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Infrared Spectra of Spermine Phosphate Hexahydrates

Yoshimasa Kyogoku[1a] and Yukiko Fuse[1b,c]

Faculty of Pharmaceutical Sciences, University of Tokyo[1a] *and Institute of Food Microbiology, Chiba University*[1b]

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Infrared spectra of spermine phosphate hexahydrates $C_{10}H_{26}N_4 \cdot 2H_3PO_4 \cdot 6H_2O$ and its N and O deuterated product have been measured. From the comparison of the spectra with those of the related simple molecules it is concluded that the salt is in the ionized form written $[NH_3^+(CH_2)_3NH_2^+(CH_2)_2]_2 \cdot 2[HPO_4]^{2-} \cdot 6H_2O$.

Spermine is an aliphatic polyamine which associates with nucleic acids and membrane components in a cell. Recently the crystal structure of spermine phosphate hexahydrates has been determined by Iitaka and Fuse.[2] From the interatomic distances in the analysis it is shown that hydrogen bonds are formed between the nitrogen atoms of the spermine molecule and the oxygen atoms of the phosphate molecules, though the positions of the hydrogen atoms could not be determined. The purpose of this work is to investigate the state of the hydrogen atoms of the amino, imino and phosphate groups of the compound by the infrared spectra measurement.

Results and Discussion

Absorption Bands due to the Amino and Imino Groups

The broad band around 3000 cm^{-1} and fairly sharp bands at 1660, 1570, 1560, 1480, 830 and 660 cm^{-1} are related to the motions of the NH and OH groups, since they disappear on deuteration. From the assignments of some hydrate crystals,[3] the shorter wavelength side of the broad band at 3000 cm^{-1} and the 1660 and 660 cm^{-1} bands can be ascribed to adsorbed water. The strong band tailing to 2000 cm^{-1} is typical of the spectra of compounds like amino acids and methylamine hydrochloride[4] which have a protonated amino group $-NH_3^+$. The bands at 1570, 1560 and 1480 cm^{-1} should be due to the asymmetric and symmetric deformation vibrations of the protonated amino group. They move to 1200—1100 cm^{-1} on deuteration.

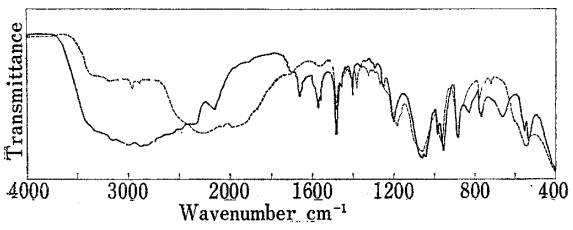

Fig. 1. Infrared Spectra of Spermine Phosphate Hexahydrates in Nujol and Hexachlorobutadiene Mulls

Solid line; undeuterated compound; Dotted line; N and O deuterated product

The spectrum of dimethylamine hydroiodide which has the NH_2^+ group shows a strong rocking band at 878 cm^{-1},[5] while di-*n*-propylamine containing the NH group gives a weak broad band at 735 cm^{-1} due to the out-of-plane bending vibration[6] (Table I). It is reason-

1) Location: *a*) *Hongo, Bunkyo-ku, Tokyo*; *b*) *Izumicho, Narashino, Chiba*; *c*) Present address: *Department of Biochemistry, School of Medicine, Keio University, Shinanomachi, Shinjuku-ku, Tokyo.*
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Table I. Characteristic Frequencies of the Absorption Bands of the Normal and Protonated Primary and Secondary Aliphatic Amines.

Type (Compound)	Frequency (cm^{-1}) Non-deuterated	Deuterated	Assignment
$-NH_3^+$ (amino acids[4])	~2800	2200	str.
	1650—1580	1180—1150	asym. def.
	1530—1450		sym. def.
	1200—1100	900— 770	rock.
$-NH_2$ (*n*-propylamine, liquid[6])	~3350		str.
	1600		scissors
	815		bend.
	763		bend.
$\overset{\vert}{N}H_2^+$ (dimethylammonium iodide[5])	3000—2700	2300—2100	str.
	1582	1157	scissors
	1421	1055	wag.
	878	689	rock.
$\overset{\vert}{N}H$ (di-*n*-propylamine, liquid[6])	3350		str.
	1650		bend. (in-plane)
	735		bend. (out-of-plane)

able, therefore, to assign the 830 cm^{-1} band of spermine phosphate to the motion of the NH_2^+ group. A shoulder of the 1590—60 cm^{-1} band and a part of the 1480—60 cm^{-1} band are responsible for the scissoring and wagging modes of the NH_2^+ group respectively. These evidences clearly show that both the amino and imino groups of the spermine molecule are in the protonated form.

Absorption Bands due to the Methylene and Phosphate Groups

Spermine has four methylene groups which give the CH stretching band at 2965 cm^{-1}, the CH_2 scissoring band at 1480 cm^{-1}, the wagging band at 1400 cm^{-1} and the rocking band at 770 cm^{-1}.

Table II. Position of the Infrared Absorption Bands of Orthophosphates[7-9]

Type	Frequency (Intensity)	Assignment
H_3PO_4	1170 (vs)	P=O str.
	1075—1065 (w)	PO_3 deg. str.
	1010 (vs)	PO_3 deg. str.
	880 (w)	PO_3 sym. str.
	500 (w)	OPO bend.
$H_2PO_4^-$	1200—1140 (s)	PO_2^- anti. str.
	1090—1050 (vs)	PO_2^- sym. str.
	990— 925 (vs)	PO_2 (H) anti. str.
	870 (m)	PO_2 (H) sym. str.
	570— 500 (s)	PO_2 (H) bend.
	420 (w)	PO_2^- bend.
HPO_4^{2-}	1100—1040 (vs)	PO_3^{2-} deg. str.
	990— 950 (s, sharp)	PO_3^{2-} sym. str.
	880— 820 (s)	PO (H) str.
	575— 520 (m)	OPO bend.
PO_4^{3-}	1005	PO str.

7) A.C. Chapman and L.E. Thirlwell, *Spectrochim. Acta*, **20**, 937 (1967).
8) T. Shimanouchi, M. Tsuboi and Y. Kyogoku, "Advances in Chemical Physics," Vol. VII, Intersciences, New York, N.Y., p. 435.
9) M. Tsuboi, *J. Am. Chem. Soc.*, **79**, 1351 (1957).

The other strong bands unaffected on deuteration are mainly due to the motion of the phosphate group. Phosphoric acid can take one of the four states which are shown in Table II. When it takes the tribasic form, PO_4^{3-}, only a strong band at 1005 cm^{-1} is expected in the infrared spectrum. On the other hand H_3PO_4 should give two very strong bands at about 1170 and 1010 cm^{-1} and a strong band around 1250 cm^{-1}.[7] The feature of the observed spectrum is different from such spectral feature. It shows a strong broad band at 1060—40 cm^{-1} and a sharp band at 953 cm^{-1} which are typical of the spectrum of the compound which has a $-PO_3^{2-}$ group.[8,9] Therefore the phosphoric acid is in the dibasic form HPO_4^{2-}. If it takes the monobasic form $H_2PO_4^-$, it should give four strong bands around 1170, 1070, 950 and 870 cm^{-1}. Although the feature of the observed spectrum appears to resemble that of $H_2PO_4^-$, the lack of the 1170 cm^{-1} band eliminates the possibility of the existence of the monobasic form. The band at 1200 cm^{-1} may be due to the NH_3^+ rocking vibration and the 883 cm^{-1} band comes from the P–O(H) stretching mode.

Comparison with the Results of the Crystal Analysis

As shown in the crystal analysis of spermine phosphate hexahydrates,[2] one of the four P–O bonds of the phosphate molecule is 1.59 A, which is fairly longer than the other three P–O bonds ranging from 1.53 to 1.52 A. As the P–O(H) bond is generally longer than the P–O^- bond, the phosphate molecule should be in the $(H)OPO_3^{2-}$ form, though the position of the hydrogen atom was not determined.

The amino nitrogen atom of the spermine molecule tetrahedrally coordinates with three ionized oxygen atoms of the phosphate molecules. Similarly the imino nitrogen atom coordinates with one of the ionized oxygen atoms of the phosphate molecule and with the oxygen

Table III. Assignments of the Infrared Absorption Bands of Spermine Phosphate Hexahydrates

Non-detuerated			Deuterated		
Freq.	Int.	Assignment	Freq.	Int.	Assignments
3400—2320	vs	NH_3^+, NH_2^+, OH, CH, str.	2965		CH str.
2160	s		2600—1900		ND_3^+, ND_2^+, OD str.
1700	w				
1660	m	H_2O bend.			
1590	w	NH_2^+ scissors			
1570	m	NH_3^+ asym. def.			
1560	m				
1480	s	NH_3^+ sym. def., CH_2	1480	m	CH_2 scissors
1460	w	scissors, NH_2^+ wag.	1460	w	
1410	w	CH_2 wag.			
1400	w		1400	w	CH_2 wag.
1357	vw		1380	w	(NH_2D^+ def.)
1290	vw		1325	vw	
1250	w	POH in-plane bend.	1240	w	D_2O bend.
1200	s	NH_3^+ rock.	1200	s	ND_3^+ asym. def., ND_2^+ scissors
1060	vs	PO_3^{2-} deg. str.	1180	s	ND_3^+ sym. def.
1040	vs		1060	vs	PO_3^{2-} deg. str.
981	m	PO_3^{2-} sym. str.			
953	s		960	s	PO_3^{2-} sym. str.
883	s	P-O str.	880	s	P-O str.
830	m	NH_2^+ rock.			
768	m	CH_2rock.	775	m	CH_2 rock.
660	m	H_2O libration			
552	w	PO_3^{2-} def.			ND_2^+ rock.
532	m		600	m	D_2O libration
					PO_3^{2-} def.

atom of one of the water molecules. Thus the results of the X-ray analysis on the states of the amino, imino and phosphate groups are consistent with those of the present work.

Experimental

Commercial spermine phosphate (L. Light & Co. Ltd., England) was recrystallized from a hot aqueous solution. N and O deuterated samples were prepared by recrystallization from hot deuterium oxide. Infrared spectra were measured with a Perkin Elmer 621 infrared spectrophotometer for mulls in Nujol and hexachlorobutadiene.

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