $\mu = 6.2\text{ cm}^{-1}$ ). The intensities were placed on an approximate absolute scale by the method of Wilson (1942).

Table 2. Final atomic parameters and their estimated standard deviations\*

| Atom  | x         | y          | z         | $B_{11}(B)$ | $B_{22}$ | $B_{33}$ | $B_{12}$ | $B_{13}$ | $B_{23}$ |
|-------|-----------|------------|-----------|-------------|----------|----------|----------|----------|----------|
| Cl(1) | 17909(8)  | -24537(3)  | 20870(5)  | 1616(10)    | 163(2)   | 752(6)   | 58(4)    | 45(7)    | 9(3)     |
| Cl(2) | 20918(8)  | 1219(3)    | 712(3)    | 1581(11)    | 203(2)   | 803(6)   | -125(4)  | 272(7)   | -60(3)   |
| O(1)  | 68493(26) | 6673(8)    | 6130(16)  | 1946(37)    | 208(5)   | 824(18)  | 30(13)   | -373(24) | 90(8)    |
| O(2)  | 83253(28) | 7233(8)    | 28128(16) | 3042(49)    | 146(5)   | 845(18)  | 100(13)  | -517(29) | -33(8)   |
| N(1)  | 97728(25) | -7396(9)   | 26391(19) | 1146(34)    | 145(5)   | 727(20)  | 3(11)    | 25(23)   | -4(9)    |
| N(2)  | 67276(26) | -25142(10) | 13869(18) | 1476(37)    | 208(6)   | 640(18)  | -74(16)  | -17(24)  | -36(9)   |
| N(3)  | 70840(31) | -31924(10) | 34121(21) | 1935(44)    | 175(6)   | 1066(24) | -20(15)  | 83(31)   | 144(10)  |
| C(1)  | 76825(29) | 3412(11)   | 16198(20) | 1209(42)    | 149(6)   | 596(21)  | -32(13)  | 49(25)   | 26(10)   |
| C(2)  | 80651(32) | -5872(10)  | 16929(19) | 1214(38)    | 135(6)   | 470(19)  | -3(14)   | -35(26)  | -2(9)    |
| C(3)  | 62772(28) | -10412(11) | 22808(23) | 1022(38)    | 168(7)   | 756(24)  | 6(13)    | -22(27)  | 14(11)   |
| C(4)  | 66124(26) | -19423(11) | 25243(21) | 930(38)     | 156(6)   | 665(22)  | -61(12)  | 25(24)   | -29(9)   |
| C(5)  | 70175(35) | -32602(12) | 19571(25) | 1630(47)    | 155(7)   | 1137(29) | -48(17)  | 35(37)   | -95(12)  |
| C(6)  | 68394(34) | -23726(11) | 38027(22) | 1601(45)    | 209(7)   | 662(22)  | -51(18)  | 99(31)   | 24(11)   |
| H(1)  | 661(4)    | -238(1)    | 47(3)     | 3.3(5)      |          |          |          |          |          |
| H(2)  | 716(4)    | -375(1)    | 144(3)    | 3.1(5)      |          |          |          |          |          |
| H(3)  | 712(4)    | -362(1)    | 397(3)    | 4.2(6)      |          |          |          |          |          |
| H(4)  | 685(4)    | -216(1)    | 476(2)    | 3.2(5)      |          |          |          |          |          |
| H(5)  | 529(3)    | -97(1)     | 160(2)    | 1.9(4)      |          |          |          |          |          |
| H(6)  | 591(3)    | -79(1)     | 322(3)    | 2.9(5)      |          |          |          |          |          |
| H(7)  | 837(3)    | -78(1)     | 68(2)     | 1.6(4)      |          |          |          |          |          |
| H(8)  | 955(3)    | -55(1)     | 353(2)    | 2.5(5)      |          |          |          |          |          |
| H(9)  | 1002(4)   | -131(1)    | 262(3)    | 3.9(5)      |          |          |          |          |          |
| H(10) | 1069(3)   | -48(1)     | 229(2)    | 1.5(4)      |          |          |          |          |          |
| H(11) | 820(4)    | 120(2)     | 283(3)    | 4.6(6)      |          |          |          |          |          |

\* The form of the anisotropic ellipsoid is:

$$\exp[-(B_{11}h^2 + B_{22}k^2 + B_{33}l^2 + 2B_{12}hk + 2B_{13}hl + 2B_{23}kl)].$$

The heavy-atom parameters have been multiplied by  $10^5$ . The hydrogen atom positional parameters have been multiplied by  $10^3$ .

### Solution and refinement of the structure

The positions of the two independent chloride anions were determined from an unsharpened Patterson synthesis. A subsequent structure factor-Fourier calculation based on the phases derived from the positions of the chlorine atoms yielded coordinates for the remaining 11 heavy atoms in the asymmetric unit. Six cycles of isotropic least-squares refinement, the quantity minimized being  $\sum w(|F_o| - |F_c|)^2$  where  $w = 1/\sigma^2(F_o)$ , reduced  $R (= \sum \|F_o| - |F_c|\| / \sum |F_o|)$  to 0.093. A difference-Fourier map at this stage revealed the positions of the 11 hydrogen atoms.

The refinement was continued, adding successively to the parameter list: (1) anisotropic thermal factors for the heavy atoms; (2) coordinates for the hydrogen atoms; (3) isotropic temperature factors for the hydrogen atoms. The (200) reflection was excluded from these final cycles of refinement,  $|F_o| = 136e$ ,  $|F_c|$  about 181e; the relatively large difference in  $|F_o|$  and  $|F_c|$  being attributed to extinction effects. The final  $R$ -value is 0.027 for all reflections. The final weighted  $R$ -value  $[(\sum w(F_o - F_c)^2 / \sum w F_o^2)^{1/2}]$  and goodness-of-fit  $[(\sum w(F_o - F_c)^2 / (n - p))^{1/2}]$  for  $n = 1358$  observations of non-zero weight and  $p = 162$  are 0.029 and 1.9, respectively.

The scattering curves for Cl, O, N and C were taken from the compilation of Hanson, Herman, Lea & Skillman (1964). The scattering curve for H was that of Stewart, Davidson & Simpson (1965). The real part of the scattering curve for Cl was corrected for anomalous dispersion effects (Cromer, 1965). Final atomic coordinates and thermal parameters are given in Table 2. In the final cycle of refinement, no shift over error exceeded 1.0 for any parameter.

The structure factor and Fourier calculations were done using the X-RAY 67 series of programs (Stewart, 1967); the least-squares refinements were performed with an extensively modified version of ORFLS (Busing, Martin & Levy, 1962); best planes were computed with the program of Pippy & Ahmed (1968); the illustrations were prepared with the aid of the computer program ORTEP (Johnson, 1965).

### Discussion

A perspective view of the diprotonated *L*-histidine dication is presented in Fig. 1. The dication exists in the crystal in an open and extended conformation with protonation occurring on the primary amine, N(1), and the imidazole ring. It is well known that histidine, like other amino acids, generally exists in the crystal as a zwitterion with the proton on the carboxylic acid function moving to the primary amine nitrogen (Madden, McGandy & Seeman, 1972; Madden, McGandy, Seeman, Harding & Hoy, 1972). The mono-protonated histidine moiety (Donohue & Caron, 1964; Bennett, Davidson, Harding & Morelle, 1970) exhibits protonation at the imidazole ring as well as

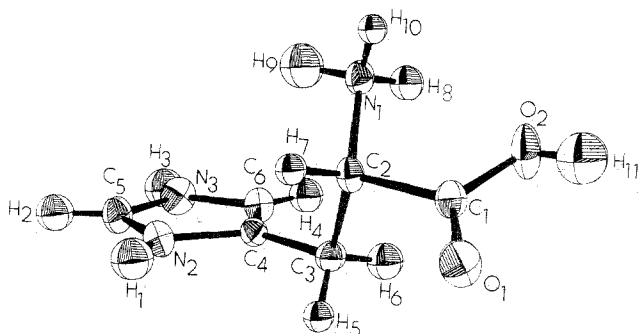


Fig. 1. Perspective view of the diprotonated *L*-histidine dication in histidine dihydrochloride. The thermal ellipsoids are drawn at the 40% probability level.

at the primary amine nitrogen. The existence of the protons on the primary amine and imidazole ring in histidine dihydrochloride is consistent then with the protonation pattern observed in other studies. Furthermore, the carboxylate group is also protonated in histidine dihydrochloride as is expected. These sites of available hydrogens for the formation of hydrogen bonds have played an important role (see below) in the conformational properties of histidine derivatives (Kistenmacher & Marsh, 1971).

(i) Bond lengths and angles

The distances and angles involving the heavy atoms are collected in Table 3. An extensive review of the molecular parameters in other crystalline histidine studies has recently been given (Carlstrom, Bergin & Falkenberg, 1973); consequently, we will restrict our discussion to geometrical features of the histidine dication in *L*-histidine dihydrochloride which are related to conformational or protonation effects. In general, the bond lengths in the histidine diprotonated cation are in excellent agreement with the values found in the monoprotinated cation (Table 3, Oda & Koyama, 1972). The only unexpected difference is in the N(2)–C(5) bond, 1.325(3)Å in the dication and 1.340(4)Å in the monocation. The value in the dication is, however, comparable to that found in *L*-*N*-acetylhistidine, 1.324(2)Å. It is unlikely that the shortening of the N(2)–C(5) bond is a result of conformational differences between the histidine moiety in histidine hydrochloride monohydrate and histidine dihydrochloride, since the two independent molecules in the structure of *L*-*N*-acetylhistidine have very different conformations (Kistenmacher, Hunt & Marsh, 1972), yet their N(2)–C(5) bond lengths differ by only 0.003Å. The small, but significant, difference in the N(2)–C(5) bond lengths must then be related to the effect of the proton on the carboxylate group or

Table 3. Distances and angles involving only heavy atoms

|                | orthorhombic<br><i>L</i> -histidine <sup>a</sup> | histidine<br>dihydrochloride <sup>b</sup> | histidine<br>hydrochloride<br>monohydrate <sup>c</sup> |
|----------------|--|---|--|
| N(1)–C(2)      | 1.493Å   | 1.482Å                                    | 1.481Å   |
| N(2)–C(4)      | 1.382  | 1.379                                     | 1.384  |
| N(2)–C(5)      | 1.327  | 1.325                                     | 1.340  |
| N(3)–C(5)      | 1.339  | 1.310                                     | 1.313  |
| N(3)–C(6)      | 1.374  | 1.380                                     | 1.377  |
| C(1)–O(1)      | 1.247  | 1.196                                     | 1.228  |
| C(1)–O(2)      | 1.250  | 1.313                                     | 1.266  |
| C(1)–C(2)      | 1.545  | 1.525                                     | 1.540  |
| C(2)–C(3)      | 1.536  | 1.539                                     | 1.532  |
| C(3)–C(4)      | 1.505  | 1.491                                     | 1.493  |
| C(4)–C(6)      | 1.361  | 1.351                                     | 1.355  |
| C(4)–N(2)–C(5) | 104.9°   | 109.5°                                    | 108.7°   |
| C(5)–N(3)–C(6) | 106.9  | 109.2                                     | 109.6  |
| O(1)–C(1)–O(2) | 126.7  | 125.0                                     | 127.3  |
| O(1)–C(1)–C(2) | 117.1  | 123.4                                     | 118.8  |
| O(2)–C(1)–C(2) | 116.3  | 111.6                                     | 113.7  |
| N(1)–C(2)–C(1) | 109.5  | 109.2                                     | 109.3  |
| N(1)–C(2)–C(3) | 109.8  | 112.0                                     | 111.6  |
| C(1)–C(2)–C(3) | 110.6  | 110.0                                     | 112.8  |
| C(2)–C(3)–C(4) | 112.7  | 112.9                                     | 115.2  |
| N(2)–C(4)–C(3) | 120.5  | 123.8                                     | 122.1  |
| N(2)–C(4)–C(6) | 109.6  | 105.9                                     | 106.4  |
| C(3)–C(4)–C(6) | 129.9  | 130.2                                     | 131.4  |
| N(2)–C(5)–N(3) | 112.2  | 108.2                                     | 108.1  |
| N(3)–C(6)–C(4) | 106.4  | 107.1                                     | 106.9  |

<sup>a</sup>Madden, McGandy and Seeman (1972). Bond length e.s.d.'s about 0.002–3Å, bond angle e.s.d.'s about 0.2°.

<sup>b</sup>This study. Bond length e.s.d.'s about 0.002–3Å, bond angle e.s.d.'s about 0.2°.

<sup>c</sup>Oda and Koyama (1972). Bond length e.s.d.'s about 0.004Å, bond angle e.s.d.'s about 0.2°.

packing effects. There are, as expected, highly significant differences in the C–O and C–C bond lengths in the carboxylic acid portion of the molecule. The parameters in the histidine dication are in good agreement with those from other X-ray studies (see, for example, Kistenmacher & Marsh, 1972).

The heavy-atom bond angles in histidine dihydrochloride are compared to those in histidine hydrochloride and orthorhombic *L*-histidine in Table 3. As noted previously (Bennett, Davidson, Harding & Morelle, 1970; Madden, McGandy & Seeman, 1972), the angles C(1)–C(2)–C(3) and C(2)–C(3)–C(4) are significantly dependent on the molecular conformation of the histidine

moiety. The close agreement between these two bond angles in *L*-histidine and *L*-histidine dihydrochloride reflects their very similar molecular conformations (see below). It has also been noted (Bennett, Davidson, Harding & Morelle, 1970; Madden, McGandy and Seeman, 1972) that three endocyclic bond angles in the imidazole ring [C(4)–N(2)–C(5), C(5)–N(3)–C(6) and N(2)–C(5)–N(3)] are significantly altered on protonation. Thus, the above three bond angles are very similar in *L*-histidine dihydrochloride and *L*-histidine hydrochloride monohydrate and strikingly different than those found in *L*-histidine (Table 3). There are also significant adjustments in the bond angles at C(1) and C(2) due to the protonation of the carboxylate group (Table 3).

Table 4. Bond lengths and angles involving the hydrogen atoms

| bond length e.s.d.'s about 0.03Å,<br>bond angle e.s.d.'s about 2.0° |                 |                 |        |
|---|-----------------|-----------------|--------|
| N(2)–H(1)   | 0.85Å           | C(3)–H(6)       | 0.96Å  |
| C(5)–H(2)   | 0.93            | C(2)–H(7)       | 0.98   |
| N(3)–H(3)   | 0.85            | N(1)–H(8)       | 0.87   |
| C(6)–H(4)   | 0.92            | N(1)–H(9)       | 0.93   |
| C(3)–H(5)   | 0.93            | N(1)–H(10)      | 0.83   |
|   | O(2)–H(11)      | 0.77Å           |        |
| C(4)–N(2)–H(1)  | 122.6°          | C(4)–C(3)–H(6)  | 108.5° |
| C(5)–N(2)–H(1)  | 127.8           | H(5)–C(3)–H(6)  | 109.2  |
| N(2)–C(5)–H(2)  | 127.2           | N(1)–C(2)–H(7)  | 107.7  |
| N(3)–C(5)–H(2)  | 124.5           | C(1)–C(2)–H(7)  | 108.0  |
| C(5)–N(3)–H(3)  | 120.9           | C(3)–C(2)–H(7)  | 109.8  |
| C(6)–N(3)–H(3)  | 129.4           | C(2)–N(1)–H(8)  | 109.2  |
| N(3)–C(6)–H(4)  | 126.2           | C(2)–N(1)–H(9)  | 107.6  |
| C(4)–C(6)–H(4)  | 126.8           | C(2)–N(1)–H(10) | 108.8  |
| C(2)–C(3)–H(5)  | 108.3           | H(8)–N(1)–H(9)  | 112.9  |
| C(4)–C(3)–H(5)  | 109.6           | H(8)–N(1)–H(10) | 107.8  |
| C(2)–C(3)–H(6)  | 108.4           | H(9)–N(1)–H(10) | 110.6  |
|   | C(1)–O(2)–H(11) | 116.4°          |        |

The refinement of the hydrogen atoms has led to positions which give geometrically acceptable bond lengths and angles (Table 4). The five independent C–H and N–H distances average to 0.94(3) and 0.87(3)Å, respectively. These values are in excellent agreement with other X-ray diffraction studies (Churhill, 1973). The O(2)–H(11) distance of 0.77(3)Å is in the expected range for carboxylic acids (Kistenmacher & Marsh, 1972; Nardelli, Fava and Giraldi, 1962).

The atoms of the protonated imidazole ring are coplanar within experimental error (Table 5). The relatively small deviation of C(3) from the plane of the imidazole ring is indicative of the lack of strain in the relatively open conformation of the histidine dication (Kistenmacher, Hunt & Marsh, 1972).



Table 5. *Least-squares planes and the deviations of individual atoms from these planes*

In each of the equations of the planes, the  $X$ ,  $Y$  and  $Z$  are coordinates (Å) referred to the unit-cell axes. Atoms indicated by an asterisk (\*) were given zero weight in calculating the planes; other atoms were weighted equally.

(a) *imidazole plane* ( $0.9886X + 0.1424Y - 0.0494Z = 3.9971\text{Å}$ )

|      |         |      |         |
|------|---------|------|---------|
| N(2) | -0.001A | C(3) | -0.012* |
| N(3) | -0.002  | H(1) | -0.002* |
| C(4) | 0.000   | H(2) | 0.000*  |
| C(5) | 0.002   | H(3) | 0.002*  |
| C(6) | 0.001   | H(4) | 0.001*  |

(b) *Carboxylic acid group* ( $0.8748X + 0.1442Y - 0.4626Z = 4.0815\text{Å}$ )

|      |        |       |         |
|------|--------|-------|---------|
| C(1) | 0.001A | H(11) | -0.004* |
| C(2) | -0.003 | N(1)  | 0.610*  |
| O(1) | -0.004 | C(3)  | -1.443* |
| O(2) | -0.004 |       |         |

The four atoms of the carboxylic acid group are approximately coplanar (Table 5). The deviation of the primary amine nitrogen N(1) from the carboxylic acid group is typical of amino acids (Marsh & Donohue, 1967).

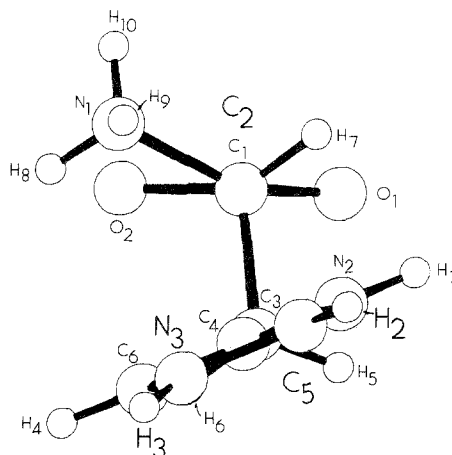


Fig. 2. *Conformational view of the L-histidine dication down the C(2)-C(1) bond. The torsion angle N(1)-C(2)-C(1)-O(2) is  $-27.1(2)^\circ$ .*

(ii) *Molecular conformation*

As can be seen from Fig. 1 and Fig. 2, which is a projection of the histidine dication down the C(2)–C(1) bond, the molecular conformation of the histidine fragment in *L*-histidine dihydrochloride is open and extended. There are, in general, three single bonds in the histidine moiety which permit conformational freedom: C(4)–C(3), C(3)–C(2) and C(2)–C(1). A comparison of the torsion angles about these three bonds in the histidine molecule in crystals of *L*-histidine hydrochloride monohydrate, *L*-histidine dihydrochloride and the orthorhombic form of *L*-histidine is given in Table 6. The differentiating torsion angle [between the 'open' and 'closed' conformations (Kistenmacher & Marsh, 1971)] C(4)–C(3)–C(2)–C(1) are within 6° of each other (Table 6) in orthorhombic *L*-histidine and *L*-histidine dihydrochloride, consistent with the occurrence of 'open' conformations in each case; the molecular conformation in *L*-histidine hydrochloride monohydrate is 'closed' with the torsion angle about C(4)–C(3) equal to –52.8° (Oda & Koyama, 1972).

Table 6. *Torsion angles*

| Angle               | LHCL    | LHDCL  | OLH   |
|---------------------|---------|--------|-------|
| N(2)–C(4)–C(3)–C(2) | –120.3° | –75.1° | 56.8° |
| C(4)–C(3)–C(2)–C(1) | –52.8   | –174.3 | 179.8 |
| N(1)–C(2)–C(1)–O(2) | –1.7    | –27.1  | –26.8 |

A positive angle corresponds to a right-handed screw. Standard deviations are about 0.2° in each of the three structure determinations. Values for the torsion angles in other histidine derivatives are given by Madden, McGandy, Seeman, Harding & Hoy (1972) and Oda & Koyama (1972).

LHCL: *L*-histidine hydrochloride monohydrate (Oda & Koyama, 1972)

LHDCL: *L*-histidine dihydrochloride (this study)

OLH: orthorhombic *L*-histidine (Madden, McGandy & Seeman, 1972)

The difference in the torsion angle about C(4)–C(3) in orthorhombic *L*-histidine and *L*-histidine dihydrochloride is related to the formation of an intramolecular hydrogen bond between the unprotonated imidazole ring nitrogen N(2) and the protonated primary amine nitrogen N(1) in orthorhombic *L*-histidine (Madden, McGandy & Seeman, 1972). The value for the torsion angle about C(4)–C(3) in *L*-histidine dihydrochloride, –75.1(2)°, is, however, within about 12° of that observed in the two independent molecules in *L*-*N*-acetylhistidine (Kistenmacher, Hunt & Marsh, 1972) and *DL*-histidine hydrochloride (Bennett, et al, 1972) where no intramolecular hydrogen bond exists.

As has been noted previously (Kistenmacher, Hunt & Marsh, 1972; Madden, McGandy & Seeman, 1972; Bennett, et al, 1972) the range of magnitudes for the torsion angles in the histidine fragment in various crystal structures not only affect certain bond lengths and angles, but also indicate that the conformation of a histidine residue in a polypeptide may be dictated by its local environment (hydrogen bond formation, solvation, etc.).

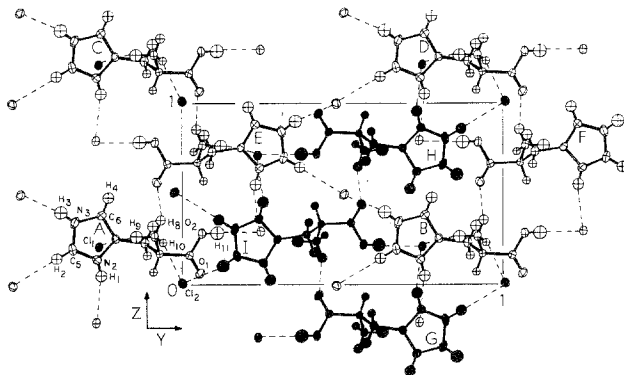


Fig. 3. View down the  $a$ -axis of the crystal packing in histidine dihydrochloride. Hydrogen bonds are indicated by dashed lines. The labeled chloride ions, Cl(1) and Cl(2), are at  $1 + x, y, z$  relative to Table 2. The nine histidine dications have the following symmetry transforms relative to Table 2:

- |                       |                               |
|-----------------------|-------------------------------|
| (A) $x, y, z$         | (E) $3/2 - x, -y, 1/2 + z$    |
| (B) $x, 1 + y, z$     | (F) $3/2 - x, 1 - y, 1/2 + z$ |
| (C) $x, y, 1 + z$     | (G) $1/2 + x, 1/2 - y, -z$    |
| (D) $x, 1 + y, 1 + z$ | (H) $1/2 + x, 1/2 - y, 1 - z$ |
|                       | (I) $2 - x, 1/2 + y, 1/2 - z$ |

### (iii) Crystal packing and hydrogen bonding

A view down the  $a$ -axis of the crystal packing in  $L$ -histidine dihydrochloride is shown in Fig. 3. The histidine dications lie essentially in parallel channels along a crystallographic axis ( $b$ ) which seems to be typical of other crystal structures where the molecular conformation is extended (orthorhombic  $L$ -histidine, monoclinic  $L$ -histidine,  $DL$ -histidine hydrochloride dihydrate, and one of the two independent molecules in  $L$ - $N$ -acetylhistidine monohydrate). The head-to-tail alignment of the molecules is stabilized by a series of hydrogen bonds [O(2)—H(11)...Cl(1), Cl(1)...H(9)—N(1), N(1)—H(10)...Cl(2), Cl(2)...H(2)—C(5)] running approximately parallel to the  $b$ -axis. In the structures of orthorhombic and monoclinic  $L$ -histidine,  $DL$ -histidine hydrochloride dihydrate and  $L$ - $N$ -acetylhistidine, the parallel arrangement of molecules is stabilized by intermolecular hydrogen bonds of the type  $-\text{CO}_2^- \dots \text{H}-\text{N}$  (2 or

3) of the imidazole ring. In *L*-histidine dihydrochloride, crosslinkages between the histidine dications are provided in the *a*- and *c*-axis directions by a series of three hydrogen bonds: N(2)–H(1)...Cl(1), N(3)–H(3)...Cl(2), N(1)–H(8)...O(1) [Fig. 3].

Table 7. Distances and angles in the intermolecular contacts of the type, D–H...A

| D    | H     | A                  | D...A | H...A | ∠D–H...A |
|------|-------|--------------------|-------|-------|----------|
| N(2) | H(1)  | Cl(1) <sup>a</sup> | 3.11Å | 2.32Å | 157°     |
| C(5) | H(2)  | Cl(2) <sup>a</sup> | 3.52  | 2.60  | 173      |
| N(3) | H(3)  | Cl(2) <sup>b</sup> | 3.10  | 2.28  | 162      |
| N(1) | H(8)  | O(1) <sup>c</sup>  | 2.90  | 2.12  | 150      |
| N(1) | H(9)  | Cl(1) <sup>d</sup> | 3.15  | 2.28  | 155      |
| N(1) | H(10) | Cl(2) <sup>d</sup> | 3.14  | 2.42  | 146      |
| O(2) | H(11) | Cl(1) <sup>e</sup> | 2.95  | 2.18  | 173      |

<sup>a</sup>  $1/2 + x, -1/2 - y, -z$

<sup>b</sup>  $1 - x, -1/2 + y, 1/2 - z$

<sup>c</sup>  $3/2 - x, -y, 1/2 + z$

<sup>d</sup>  $1 + x, y, z$

<sup>e</sup>  $1 - x, 1/2 + y, 1/2 - z$

The bond distances and angles in these hydrogen bonds are given in Table 7. All protons attached to nitrogen or oxygen atoms are involved in hydrogen bonds, and we include in the hydrogen bond list the C–H...Cl<sup>−</sup> interaction for the proton off C(5). The H(2)...Cl(2) distance for this interaction, 2.60Å, is considerably less than the sum of the van der Waals radii for H and Cl [3.0Å, Pauling (1960), using a van der Waals radius of 1.2 for H and 1.8Å for Cl]. Several authors (see Baur, 1972, for example) have argued that a more realistic value for the van der Waals radius for H is 1.0Å. Even using this more restrictive criterion, the H(2)...Cl(2) distance is about 0.2Å less than the sum of the van der Waals radii. Hamilton (1968), for example, has suggested that a hydrogen bond may be said to exist when the H...A distance is about 0.2Å less than the sum of the van der Waals radii of H and A. Furthermore, the C(5)–H(2)...Cl(2) angle, 173°, is suitably linear, and the crystal packing (Fig. 3) suggests that this interaction may play some role in the stabilization of the crystal structure.

The environments about the chloride anions are similar. Cl(2) accepts two N–H hydrogen bonds as well as the C–H...Cl<sup>−</sup> interaction, while Cl(1) acts as a hydrogen bond acceptor in two N–H...Cl<sup>−</sup> hydrogen bonds and a single O–H...Cl<sup>−</sup> hydrogen bond. Cl(1) also sits about directly above the protonated imidazole ring of the histidine moiety (and accepts a hydrogen bond from the primary amine N(1)) in a manner similar to that observed in *L*-histidine hydrochloride monohydrate.

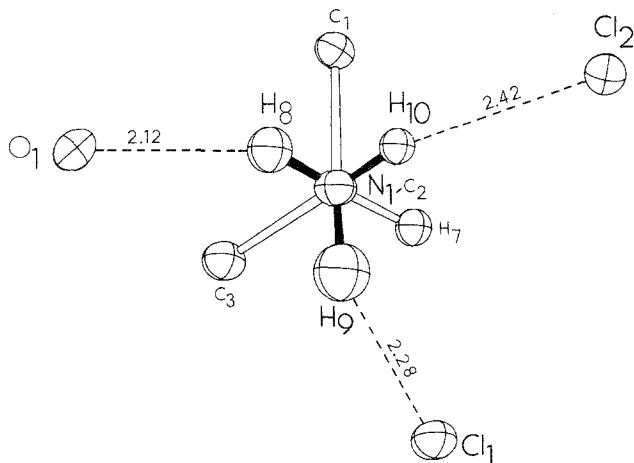


Fig. 4. *Newman projection down the N(1)–C(2) bond.* The substituents on N(1) and C(2) are in a nearly staggered conformation. The hydrogen bond acceptors in the hydrogen bonds involving the  $\alpha$ -amino nitrogen (N(1)) are also shown (H...A distances given).

Figure 4 illustrates the environment about the three hydrogen atoms on N(1). The three protons are arranged in an approximately staggered conformation relative to the substituents on C(2). This conformation about the N(1)–C(2) bond is very similar to that observed in *DL*-histidine hydrochloride dihydrate (Bennett, et al, 1970), but it is in direct contrast to the nearly eclipsed conformation observed in *L*-histidine hydrochloride monohydrate (Donohue, Lavine & Rollett, 1956).

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