mediate II is formed in the aqueous aminolysis reaction *it occurs following the rate-controlling transition state.* Certainly the rate of disappearance of II in aqueous solution is too fast to follow using conventional stopped flow techniques ($t_{1/2} < 10$ msec at 25° in 50% DMSO–water). It is our contention that a similar situation exists for other amines and also for oxygen nucleophiles (Figure 1), with k_1 rate controlling, even though I represents a highly acyl-activated ester containing a "poor" leaving group.

Finally, we note (footnotes *e*, *f* to Table I) that the 10^{11}-fold catalysis of HO^- compared to H_2O is provided entirely by the large increase in $\Delta S^{\pm}$. A similar large positive $\Delta S^{\pm}$ (14 cal mol^{-1} deg^{-1}) accompanies formation of the intermediate II in dimethyl sulfoxide. These large values suggest that solvation plays an important role in determining the overall rate and differences in rate for such reactions and this question is being examined in more detail.

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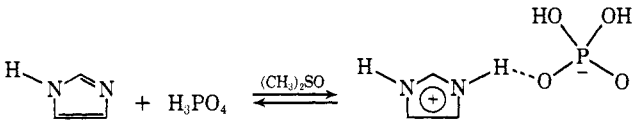
Base Stacking and Hydrogen Bonding in Crystals of Imidazolium Dihydrogen Orthophosphate

Sir:

Nuclear magnetic resonance effects accompanying the titration of imidazole with phosphoric acid in dilute solution in dimethyl sulfoxide have been attributed to a strong hydrogen bonding association.[1]



This hypothesis was, in turn, applied to the interpretation of nmr studies of the active site binding in ribonuclease, leading to a mechanism which involves hydrogen bonding between the imidazolyl side chain of the enzyme's histidyl residue 119 and a substrate phosphate moiety.[1,2] We have prepared the salt, imidazolium dihydrogen orthophosphate, and determined its crystal structure, and our results reveal short, strong (N–H···O–P) hydrogen bonds. Furthermore, we find structural evidence for stacking interactions between the imidazolium rings. This suggests to us that such interactions might occur between imidazolyl side chains in proteins and the purine or pyrimidine bases in nucleic acids.

The salt crystallized spontaneously on evaporation *in vacuo* of a very concentrated, equimolar, aqueous solution of imidazole and phosphoric acid. The structure analysis proved the salt to be anhydrous and confirmed the expected stoichiometry, *viz.*, $(C_3N_2H_5)^+$-$(H_2PO_4)^-$. The colorless, deliquescent crystals are monoclinic[3] with lattice dimensions $a = 8.53 \pm 0.01$ Å, $b = 12.72 \pm 0.02$ Å, $c = 15.93 \pm 0.02$ Å, $\beta = 126.13 \pm 0.06°$ (goniostat measurements, 20 ± 2°, λ(Mo Kα) = 0.7107 Å); space group $P2_1/c$ (No. 14); $\rho_{measd} = 1.59$ g cm^{-3} (by flotation), $Z = 8$ formulas per unit cell (two per crystallographically asymmetric unit), and $\rho_{X\text{-ray}} = 1.59$ g cm^{-3}. The crystal structure was solved by heavy atom methods and refined by full-matrix least-squares iteration using 3382 (observed) structure factor magnitudes, which were measured on a four-circle diffractometer (Picker FACS-I) using graphite monochromated Mo Kα X radiation. At an intermediate stage of the refinement, all the hydrogen atoms were located by a difference electron density synthesis and included in the subsequent final refinement which converged with (conventional) $R = 0.046$. Final atomic coordinates are listed in Table I. Observed and calculated structure factor data are listed in Table II.[4]

Table I. Atomic Coordinates (and Least-Squares Inverse-Matrix Estimated Standard Deviations)[a]

Atom	x/a	y/b	z/c
N(11)	18751 (31)	43783 (16)	44154 (15)
C(12)	34828 (41)	42929 (21)	53670 (20)
N(13)	38223 (33)	52045 (18)	58475 (16)
C(14)	23920 (43)	58884 (20)	51942 (22)
C(15)	11540 (39)	53775 (21)	42884 (20)
N(21)	−31674 (30)	3338 (15)	47822 (18)
C(22)	−27397 (33)	6870 (19)	56708 (19)
N(23)	−38152 (28)	15260 (14)	54893 (14)
C(24)	−49804 (41)	17095 (19)	44386 (19)
C(25)	−45593 (42)	9729 (20)	39981 (19)
P(1)	24964 (7)	34331 (4)	16910 (4)
O(11)	20857 (25)	35267 (13)	6378 (12)
O(12)	27986 (31)	44474 (14)	22420 (15)
O(13)	7804 (32)	28072 (18)	15390 (15)
O(14)	43095 (36)	27512 (24)	24297 (18)
P(2)	−11424 (7)	20832 (4)	29686 (4)
O(21)	3128 (23)	27930 (13)	30052 (12)
O(22)	−32212 (21)	22915 (13)	20687 (11)
O(23)	−10384 (24)	22087 (14)	39739 (12)
O(24)	−5461 (24)	9264 (12)	29403 (14)
H(N11)	1341 (60)	3812 (32)	3949 (31)
H(C12)	4378 (58)	3668 (33)	5707 (32)
H(N13)	4984 (57)	5351 (32)	6600 (31)
H(C14)	2318 (48)	6659 (25)	5346 (25)
H(C15)	46 (53)	5637 (31)	3611 (29)
H(N21)	−2701 (48)	−245 (25)	4728 (26)
H(C22)	−1827 (51)	317 (29)	6357 (28)
H(N23)	−3700 (39)	1912 (20)	5987 (21)
H(C24)	−5828 (41)	2273 (21)	4134 (21)
H(C25)	−5240 (54)	750 (30)	3201 (30)
H(O13)	874 (52)	2767 (27)	2119 (28)
H(O14)	5157 (67)	2709 (35)	2269 (34)
H(O23)	−80 (49)	2056 (24)	4459 (26)
H(O24)	−1338 (54)	512 (31)	2838 (29)

[a] ×10⁵ for nonhydrogen atoms; ×10⁴ for hydrogen atoms.

(1) J. S. Cohen, *Biochem. Biophys. Res. Commun.*, 33, 467 (1968).
(2) G. C. K. Roberts, E. A. Dennis, D. H. Meadows, J. S. Cohen, and O. Jardetzky, *Proc. Nat. Acad. Sci. U. S.*, 62, 1151 (1969); G. C. K. Roberts and O. Jardetzky, *Advan. Protein Chem.*, 24, 448 (1970).
(3) At the kind suggestion of a referee, we note that the unit cell that we report in the text, and on which our results and discussion are based, is not the Bravais-reduced cell. The lattice of the latter cell has space group symmetry $P2_1/n$ and dimensions $a_B = 8.53$ Å, $b_B = 12.72$ Å, $c_B = 12.90$ Å, $\beta_B = 93.80°$. The coefficients of the system of transformation equations which gives fractional atomic coordinates in the Bravais-reduced cell from coordinates in our unreduced cell are $10\bar{1}/010/001$.
(4) Observed and calculated structure factor data will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D.C. 20036, by referring to code number JACS-72-4034. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche.

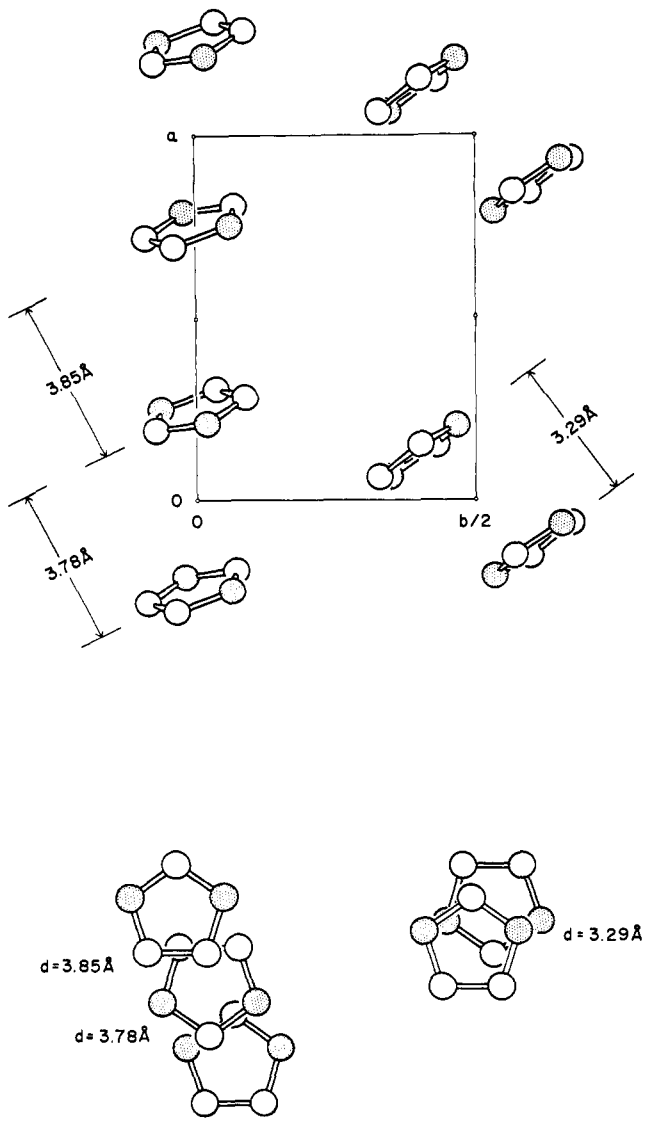


Figure 1. Stacking of the imidazolium rings viewed from the $-c^*$ direction. The stippled spheres represent the nitrogen atoms; hydrogen atoms are not shown. The distances indicated are the perpendicular ring plane to ring plane distances.

A detailed account of the structure analysis and crystal chemistry will be published soon.

In the crystal structure the phosphate anions are linked together by short, strong (P–O–H···O–P) hydrogen bonds to form a three-dimensional network, one feature of which is that there are large channels in the phosphate network along the lines $[x,0,0]$, $[x,{}^1/_2,{}^1/_2]$, $[x,{}^1/_2,0]$, and $[x,0,{}^1/_2]$. The imidazolium cations are stacked in columns filling these channels and are linked to the surrounding phosphate network by (N–H···O–P) hydrogen bonds.

The columns of stacked imidazolium rings are illustrated in Figure 1. Within each column any two adjacent rings are related by a center of symmetry. The degrees of overlapped contact between the stacked rings are shown at the bottom of Figure 1, where centrosymmetrically related pairs of rings are illustrated as viewed in projection along the directions normal to their mean planes. The atoms in each ring are, within experimental error, coplanar.

We observe close agreement between corresponding interatomic distances and angles in the two independent

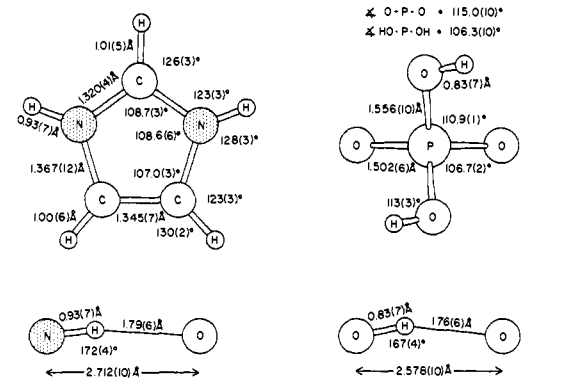


Figure 2. Averaged interatomic distances and angles. The estimated standard deviation given in parentheses is the larger of the two values from (1) the least-squares refinement and (2) the sample averaging (see text).

cations and anions and in the four independent examples of each of the two chemical kinds of hydrogen bonds in the crystallographically asymmetric unit. Moreover, in accord with chemical intuition, the two imidazolium cations very nearly conform to the symmetry of point group $2m$ (C_{2v}); similarly, the two independent tetrahedral phosphate anions are close to point group 2 (C_2). Interatomic distances and angles averaged over this noncrystallographic symmetry as well as over the asymmetric unit are given in Figure 2. When compared with values reported for other (N–H···O) hydrogen bonds,[5] the distances and angles in the hydrogen bonds between the imidazolium rings and phosphate groups in this structure indicate that these hydrogen bonds are among the strongest (N–H···O) bonds yet reported.

(5) G. C. Pimentel and A. L. McClellan, "The Hydrogen Bond," W. H. Freeman, San Francisco, Calif., 1960, pp 282–285; W. C. Hamilton and J. A. Ibers, "Hydrogen Bonding in Solids," W. A. Benjamin, New York, N. Y., 1968, pp 259–265.

(6) We are grateful for the financial support of this work by the National Institutes of Health (Training Grant No. GM-01728) and by the Health Research and Services Foundation of Pittsburgh (Grant No. L71).

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Enzymatic Synthesis of Cephalosporins

Sir:

Cephalosporins have been synthesized by chemical N-acylation of 7-amino-3-cephem compounds with the corresponding organic acids. For the preparation of cephalosporins bearing a free amino group in the side chain, the amino group of the amino acids employed for N-acylation has usually been blocked by a suitable protecting group before use. For example, cephalexin has been prepared by N-acylation of 7-aminodeacetoxycephalosporanic acid trichloroethyl ester with *N-tert*-butoxycarbonyl-D-α-phenylglycine, followed by stepwise deblocking of the doubly protected form of cephalexin.[1] We wish to report an enzymatic

(1) R. R. Chauvette, P. A. Pennington, C. W. Ryan, R. D. G. Cooper,