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Studies of vibrational spectra of 6-fluorothymine and its methyl derivatives

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Abstract—The solid and the argon-matrix isolated samples of 6-fluorothymine, 1-methyl-6-fluorothymine, 1,3-dimethyl-6-fluorothymine and 3-methylfluorothymine are studied at low temperatures by i.r. spectroscopy. The spectra obtained are interpreted using the calculation of planar normal vibration frequencies. The electronegative fluorine atom in the 6-position is shown to have a strong influence on the normal vibration frequency changes of crystalline and isolated thymine. N–H · · · F hydrogen bonds are evidenced to exist in crystalline samples.

INTRODUCTION

Infrared spectroscopy is widely employed to investigate the structure of nucleic acid bases and related compounds. The special interest in the derivatives of the pyrimidine base of nucleic acids—thymine and in particular in 6F substitutes and their methylated analogues is connected with their antimetabolic properties [1–3]. The spectra of these compounds are not clearly understood yet. Reference 4 shows the i.r. spectra of 6-fluorothymine solutions in the regions of the stretching vibrations of NH and C=O groups.

This work is therefore aimed at studying the vibrational spectra of 6-fluorothymine, 1-methyl-6-fluorothymine and 1,3-dimethyl-6-fluorothymine in the crystalline state and isolated in argon matrices. The experimental results are interpreted on the basis of calculated normal vibrations.

EXPERIMENTAL

The spectra of crystalline samples were registered with an IKS-29 spectrometer on films in Vaseline and perfluorovaseline oils. The method of low temperature matrix isolation in solid argon [5] was used to record spectra of the isolated molecules of 6-fluorothymine and its methylated derivatives. The spectra were registered with a "Specord IR-75" spectrometer, the resolution being 1 cm^{-1} at 1800–400 cm^{-1} and 4 cm^{-1} at 3600 cm^{-1}. To avoid the band splitting due to nitrogen impurities in the argon matrix, it was especially important to use leak-proof vacuum joints (the vacuum was greater than 10^{-6} torr). The argon purity was 99.99%. The concentration of the substances investigated in the matrix was controlled with quartz microbalances and was one molecule per 1000 argon atoms. The detailed description of the measurement method and sample preparation can be found in Ref. 6.

MATERIALS

The samples of 6-fluorothymine were prepared by alkali hydrolysis of 2,4,6-trifluoro-5-methylpyrimidine in the form of colourless crystals: $\lambda_{max} = 270$ nm ($\varepsilon = 3.98$).

1-Methyl-3-methyl-1,3-dimethyl-6-fluorothymine were prepared through an interaction between 6-fluorothymine and dimethylsulphate in water in the presence of alkali with the molar proportions 1 : 1, 3 : 1, 3 at 20–25°C for 2 h. Pure methylated derivatives of 6-fluorothymine were obtained after fractional crystallization of the precipitate from chloroform:

1-methyl-6-fluorothymine—$\lambda_{max} = 257$ nm ($\varepsilon = 3.89$);
3-methyl-6-fluorothymine—$\lambda_{max} = 256$ nm ($\varepsilon = 3.83$);
1,3-dimethyl-6-fluorothymine—$\lambda_{max} = 250$ nm ($\varepsilon = 3.93$).

CALCULATION

The vibrational spectra were calculated using the programs compiled by GRIBOV and DEMENTIEV [7, 8] and the force constants of thymine [9]. The geometrical sizes of the crystalline 6-fluorothymine molecule were similar to those of thymine [8]. The C–F bond length was taken to be the same as in the fluorobenzene molecule, i.e. 1.29 Å [10]. The vibrational spectra of 6-fluorothymine in the argon matrix were calculated using the same bond lengths and valence angles as obtained by the quantum-chemical method for the thymine molecule with complete geometrical optimization [11]. Calculations involved the valence-force field method.

RESULTS AND DISCUSSION

NH- *stretching vibrations*

A comparison of the i.r. spectra for thymine, 6-fluorothymine and its methyl derivatives (Fig. 1, Table 1) shows that a fluorine atom introduced into the 6-position induces significant changes in the region of the NH stretching vibrations.

Firstly, instead of the two intensive bands observed at 3180 and 3033 cm^{-1} in the spectra of solid samples (N_1H_{str} and N_3H_{str} of thymine [9], respectively), the spectrum of 6-fluorothymine displays doublets at

Table 1. Observed and calculated frequencies of planar vibrations of 6-fluorothymine and its methylated derivatives*

6-Fluorothymine		1-Methyl-6-fluorothymine	3-Methyl-6-fluorothymine	1,3-Dimethyl-fluorothymine	
Observed	Calculated	Observed	Observed	Observed	Type
3210	3203	3175	—	—	N_3H str
3105	3096	3120	3125	—	N_1H str
3081			3105		
785	795	760	762	750	C_5–R (CH_3) str
1150	1138	1165	1150	1170	C_6–F str
1745	1741	1725	1745	1715	C_2=O str, C_2–N str, C=C str
1730			1738		
1702	1697	1690	1698	1686	C_4=O str, C=C str
1652	1648	1670	1625	1645	C_4=O str, C=C str
1524	1525	1490	1555	1450	Ring str
1400	1403	1425	1390	—	Ring str, N_3H bend
1250	1237	1245	1240	1265	Ring str, N_1H bend
985	964	965	975	925	Ring str, ring bend
1025	1009	—	1048	1030	Ring str, ring bend
1310	1306	—	1305	—	N_1H bend
1466	1442	1455	—	—	N_3H bend
345	333	—	354	—	C_5–R str
—	314	—	—	—	C_6–R str
—	596	610	—	—	C=O bend
382	383	—	382	—	C=O bend
728	728	728	720	722	Ring bend
645	681	668	655	652	Ring bend
530	542	532	—	—	Ring bend
2900	3000	3020	3000	3040	CH_3 str as
2964	2927	2920	2960	2940	CH_3 str s
1492	1478	1480	1450	1475	CH_3 bend sciss.
1305	1336	1312	1315	—	Ring str, C–CH_3 str

*Abbreviations: str, stretching; bend, bending. Frequency in cm^{-1}.

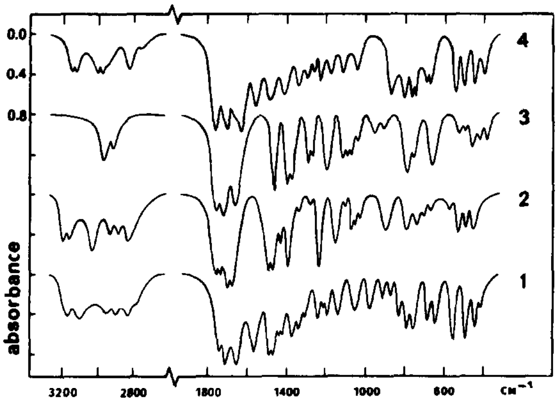

Fig. 1. Infrared spectra of solid samples of 6-fluorothymine and its methylated derivatives: (1) 6-fluorothymine, (2) 1-methyl-6-fluorothymine, (3) 1,3-dimethyl-6-fluorothymine, (4) 3-methyl-6-fluorothymine.

3105, 3081 cm^{-1} and a broad band at 3210 cm^{-1}. The spectra of 1-methyl- and 3-methyl-derivatives of 6-fluorothymine also have doublets in this region: 3175, 3120 cm^{-1} and 3125, 3105 cm^{-1}, respectively. In addition, the calculation shows that, unlike thymine [9], the high-frequency band in the vibrational spectrum of 6-fluorothymine is due to N_3H_{str} vibrations and the low-frequency one to N_1H_{str} vibrations, i.e. the N_1H_{str} and N_3H_{str} bands are observed to reverse (Table 1). The significant lowering of the N_1H_{str} frequency (by *ca* 60–80 cm^{-1}) in the 6-fluorothymine and 3-methyl-6-fluorothymine spectra is accounted for by the formation of the hydrogen bond $N_1H \cdots F$ in the intermolecular complexes of various structures (Fig. 2). The probability of two types of complex is supported by the NH_{str} band splitting in the spectra of all the 6-fluorothymines studied. In the absence of a strong

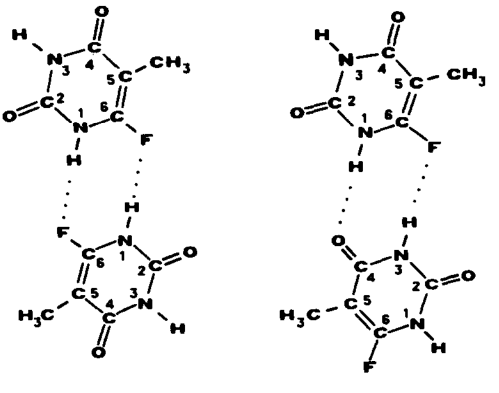

Fig. 2. The structure of 6-fluorothymine complexes with N–H ⋯ F hydrogen bonds.

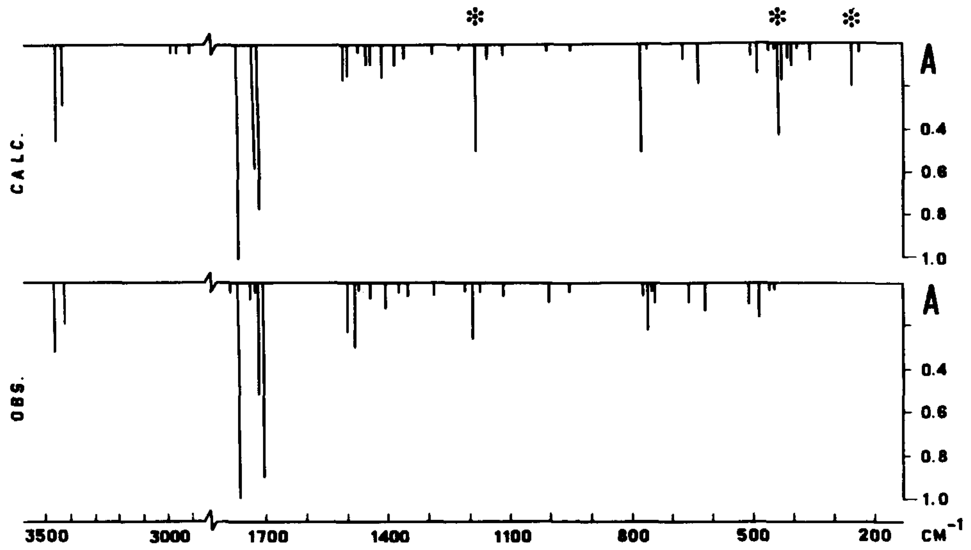

Fig. 3. Calculated (top) and observed (argon matrix, 11 K) vibrational spectra of 6-fluorothymine. The bands assigned to C–F bond vibrations (stretching, bending and out-of-plane) are marked.

molecular interaction the N_1H_{str} and N_3H_{str} frequencies (3463 and 3438 cm^{-1}) of matrix-isolated 6-fluorothymine (Fig. 3 and Table 2) are in good agreement with the corresponding frequencies of matrix-isolated thymine (3478 and 3431 cm^{-1}) (Table 3). The 15 cm^{-1} decrease of the N_1H_{str} frequency is due to the lowering of the N_1H bond order by the fluorine atom introduced into the 6-position. Conclusions on the effect of molecular interaction upon the particular structural fragments may be drawn from a comparison of the force constants for solid and isolated 6-fluorothymines (Table 4).

NH *deformation vibrations*

Our assumptions on the effects produced by the fluorine atom substitution are also supported by the changes in the i.r. spectra occurring in the region of N_1H and N_3H_{def} vibrations. While in the series thymine, 6-fluorothymine, 1-methyl- and 6-fluorothymine the N_3H_{def} frequency changes only slightly, the N_1H_{def} frequency increases by 150 cm^{-1} in the spectra of solid samples (due to the formation of hydrogen bonds, $N_1H \cdots F$) and decreases in the spectra of matrix-isolated molecules (Tables 1 and 2).

C–F *bond vibrations*

The formation of the $N_1H \ldots F$ hydrogen bond is also suggested by the changes in the $C–F_{str}$ vibrations. They are similar in the i.r. spectra of solid 6-fluorothymine and 3-methyl-6-fluorothymine and more appreciable in the i.r. spectra of 1-methyl-6-fluorothymine and 1,3-dimethyl-6-fluorothymine, which cannot form hydrogen bonds involving fluorine.

The spectra of all the matrix-isolated 6-fluorothymine molecules contain intensive bands with close magnitudes (Fig. 3, Table 2), which correspond to stretching and deformation vibrations of the C–F bond. The C–F vibrations are beyond the spectral capabilities of the instruments used. A strong 450 cm^{-1} band was observed only in the i.r. spectrum of 1-methyl-6-fluorothymine.

Carbonyl group vibrations

The fluorine atom introduced into the 6-position of thymine appreciably affects the formation of intermolecular hydrogen bonds between the carbonyl groups and NH bonds. It has been shown [2] that the presence of F at C_6 suppresses the proton-acceptor ability of the $C_4{=}O$ group but does not influence the $C_2{=}O$ group. The stretching vibration frequency of the fragment $O{=}C_4{-}C_5{=}C_6$ is observed to increase as compared to that of thymine (Table 3). The doublets observed in the region of $C{=}O_{str}$ vibrations (Fig. 1) in the spectra of both solid samples and solutions [1] suggest that in addition to the hydrogen bonds $N_1{-}H \cdots F$, the group N_1H forms the hydrogen bonds $C_2{=}O \cdots HN_1$.

The data in Table 4 indicate that methylation leads to a low-frequency shift of $C{=}O_{str}$ vibrations in the spectra of matrix-isolated molecules, the $C_2{=}O_{str}$ vibrations being shifted by N_1-methylation, and $C_2{=}O_{str}$ and $C_4{=}O_{str}$ vibrations by N_1- and N_3-methylation.

Ring vibrations

Calculation of the normal vibration modes shows that many vibrations of the pyrimidine cycle are contributed by NH deformation vibrations sensitive to molecular interactions. The transition from an associated molecule to a matrix-isolated one is therefore accompanied by a sharp increase in the vibration

Table 2. Observed (argon matrix, 11 K) and calculated vibrational spectra of 6-fluorothymine and its methylated derivatives*

6-Fluorothymine				1-Methyl-6-fluorothymine			1,3-Dimethyl-6-fluorothymine			
Observed		Calculated		Observed		Calculated	Observed		Calculated	
Frequency	I	Frequency	I	Frequency	I	Frequency	Frequency	I	Frequency	Type
3463	30	3454	42							N_1H str
3438	21	3434	28	3436	18	3434				N_3H str
				3425	1					1714+1711
		3012	2			3012			3012	
		2989	2			2989			2989	$CH_3(C_5)$ str
		2936	2			2936			2936	
						2995			2995	
						2984			2984	$CH_3(N_1)$ str
						2921			2921	
									2994	
									2984	CH_3 (N_3) str
1779	3			1750	sh				2920	
1764	99	1769	99	1745	62	1741	1723	42	1719	C_2O str
1744	6			1738	sh					
1727	3									
1717	43	1724	54	1714	77	1720				C_4O str, C=C str
1712	90	1706	75	1711	99	1703	1707	57	1712	C_5C_6 str
				1693	sh					
				1690	40	1695	1677	99	1682	Ring str
1501	21	1512	15	1487	25	1497				Ring str, N_3H bend
1488	27	1495	13							Ring str
1482	sh	1475	3	1482	25	1470	1487	42	1494	Ring str
				1460	11	1450	1460	15	1450	
1443	6	1450	9	1445	25	1448	1447	57	1448	
		1438	9			1438	1442	sh	1447	CH_3 bend
						1436			1438	
									1436	
									1430	
1406	12	1415	14							Ring str
1375	3	1382	9	1379	7	1384				N_3H bend
				1368	3	1372	1386	5	1392	Ring str
1355	6	1366	5	1365	7	1366	1366	10	1366	CH_3 (C_5) bend
									1363	CH_3 (N_3) bend
				1355	11	1360			1360	$CH_3(N_1)$ bend

1282	6	1287	3	1289	7	1287	1287	5	1289	C_5–CH_3 str
1212	3	1226	3							N_1H, bend
				1233	7	1278	1276	10	1278	N_1–CH_3 str
									1273	N_3–CH_3 str
1194	24	1182	47	1174	29	1180	1175	42	1182	C–F str
1175	3	1153	5							N_3H bend, N_1H bend
1123	6	1109	3	1140	11	1152				N_1H ring bend
							1074	5	1089	Ring bend, C–F str
							1069	10	1060	Ring str, N_3–CH_3 str
							1057	5	1052	Ring str, ring bend
							1052	21	1047	Ring str, N_1–CH_3 str
				1053	3	1036			1032	CH_3 bend
1003	6	1012	1	1051	1	1031			1027	CH_3 bend
958	3	969	1			969			969 }	
						963			962 }	CH_3 bend
									954 }	
							905	5	918	Ring bend
760	6									
757	21	760	47	758	18	763	757	31	762	C_2O bend
752	3	758	1	751	3	762			770	Ring bend
748	9			722	1	729			738	CH bend, C=O bend
666	9						654	5		
623	12			627	7					
				611	3	600				Ring bend, C–F bend
518	9	509	4							Ring bend
480	15									
				490	sh	503			490	Ring bend
460	1			486	3		462	5		
450	1						456	1		
		440	54	450	40	438			430	C–F bend
		403	9	389					394	C_4O bend
		394	2	396					399	C–CH_3 bend
				375					380	N_1–CH_3 bend
									367	N_3–CH_3 bend

*Frequency in cm^{-1}; I, relative intensity; sh, band shoulder; braces indicate merged bands; str, stretching; bend, bending.

Table 3. Observed (argon matrix, 11 K) and calculated vibrational spectra of thymine*

Observed [12]		Calculated		Observed [12]		Calculated	
Frequency	I	Frequency	Type	Frequency	I	Frequency	Type
3478	19	3480	N_1H str	1311	1		661 + 661
3431	15	3433	N_3H str	1305	1		543 + 762
		3013	C_m-H str†	1294	1	1288	C_5C_m str
		2990		1198	3	1205	C_6H bend
		2934		1182	31	1177	N_1H bend, C_6H bend
1766	74	1761	C_2O str	1086	5	1096	Ring bend, ring str
1749	57					1050	C_mH bend
1744	50	1740	C_4O str, $C_5{=}C_6$ str	959	3	963	C_mH bend
1722	36			890	4	902	Ring str, ring bend
1710	100	1703	$C_5{=}C_6$ str, C_4O str	762	11	756	Ring bend, C_6H bend
1681	15	1676	Ring str, N_1H bend	752	10	747	Ring bend
1667	11	1653	Ring str, N_3H bend			724	C_mH bend
1470	23	1483	Ring str, C_6H bend	661	17		
1456	2	1454	C_mH bend	543	21	534	C_2O bend, ring bend
1451	2	1443	C_6H bend, ring str	453	6	456	C_2O bend, C_4O bend
1434	2	1439	C_mH bend			387	C_5C_m bend
1404	21	1411	N_3H bend				
1388	4	1382	Ring str				
1365	1	1366	C_mH bend				
1354	1						
1344	1						

*See Table 2, †m, methyl.

Table 4. Force constants of 6-fluorothymine and its methylated derivatives (10^6 cm^{-2})

	6-Fluorothymine		1-Methyl-6-fluorothymine	1,3-Dimethyl-6-fluorothymine
K of bond	Solid	Argon matrix	Argon matrix	Argon matrix
$C_2{=}O$	18.5	18.12	18.7	18.4
$C_4{=}O$	17.83	16.8	17.9	17.3
C–F	11.6	12.8	12.8	12.8
$N_1–C_2$	10.3	10.4	10.4	10.35
$C_2–N_3$	9.1	10.4	10.4	10.35
$N_3–C_4$	10.3	10.4	10.4	10.4
$C_4–C_5$	9.5	10.6	10.6	10.6
$C_5–C_6$	14.0	14.9	14.8	14.8
$C_6–N_1$	10.3	10.9	10.7	10.7

frequencies of the ring, which are contributed by the NH_{def} vibrations.

The ring vibrations in the region from 1500 to 400 cm^{-1} involving the bonds of the $C_5{=}C_6–N_1$ fragment, are highly sensitive to the substitution of fluorine for the proton at C_6, to N_1- and N_3-methylation and to the transition from the solid state to the isolated one.

Methyl group vibrations

In the 1500–900 cm^{-1} region many bands in the experimental spectra refer to the deformation vibrations of the methyl groups and are of medium intensity. According to the calculation, many vibrations of the ring are mixed with those of $CH(methyl)_{def}$ in this region. The medium intensity bands in the 1270–1290 cm^{-1} region correspond to $C–CH_{3\,str}$ and $N–CH_{3\,str}$ vibrations.

CONCLUSIONS

A comparison of the spectra taken on solid and matrix-isolated samples of thymine, 6-fluorothymine and its methyl derivatives and the calculation of their vibrations permit the following conclusions.

(1) The frequencies and modes of normal vibrations of thymine are strongly influenced by the electronegative fluorine atom introduced into the 6-position. The suppression of the electron-acceptor properties of the C_4O group leads to an increase in the frequency of the vibrations corresponding to the stretching vibrations of the $O{=}C_4–C_5{=}C_6$.

(2) The formation of the hydrogen bond $N_1H \cdots F$ significantly decreases the N_1H_{str} frequency and increases the N_1H_{def} frequency.

(3) Intermolecular complexes of various structures may be formed in the condensed state with hydrogen

bonds, N_1–H ··· F or N_1–H ··· O–C_2, which leads to the splitting of the N_1H_{str} and C_2O_{str} bands.

(4) On transition from the solid state to the matrix-isolated one, the frequencies of the ring stretching vibrations involving the NH groups and of the $C{=}O_{str}$ vibrations are observed to increase significantly.

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