LETTERS TO THE EDITOR

STRUCTURE AND NATURE OF WATER IN A HUMAN BLADDER STONE: PROTON MAGNETIC RESONANCE AND INFRARED STUDIES

THE mechanism of formation of crystalline deposits (stones) in urinary bladder is complex and poorly understood. In order to understand more in detail the intricate process that takes place in the formation of stone, it is of importance to know the local arrangements and environments of the constituent atoms and molecules. Proton nuclear magnetic resonance technique has been used in a number of studies of water molecules and hydroxyl groups in the tissues[1,2]. It has been suggested that there are three types of water protons in dental enamel[3]. In the present investigation, the NMR and IR studies were undertaken on a bladder stone powder with a view to identifying the nature and structure of its water.

A random sample of urinary bladder stone from this Institute Hospital, was dried under vaccuum at room temperature and powdered finely. The PMR spectrum of the powder was recorded by using Varian wide line spectrometer operating at an R.F. frequency of 6·95 MHz. The modulation frequency employed in the experiment was 80 Hz. The signals were recorded using continuous averaging technique on a small computer having 1024 channels. Accumulated data were tansferred to an X-Y recorder. The first derivative of the PMR absorption spectrum of the bladder stone powder was recorded at 77 K and at 298 K.

The IR spectrum of the sample in KBr pellet was recorded at 25° C in the wave number range 600–4000 cm^{-1} using a Perkin-Elmer spectrophotometer.

The bands observed in the IR spectrum of the stone powder were superimposable on the bands found in the spectrum of pure calcium oxalate monohydrate[4]. In the wave number region 3000 to 3500 cm^{-1} several bands were observed (3420, 3300, 3200 cm^{-1}). The bands observed in the region 3000 to 3500 cm^{-1} may be attributed to the different types of hydrogen bonding present in the sample. The stone was identified as calcium oxalate monohydrate ($CaC_2O_4 \cdot H_2O$) on the basis of IR studies.

The line width of PMR spectrum at 77 K was 10·50 G and at room temperature it consisted of two signals superimposed, one of them being broad and the other narrow. The line width of the broad signal was 7·50 G and that of narrow line was 1·01 G. Since the IR evidence indicates that the sample is mainly calcium oxalate monohydrate, the origin of these signals must be from the protons of water molecules in the sample. The presence of two signals at room temperature suggests that there are two types of water present in this sample. The broad signal of line width 7·50 G may be due to the protons of crystalline water and the narrow signal of line width 1·01 G may be attributed as due to protons of mobile water which may be 'caged' inside the lattice. Earlier PMR studies in human enamel showed the presence of such thermally stable 'caged' water component. The PMR signal arising from these protons of the 'caged' water was narrow indicating that the molecules of water were freely tumbling[2,3]. At 77 K, the mobile caged water becomes rigid in structure causing additional broadening of PMR line to 10·50 G.

From the recorded derivative of the absorption spectrum, the experimental value of the second moment was computed readily by graphical integration[5]. For the present case, the second moment at 77 K comes out to be 27·56 Gauss^2. Using this experimental value of the second moment and the Van Vleck's, second moment formula for rigid lattice configuration[6] the nearest proton-proton (H-H) distance was calculated to be 1·53 Å. This compares well with the H-H distance of free rigid water molecule. Second moment corresponding to the broad line (7·50 G) at room temperature comes out to be 14·06 Gauss^2 and the nearest H-H distance was calculated to be 1·71 Å which agrees fairly well with those obtained in hydrated salts[7]. The slight increase in the nearest H-H distance in the sample at room temperature may be due to the formation of hydrogen bonds by the hydroxyl group of the water molecule in the bladder stone powder and it is known[8,9] that such formation is likely to increase the nearest H-H distance.

It is interesting to note that the present bladder stone sample also contains caged water as in the case of dental enamel. The presence of water caged in the lattice is likely to weaken the electrostatic forces between the constituent molecules as water has a large dielectric constant. This may result in an increase in the volume of the unit cell.

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1. Myers, H. M., Exptl. Cell. Res., 1965, 38, 686.
2. Myers, H. M., Nature, 1965, 206, 713.
3. Little, M. F. and Casciani, F. S., Arch. Oral Biol., 1966, 11, 565.
4. Bender, G. T., Chemical Instrumentation: A Laboratory Manual Based on Clinical Chemistry, W. B. Saunders Co., Philadelphia, 1972, p. 49.
5. Pake, G. E. and Purcell, E. M., Phys. Rev., 1948, 74, 1184.
6. Van Vleck, J. H., Phys. Rev., 1948, 74, 1168.
7. Wheatley, P. J., The Determination of Molecular Structure, Oxford University Press, London, 1969, p. 248.
8. Lippincott, E. R. and Srinivaa Rao, A., J. Chem. Phys., 1964, 41, 3006.
9. Sirnivasa Rao, A. and Srinivasa Manja, K., Ind. J. Pure and Appl. Phys., 1977, 15, 567.

MOLECULAR CONFORMATION OF ADENOSINE-5'-DIPHOSPHORIC ACID ($C_{10}N_5O_{10}H_{14}P_2.3H_2O$)

ADENOSINE-5'-diphosphate, as the phosphate acceptor in the biosynthesis of ATP, through the pyruvate kinase enzyme plays a key role in the energy transfer reactions of the cell. Metal ions Mg^{2+} and K^+ are required as cofactors for this enzymatic reaction[1]. It is therefore of interest to know the detailed geometry of the molecule in the free and metal-bound states. We have earlier reported the structure of ADP as monopotassium and monorubidium salts[2-4]. We describe here its molecular structure as a free acid, as obtained from a single crystal X-ray analysis.

Experimental

Crystals of ADP-free acid were obtained during our attempts to crystallise ADP as a K^+ salt. One of the crystallisation tubes, containing acetone layered over 1 ml of ADP-free acid + KCl (1 : 1) solution at pH 3·5 was found to have needle shaped crystals showing good optical extinction. These crystals were thought to be of ADP-K. However their mass spectrometric analysis showed that K^+ ions were not present in the crystal lattice. The crystals were of ADP-free acid as confirmed by a detailed X-ray analysis. These were then mounted inside Lindemann capillaries along with traces of mother liquor, and were examined for their mosaic spread. The crystal measuring 1·2 × 0·4 × 0·1 mm³ and showing minimum spread of X-ray spots were used in getting

the crystal data. The crystal data is summarised below :

$$a = 6\cdot714 (3), \quad b = 10\cdot989 (4), \quad c = 26\cdot320 (8) \text{ Å}$$

Space group $P22_12_1$; $d_m = 1\cdot64$, $d_c = 1\cdot64$, $z = 4$ $(\mu r) \simeq 0\cdot14$. Three-dimensional intensity data to a 2θ limit of 120° were collected at room temperature on the CAD-4 diffractometer using filtered CuKα radiation and $\omega/2\theta$ scan. Two control reflections were measured at intervals of fifty, to monitor intensity changes. These fluctuations were less than 5%, indicating that crystals were stable to X-rays. The structure was solved by a combination of direct and Fourier methods and refined in a block diagonal least squares program to a final R-factor of 7·8%.

Results and Discussion

The bond lengths and bond angles between non-hydrogen atoms can be had from the authors. The average estimated standard deviations in C-C (O, N) and P-O bond lengths are about 0·02 and 0·01 Å respectively. Estimated standard deviations in bond angles are 1·5° for C-C-C, C-C-N, C-O-C and C-N-C and about 0·9° for O-P-O and P-O-P angles

Molecular Geometry and Conformation

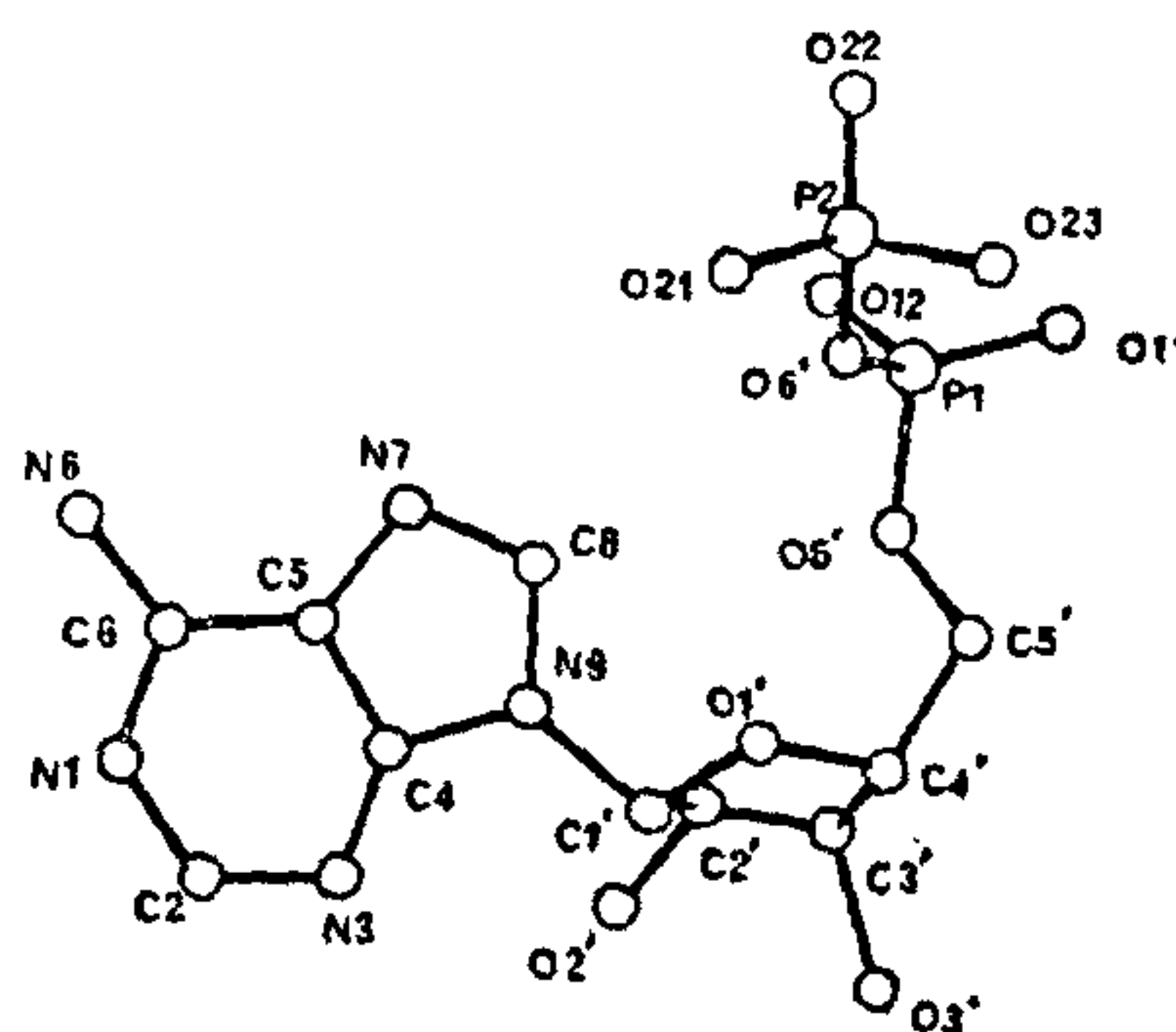A view of ADP-free acid perpendicular to the base is shown in Fig. 1. The conformational features of

FIG. 1. ADP-free acid. Viewed down the perpendicular to the base.

the molecule are : (i) anti orientation of the base with respect to sugar ($\chi_{CN} \simeq 25°$), (ii) trans orientation of pyrophosphate about C5'-O5' bond ($\phi = 152°$), (iii) gauche-gauche conformation about C4'-C5' bond and (iv) disorder of the ribose sugar between C2'-endo-C1'-exo and C3'-endo geometries. The resulting molecular shape is very similar to that found in the K^+ and Rb^+ salts of ADP.