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Out-of-plane vibration modes of nucleic acid bases

I. Pyrimidine bases

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Abstract. The out-of-plane vibration modes of uracil, cytosine and their deuterated and methylated derivatives such as 1,5-dimethyluracil (1-methylthymine), 1-methylcytosine, 5-methylcytosine and 1,5-dimethylcytosine have been computed. The calculated wavenumbers have been compared to the published Raman peak and infrared band positions observed for solid or aqueous samples. The calculations have been carried out on a non-redundant set of symmetrical coordinates and a valence force field has been used. Some characteristic modes located between 750 and 800 cm^{-1} found in the infrared spectra of 2′-deoxycytidine, 2′-deoxythymidine 5′-monophosphate and polynucleotides containing cytosine and thymine bases can be interpreted from the calculated results on 1-methylthymine and 1-methylcytosine.

Key words: Uracil, thymine, cytosine, vibration modes, nucleic acids, normal coordinate analysis

Introduction

In-plane vibration modes of nucleic acid bases have been intensively studied in recent years. In fact, the 1700-1300 cm^{-1} region of nucleic acid Raman and infrared spectra is mainly assigned to base in-plane modes. Recently, linear dichroism studies showed that the narrow and intense infrared bands observed in the 800-750 cm^{-1} region of polynucleotide spectra are polarized along the double-helix axis and arise from base out-of-plane vibration modes (Ghomi et al. 1984; Chinsky et al. 1984; Taillandier et al. 1984, 1985). In the 800-600 cm^{-1} region the ring-breathing modes located in the base planes are observed in the Raman spectra of polynucleotides (Pohl et al. 1973; Thamann et al. 1981; Nishimura et al. 1984; Benevides et al. 1984; Thomas and Peticolas 1984) but not in the IR spectra.

In this work we discuss the results obtained for pyrimidines and in another paper (Letellier et al. 1986a) those concerning purines. Our aim is to confirm by calculation, the experimental assignments of the infrared bands observed between 800 and 750 cm^{-1} as effectively corresponding to the out-of-plane vibration modes of the base residues. For this a systematic investigation has been undertaken in order to adopt a force field allowing us to reproduce the previously published data concerning the out-of-plane motions of pyrimidines.

Theoretical considerations

On the basis of X-ray diffraction data the nucleic acid bases are planar. However, the presence of a methyl-group in the 5 position of thymine makes it difficult to separate the vibrations of this molecule into two distinct families of in-plane and out-of-plane modes. It was shown previously (Susi and Ard 1974) that the bond-stretch and angular deformations of the methyl-group give rise to the modes with wavenumbers situated above 900 cm^{-1}. Thus, their interaction with the ring out-of-plane modes arising from the out-of-plane wagging (o.p.w.) or torsion modes located generally below 900 cm^{-1} should be negligible. We have verified by additional calculations (not detailed here) that even the CH3-rocking modes do not interact with the ring non-planar vibrations.

So, taking account of this fact, we have supposed that the methyl-group found in thymine, as in other methylated analogs, can be considered as a rigid dynamic unit with the mass concentrated on the carbon atom. So, one can suppose, in the framework of this approximation that all of the nucleic acid bases and their methylated derivatives studied in

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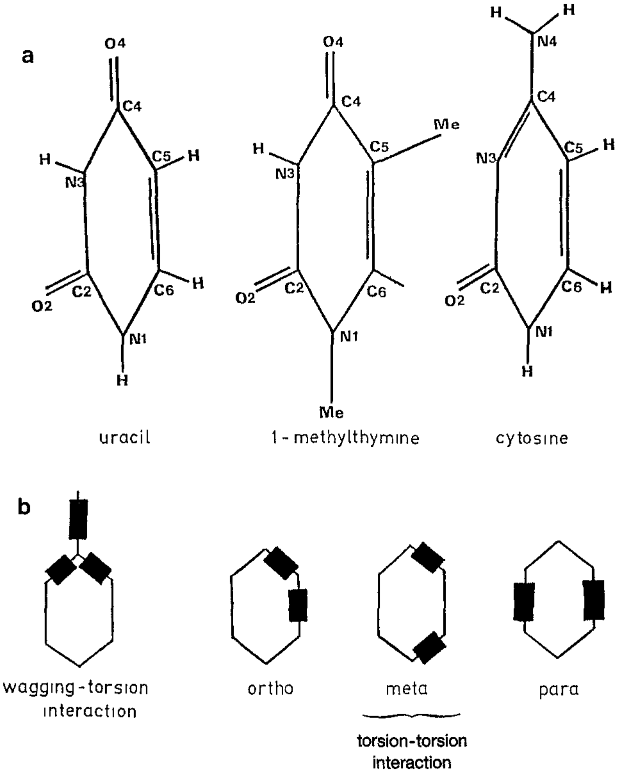

Fig. 1. **a** Representation and atom numbering for the three molecules on which the out-of-plane vibration mode calculations are detailed in this work. *Me*: methyl group. **b** Interaction range considered in the present calculations between an out-of-plane wagging coordinate and cyclic torsion coordinates on the one hand and between the cyclic torsion coordinates on the other. See Table 1b for the corresponding force constants

this work belong to the C_s point-group and each one possesses $2N-3$ in-plane (A'-symmetry) and $N-3$ out-of-plane (A''-symmetry) modes; N is the number of atoms in each molecule. Therefore, uracil, thymine and their analogs give rise to 9 and cytosine to 10 out-of-plane vibration modes (Fig. 1a).

When these bases are associated with the nucleic acid chains their symmetry is consequently lowered. Due to this fact the vibration mode couplings will be consequently modified (Lu et al. 1979) and the above assumptions will not be valid. In this case, the calculations should be done by considering the real overall symmetry of the nucleic acids. However, the results of the present calculations would be helpful in order to examine the nature of the vibration modes of the bases in terms of their local symmetry and in the absence of the perturbation induced by their connection to the nucleic acid double-helical chains.

In order to preserve the harmonic approximation of the potential field, it is necessary to perform the vibration mode calculations on a non-redundant set of internal coordinates. In uracil and thymine 12 non-planar internal coordinates (6 torsions + 6 o.p.w.) are numbered while cytosine involves 14 such coordinates (7 torsions + 7 o.p.w.). So, there are 3 redundant coordinates in uracil and thymine and 4 in cytosine.

Moreover, it is known that the force constants in a redundant molecular system are not the same as in a non-redundant one. Previously, the expression of the force constant corrections in redundant molecular coordinates have been reported (Van Zandt and Lu 1977). The numerical procedure used to eliminate the superfluous coordinates, has been published previously (Ghomi and Taillandier 1985). An independent set of non-redundant symmetrical coordinates (S) has been obtained and used in vibration mode calculations.

The interatomic interactions due to the out-of-plane motions have been expressed by means of a valence force field. The non-planar potential energy, $V_{\text{n p.}}$, has been considered as:

$$V_{\text{n p.}} = V_{\text{diag}} + V_{\text{int}} \,. \tag{1}$$

Where V_{diag} is the principal part of the potential energy including the diagonal force constants:

$$2\,V_{\text{diag}} = \sum_i K_{\omega_i}(\delta\omega_i)^2 + \sum_j K_{\tau_j}(\delta\tau_j)^2 \,, \tag{2}$$

$\delta\omega_i$ refers to an o.p.w. and $\delta\tau_j$ to a torsion internal coordinate. K_{ω_i} and K_{τ_j} represent the corresponding force constants. In order to reduce the number of necessary force constants, we have assigned the same force constant to all similar internal coordinates and considered the force field as transferable from one base to another.

V_{int} is the interaction part of potential energy involving the off-diagonal constants. It is composed by the following terms:

$$2\,V_{\text{int}} = \sum_{\substack{i,j\\ i\neq j}} K_{\omega_i,\omega_j}(\delta\omega_i)\,(\delta\omega_j) + \sum_{i,j} K_{\omega_i,\tau_j}(\delta\omega_i)\,(\delta\tau_j) + \sum_{\substack{i,j\\ i\neq j}} K_{\tau_i,\tau_j}(\delta\tau_i)\,(\delta\tau_j) \,. \tag{3}$$

The first term reflects the interaction between o.p.w.'s considered previously in benzene (Scherer 1967) or imidazole (Cordes and Walter 1968). Only the interactions between the C=O o.p.w.'s in uracil and between C–NH_2 and NH_2 have been considered in order to improve the calculated results (Table 1b). The interactions between an o.p.w. and the cyclic torsion coordinates are involved in the second term. It is found that each o.p.w. interacts mainly with its two adjacent torsion coordinates (Fig. 1b). The absolute values of the K_{ω_i,τ_j} force constants for a given value of i, are supposed to be the

Out-of-plane wagging force constants		Torsion force constants	
Coordinates	Values (mdyn.Å)	Coordinates	Values (mdyn.Å)
W(C-H)	0.370	T(C-C)	0.520
W(N-H)	0.300	T(C=C)	0.520
W(C=O)	0.334	T(C-N)	0.255
W(C-Cm)	0.334	T(C=N)	0.629
W(C-NH2)	0.334	T(C-NH2)	0.112
W(N-Cm)	0.334		
W(NH2)	0.035		

Table 1a. Diagonal force constants for the out-of-plane vibration modes of the pyrimidines and their methylated derivatives. *W* designs an out-of-plane wagging coordinate and *T* a torsion coordinate. Cm: methyl group

same. They are obviously of opposite sign because of the permanent out-of-phase motions corresponding to two adjacent cyclic torsions (Table 1b). Finally, the third term in Eq. (3) relates to the interaction between the torsion coordinates. We have separated the *ortho, meta,* and *para* contributions among all possible interactions (Fig. 1b) and given the same value of K_{τ_i,τ_j} to each family of interactions (Table 1b).

To calculate the vibrational wavenumbers and atomic displacement amplitudes the Wilson GF-method has been used (Wilson et al. 1955). The calculation program NCTB (Shimanouchi 1968) has been simplified and divided into four segments in order to obtain a version suitable for small computers. All numerical calculations have been performed on a DPS6/96 Honeywell-Bull computer. The force constants have been refined using a mean-square method (Table 1a and b). To assign the vibration modes with clarity the (PED) matrix, representing the potential energy distribution calculated on the basis of the non-redundant *S* coordinates, has been projected on the internal coordinates *R* (Letellier et al. 1986b). Moreover, by means of a simple program developed in our laboratory the molecular deformation during a given vibration mode has been drawn on a graphic screen SECAPA 770A. Hardcopies of these graphs are presented here.

Results

A. Uracil and its deuterated derivatives

To construct B_s and G_s matrices (Ghomi and Taillandier 1985) the geometry of crystalline uracil (Stewart and Jensen 1967) has been used. Raman and infrared spectra of polycrystalline uracil and its C,C-dideutero, N,N-dideutero and perdeutero derivatives have been studied by Susi and Ard (1971) and the non-planar contributions have been discussed (Table 2). Because of the existence of an inversion symmetry in uracil crystals the infrared and Raman peak positions do not coincide. However, good agreement has been obtained between the experimental and calculated results based on isolated molecules with C_s-symmetry. The changes in the uracil vibrational spectra upon selective deuterations have been satisfactorily reproduced, and are in good agreement with the experimental interpretation proposed originally by Susi and Ard (1971). Figure 2 shows the graphic representation of the atomic displacements for the 9 out-of-plane vibration modes of uracil.

B. 1,5-dimethyluracil or 1-methylthymine and its N3-deuterated derivatives

The effect of methylation of the N1 and C5 positions of uracil on its vibrational spectra were first studied by Kyogoku et al. (1967). In their work the single crystals of 1-methylthymine were cleaved along the principal crystallographic axis. The infrared spectra were then studied by a properly selected polarization of the incident beam in order to separate the planar and non-planar vibration modes.

Later, Guay et al. (1983) recorded the Raman and infrared spectra of polycrystalline samples as well as the Raman spectra of aqueous 1-methylthymine (Table 3).

The calculated results based on the structural data of 1-methylthymine obtained from X-ray diffraction patterns of crystalline samples (Hoogsteen 1963) interpret well the experimental wavenumbers and also reflect the N3-deuteration effect on the peak positions (Table 3). Graphic representation of

Table 1b. Non-diagonal force constants for the out-of-plane vibration modes of the pyrimidines and their methylated derivatives. *W* designs an out-of-plane wagging coordinate and *T* a torsion coordinate. Cm: methyl group

Interactions	uracile and its derivatives		1-methylthymine and its derivative		cytosine and its derivatives	
	values	deviations	values	deviations	values	deviations
	(mdyn.Å)		(mdyn.Å)		(mdyn.Å)	
wagging,torsion						
W(N1-H),T(N1-C2)	0.079	(0.04)			0.067	(0.041)
W(N1-H),T(N1-C6)	-0.079				-0.067	
W(C2=O),T(N1-C2)	-0.056	(0.0)	-0.056	(0.0)	-0.056	(0.0)
W(C2=O),T(N3-C2)	0.056		0.056		0.056	
W(N3-H),T(N3-C2)	0.062	(0.33)	0.002	(0.14)		
W(N3-H),T(N3-C4)	-0.062		-0.002			
W(C4=O),T(N3-C4)	-0.056	(0.0)	-0.056	(0.0)		
W(C4=O),T(C4-C5)	0.056		0.056			
W(C5-H),T(C4-C5)	-0.171	(0.02)			-0.171	(0.0)
W(C5-H),T(C5=C6)	0.171				0.171	
W(C6-H),T(C5=C6)	-0.127	(0.02)	-0.140	(0.0)	-0.127	(0.0)
W(C6-H),T(N1-C6)	0.127		0.140		0.127	
W(C5-Cm),T(C4-C5)			-0.056	(0.0)	-0.056	(0.0)
W(C5-Cm),T(C5=C6)			0.056		0.056	
W(N1-Cm),T(N1-C2)			0.056	(0.0)	0.056	(0.0)
W(N1-Cm),T(N1-C6)			-0.056		-0.056	
W(C4-N4),T(C4=N3)					-0.056	(0.0)
W(C4-N4),T(C4-C5)					0.056	
torsion,torsion						
Ortho	-0.018	(0.0)	-0.053	(0.0)	-0.018	(0.039)
Meta	-0.012	(0.0)	0.012	(0.0)	-0.012	(0.004)
Para	-0.004	(0.0)	-0.004	(0.0)	-0.004	(0.0)
wagging,wagging						
W(C2=O),W(C4=O)	0.091	(0.0)	0.0	(0.0)		
W(NH2),W(C4-N4)					0.041	(0.0)

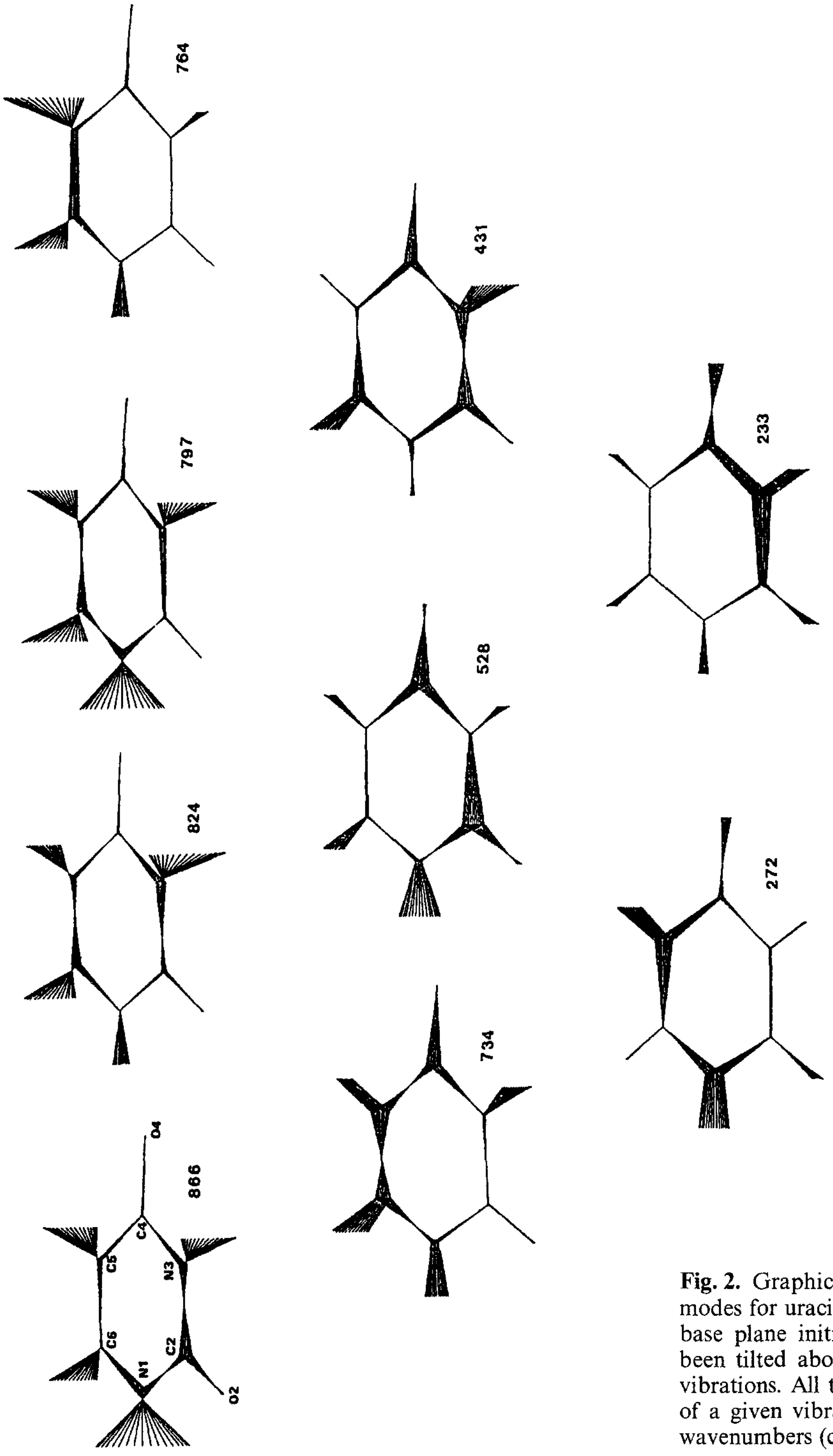

Fig. 2. Graphic representation of the 9 out-of-plane vibration modes for uracil and obtained by the present calculations. The base plane initially perpendicular to the drawing plane has been tilted about 10° in order to visualize the out-of-plane vibrations. All the situations of the base ring during a period of a given vibration mode have been drawn. The calculated wavenumbers (cm^{-1}) of each mode is noted. See also Table 2

the 1-methylthymine non-planar modes is shown in Fig. 3.

C. Cytosine and its N-deuterated derivative

The calculations are based on the crystalline structural data proposed by Barker and Marsh (1964) and the experimental wavenumbers obtained on cytosine polycrystalline samples (Table 4) (Susi et al. 1973). As the effect of the crystalline field on the vibration modes is not considered in this work, all of the originally proposed non-planar modes (Susi et al. 1973) cannot be assigned by the present calculations. However, as Table 4 shows, the calculated results are in good agreement with the selected ex-

Uracil			C,C-dideutero uracil			N,N-dideutero uracil			perdeutero uracil		
Exp.	Cal.	Assignments (PED%)	Exp.	Cal.	Assignments (PED%)	Exp.	Cal.	Assignments (PED%)	Exp.	Cal.	Assignments (PED%)
867 R 851 IR	866	W(N1-H)(40); W(N3-H)(15) T(C5=C6)(12)									
			857 IR	853	W(N1-H)(46); W(N3-H)(27)						
830 R 822 IR	824	W(N3-H)(36); W(C6-H)(24) T(C5=C6)(12); W(C5-H)(11)				829 R 821 IR	828	T(C5=C6)(34); W(C6-H)(30) W(C5-H)(25)			
			815 IR	812	W(N3-H)(40); W(N1-H)(26)						
807 IR	797	W(N1-H)(28); W(C6-H)(19) T(N1-C6)(12); W(N3-H)(11)									
782 IR 771 R	764	W(C5-H)(45); W(C6-H)(29) T(C4-C5)(18)				778 IR	771	W(C6-H)(53); W(N1-D)(16)			
760 IR	734	W(C5-H)(24); W(C4=0)(22) T(N1-C6)(16); T(C5=C6)(14)	767 R 764 IR	749	W(C6-D)(37); W(C5-D)(27)	762 IR	760	W(C5-H)(59); W(C6-H)(13) T(C4-C5)(11)	770 IR 761 R	773	W(C6-D)(27); W(C5-D)(18) W(N1-D)(18); W(N3-D)(14) W(C4=0)(11); W(C2=0)(11)
						621 IR	635	W(C2=0)(30); W(N1-D)(22) W(N3-D)(19)	622 IR	660	W(N1-D)(21); W(C5-D)(20) W(C2=0)(19); W(N3-D)(13) W(C6-D)(11)
			602 R 598 IR	607	W(C6-D)(45); W(C4=0)(20) W(C5-D)(17)				595 IR	604	W(C6-D)(48); W(C4=0)(18)
						584 IR	576	W(N3-D)(51); W(N1-D)(25)			
			559 IR	535	W(C5-D)(46); T(C5=C6)(29) T(C4-C5)(13)				580 IR sh.	568	W(N3-D)(46); W(N1-D)(23)
530 IR	528	W(C2=0)(38); W(C4=0)(25) T(N1-C6)(23)									
			527 IR	500	W(C2=0)(35); T(N1-C6)(25) W(C6-D)(21)				552 IR	530	W(C5-D)(49); T(C5=C6)(23) T(C4-C5)(16)
431 R	431	W(C2=0)(38); W(C4=0)(22) T(N3-C4)(16); T(C2-N3)(11)				431 R	442	W(N1-D)(35); T(N1-C6)(31) W(C4=0)(13); T(N1-C2)(11)			
			416 R	411	W(C4=0)(24); W(C2=0)(23) T(N3-C4)(12); T(C5=C6)(11) T(C2-N3)(10)				415 R	405	T(N1-C6)(31); W(N1-D)(30) W(C6-D)(14)
							380	W(C2=0)(30); T(N3-C4)(24) T(C2-N3)(13); W(N1-D)(12) W(N3-D)(10)		374	W(C2=0)(28); T(N3-C4)(25) T(C2-N3)(18)
	272	T(N1-C2)(27); T(C4-C5)(19) T(C2-N3)(16); W(N1-H)(10)		266	T(C4-C5)(28); T(N1-C2)(24) T(C2-N3)(13)		267	T(N1-C2)(43); T(C2-N3)(19) T(N1-C6)(13); T(C4-C5)(12)		261	T(N1-C2)(40); T(C4-C5)(17) T(N1-C6)(17); T(C2-N3)(15)
	233	W(C4=0)(37); W(C2=0)(15) T(C4-C5)(11); T(N3-C4)(11)		232	W(C4=0)(37); W(C2=0)(15) T(N3-C4)(10)		232	W(C4=0)(38); T(N3-C4)(16) W(C2=0)(15); T(C4-C5)(14)		232	W(C4=0)(39); W(C2=0)(15) T(N3-C4)(15) T(C4-C5)(12)

Table 3. Comparison between the calculated and experimental wavenumbers (cm^{-1}) for the out-of-plane vibration modes of 1-methylthymine and its N3-deuterated analog. Assignments are based on the potential energy distribution (PED in per cent) described upon the internal coordinates. *Exp.*: Experimental data: (*a*): Raman spectra of aqueous solutions (Guay et al. 1983). (*b*): Raman spectra of polycrystalline samples (Guay et al. 1983). (*c*): Infrared spectra of oriented single crystals (Kyogoku et al. 1967). (*d*): Infrared spectra of polycrystalline samples (Guay et al. 1983). *Cal.*: Calculated wavenumbers (this work). *W* designs an out-of-plane wagging coordinate and *T* a torsion coordinate. Cm: methyl group. D: Deuterium

1-Methylthymine			1-Methylthymine N3-deuterated		
Exp.	Cal.	Assignments (PED%)	Exp.	Cal.	Assignments (PED%)
883(a)					
882(c)					
881(b)	881	W(N3-H)(69); T(N3-C4)(16)			
		T(C2-N3)(11)			
764(b)					
765(c)			772(b)	768	W(C6-H)(60); W(C5-Cm)(11)
763(d)	763	W(C6-H)(69)			
			757(d)	723	W(C6-H)(33); T(C5=C6)(19)
					W(N3-D)(19); T(N1-C6)(13)
705(c)	707	T(C5=C6)(28); W(C6-H)(26)			
705(d)		T(N1-C6)(18)			
703(a)					
699(b)			650(c)	649	W(N3-D)(50); T(N3-C4)(12)
					T(C2-N3)(10)
533(a)	539	W(C2=O)(30); W(C4=O)(29)	532(a)	539	W(C4=O)(30); W(C2=O)(28)
531(b)		W(N1-Cm)(24); W(C5-Cm)(15)	528(b)		W(N1-Cm)(25); W(C5-Cm)(14)
530(c)					
455(a)					
455(c)					
452(b)					
451(d)	442	W(C2=O)(37); W(C4=O)(20)			
		T(N3-C4)(10)	424(b)	418	W(C2=O)(36); W(C4=O)(18)
			415(d)		T(N3-C4)(16); T(C2-N3)(13)
328(b)	326	W(C5-Cm)(37); W(N1-Cm)(14)	325(b)	325	W(C5-Cm)(36); W(N1-Cm)(15)
		W(C2=O)(11); W(C4=O)(10)			W(C4=O)(11); W(C2=O)(10)
285(b)	283	T(C4-C5)(27); W(N1-Cm)(21)	284(b)	280	T(C4-C5)(27); W(N1-Cm)(21)
282(a)		W(C4=O)(15); W(N3-H)(11)			W(C4=O)(14); T(N3-C4)(12)
		T(N1-C2)(10); T(N3-C4)(10)			T(N1-C2)(10)
	175	W(N1-Cm)(24); W(C5-Cm)(19)		176	W(N1-Cm)(25); W(C5-Cm)(19)
		W(N3-H)(11); W(C4=O)(10)			W(C4=O)(10); W(N3-D)(10)
	134	T(N1-C2)(35); T(N1-C6)(22)		134	T(N1-C2)(35); T(N1-C6)(22)
		T(C4-C5)(20)			T(C4-C5)(20)

◄

Table 2. Comparison between the calculated and experimental wavenumbers (cm^{-1}) for the out-of-plane vibration modes of uracil and its selectively deuterated analogs. Assignments are based on the potential energy distribution (PED in per cent) described upon the internal coordinates. *Exp.*: Experimental Raman (R) and infrared (IR) peak positions of polycrystalline uracil (Susi and Ard 1971). *Cal.*: Calculated wavenumbers (this work). *W* designs an out-of-plane wagging coordinate and *T* a torsion coordinate. D: Deuterium

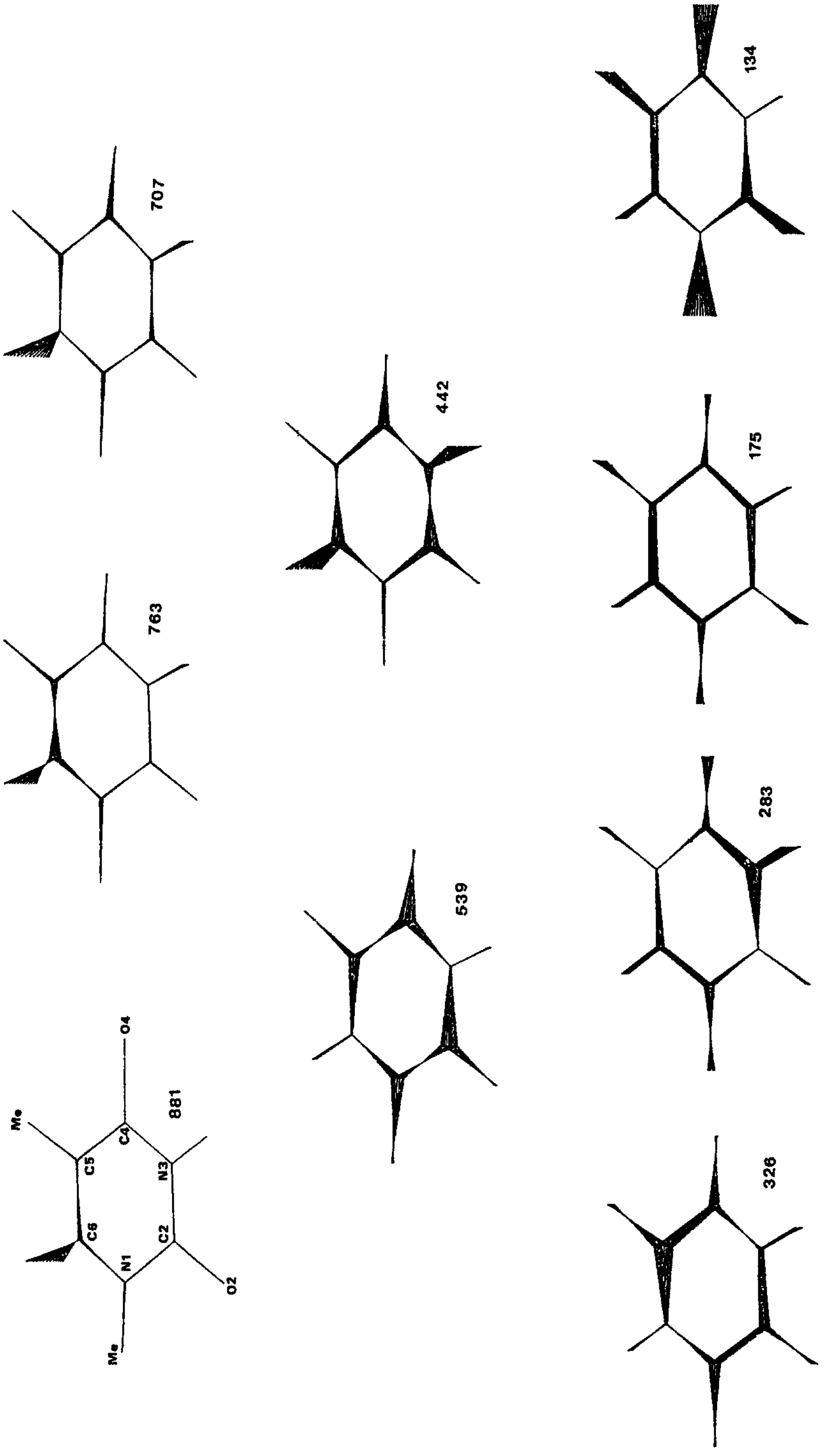

Fig. 3. Graphic representation of the 9 out-of-plane modes for 1-methylthymine. The base plane initially perpendicular to the drawing plane has been tilted about 10° in order to visualize the out-of-plane vibrations. All the situations of the base ring during a period of a given vibration mode have drawn. The calculated wavenumbers (cm^{-1}) of each mode is noted. See also Table 3

perimental data. The effect of N-deuteration (in 1 and 4 positions) has also been reproduced by the calculations. Graphic representation of the 10 calculated non-planar modes of cytosine is shown in Fig. 4.

D. Cytosine methylated derivatives

Methylation on the C5-site of cytosine has been studied by infrared spectroscopy (Goel et al. 1976). On the basis of the calculated results, the non-planar contribution of 5-methylcytosine vibration modes can be separated (Table 5). Graphic representation of these modes is shown in Fig. 5. The experimental data of the other cytosine methylated analogs is not known. However, we found it interesting to extend our force field to 1-methylcytosine and 1,5-dimethylcytosine in order to study in detail, the effect of methylation the 1 and/or 5 positions (Table 5).

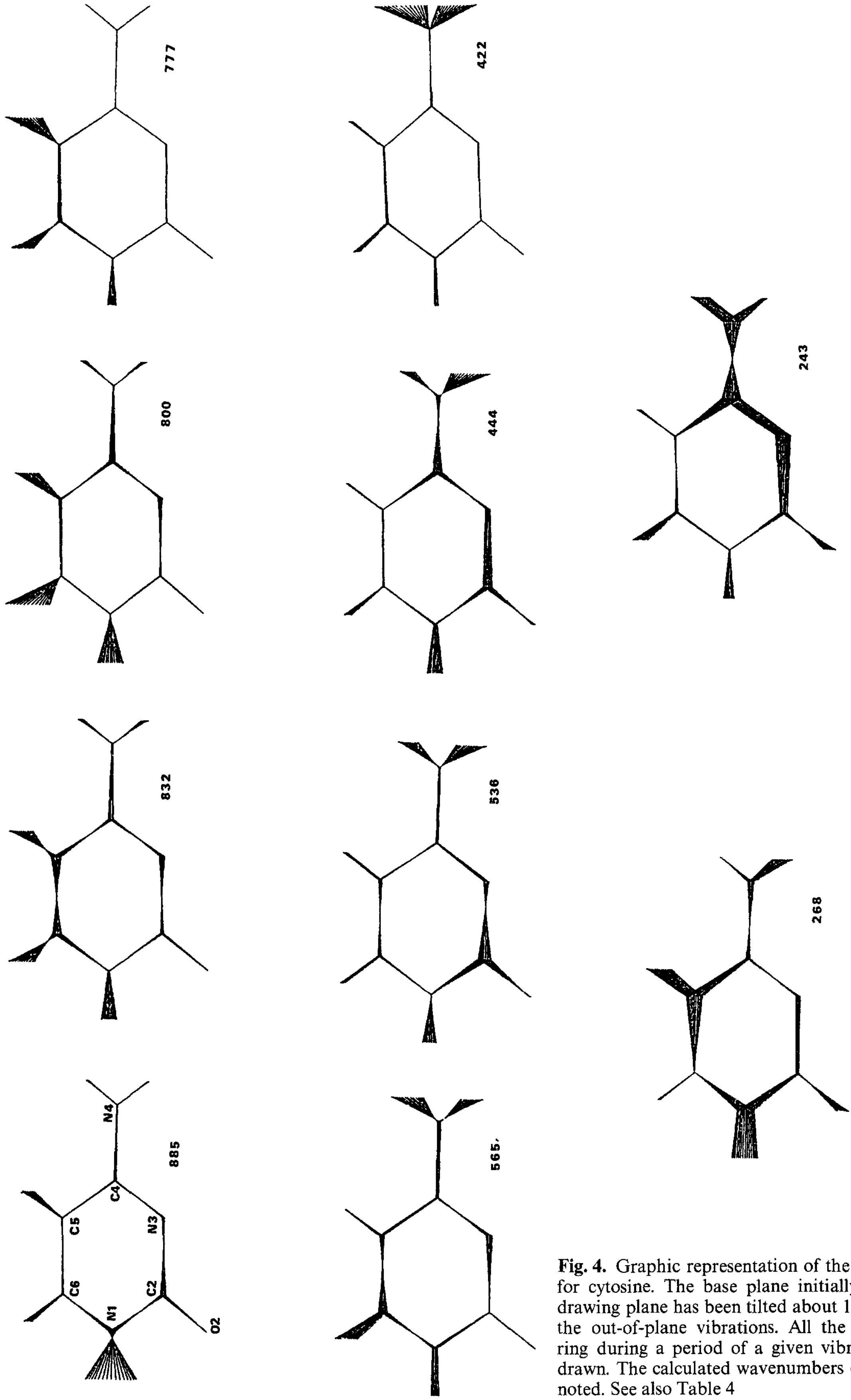

Fig. 4. Graphic representation of the 10 out-of-plane modes for cytosine. The base plane initially perpendicular to the drawing plane has been tilted about 10° in order to visualize the out-of-plane vibrations. All the situations of the base ring during a period of a given vibration mode have been drawn. The calculated wavenumbers (cm^{-1}) of each mode is noted. See also Table 4

Table 4. Comparison between the calculated and experimental wavenumbers (cm^{-1}) for the out-of-plane vibration modes of cytosine and cytosine-d$_3$ (N1 and N4 deuterated analog). Assignments are based on the potential energy distribution (PED in per cent) described upon the internal coordinate. *Exp.:* Experimental Raman (R) and infrared (IR) peak positions of polycrystalline cytosine (Susi et al. 1973). *Cal.:* Calculated wavenumbers (this work). *W* designs an out-of-plane wagging coordinate and *T* a torsion coordinate. D: Deuterium

Cytosine			Cytosine-d3		
Exp.	Cal.	Assignments (PED%)	Exp.	Cal.	Assignments (PED%)
894 R	885	W(N1-H)(76); T(N1-C2)(12)			
			850 IR		
			849 R	839	T(N3=C4)(20); W(C2=0)(18)
					T(C4-N4)(18); W(N1-D)(17)
					W(C5-H)(10); T(C2-N3)(10)
823 IR	832	W(C5-H)(41); W(C6-H)(38)			
811 R				821	W(C6-H)(52); W(C5-H)(47)
782 R	800	W(C6-H)(52); W(N1-H)(19)			
782 IR					
764 R	777	W(C5-H)(62); W(C6-H)(34)		776	W(C5-H)(49); W(C6-H)(49)
760 IR					
			611 R		
			605 IR	609	W(N1-D)(65); W(C2=0)(16)
568 R					
566 IR	565	W(NH2)(42); W(C5-H)(19)	550 R	555	T(N3=C4)(26); W(C2=0)(26)
		T(N3=C4)(12)	545 IR		W(C5-H)(16); T(C2-N3)(10)
520 IR	536	W(C2=0)(52); W(NH2)(18)			
517 R		T(N3=C4)(13)			
442 R	444	W(C4-N4)(33); W(NH2)(31)			
442 IR		W(C2=0)(22)	438 R	438	W(N1-D)(39); W(C2=0)(27)
			437 IR		T(N1-C2)(16)
421 IR	422	T(C4-N4)(93)			
			388 IR	371	W(ND2)(58); W(C4-N4)(28)
				309	T(C4-N4)(90)
	268	T(C2-N3)(40); T(N1-C2)(30)			
		W(N1-H)(12)		259	T(N1-C2)(33); T(C2-N3)(30)
					T(C4-N4)(24)
	243	W(C4-N4)(63); W(C2=0)(13)			
		T(N3=C4)(12)		238	W(C4-N4)(64); W(C2=0)(14)
					T(N3=C4)(14)

Discussion

Up to now, most theoretical work has been concentrated principally on the planar vibration modes of the pyrimidine bases or of the corresponding residues in order to separate the non-planar contributions in the vibrational spectra (Susi and Ard 1971, 1974; Susi et al. 1973; Tsuboi et al. 1973; Bowmann and Spiro 1980; Peticolas and Strommen 1980; Szczesniak et al. 1983; Ghomi et al. 1986; Tsuboi et al. 1986). Only a limited number of investigations have been devoted to the out-of-plane modes (Eyster and Prohofsky 1974; Devi Prasad and Prohofsky 1984; Ghomi et al. 1984) which deal essentially with the skeletal motions of the bases. Taking into account the importance of hydrogen motions in the vibration modes of nucleic acids (Letellier et al. 1986b) we have performed the present calculations in order to obtain a general, complete and transferable force field for all pyrimidine bases. A valence force field and non-redundant symmetrical coordinates have therefore been used. The diagonal force constants are perfectly transferable from one base to another and the alteration in the off-diagonal force constants reflects mainly the perturbation induced by the methyl group in pyrimidine rings.

Moreover, the extension of the derived force field to the methylated pyrimidines allows us to

Table 5. Calculated wavenumbers (cm^{-1}) and assignments (PED) for the out-of-plane vibration modes of 5-methylcytosine, 1-methylcytosine and 1,5-dimethylcytosine respectively. Assignments are based on the potential energy distribution (PED in per cent) described upon the internal coordinate. *Exp.*: Infrared spectrum of 5-methylcytosine (Goel et al. 1976). *Cal.*: Calculated wavenumbers (this work). *W* designs an out-of-plane wagging coordinate and *T* a torsion coordinate. Cm: methyl group

5-Methyl Cytosine			1-Methyl Cytosine		1,5-Dimethyl Cytosine	
Exp.	Cal.	Assignments (PED%)	Cal.	Assignments (PED%)	Cal.	Assignments (PED%)
887	880	W(N1-H)(77); T(N1-C2)(12)	878	W(C5-H)(38); W(C6-H)(33)		
			849	W(C6-H)(41); W(C5-H)(28)		
	839	W(C6-H)(81)			843	W(C6-H)(72)
			778	W(C5-H)(51); W(C6-H)(44)		
768	767	W(C6-H)(33); W(C4-N4)(17) T(N3=C4)(14); W(N1-H)(10)			776	W(C6-H)(43); W(N3=C4)(15) T(C4-N4)(14); W(C2=O)(13)
			579	W(N1-Cm)(34); W(NH2)(32) W(C2=O)(10)	582	W(N1-Cm)(33); W(C2=O)(33) W(NH2)(23)
575	554	W(NH2)(66); W(C2=O)(15)				
			558	W(C2=O)(58); T(N3=C4)(16)		
500	525	W(C2=O)(42); W(C5-Cm)(18) T(N3=C4)(15)			529	W(C2=O)(37); W(C5-Cm)(31) T(N3=C4)(15)
			472	W(NH2)(46); W(C4-N4)(21) W(N1-Cm)(15)	474	W(NH2)(47); W(C4-N4)(23) W(N1-Cm)(14)
445	448	W(C4-N4)(35); W(NH2)(34) W(C2=O)(18)				
410	427	T(C4-N4)(95)	424	T(C4-N4)(96)	427	T(C4-N4)(93)
	333	W(C5-Cm)(56); T(C2-N3)(18) T(N1-C2)(11)			345	W(C5-Cm)(39); T(C2-N3)(18) W(N1-Cm)(14)
			309	W(N1-Cm)(38); W(C4-N4)(26) T(C2-N3)(20)		
					267	W(C4-N4)(60); T(N3=C4)(11)
			255	W(C2=O)(10); T(N1-C2)(11) W(C4-N4)(57)		
	242	W(C4-N4)(63); T(N3=C4)(13) W(C2=O)(12)				
					187	W(N1-Cm)(28); W(C5-Cm)(21) W(C4-N4)(21); W(C2=O)(10)
	174	T(C2-N3)(28); T(N1-C2)(24) W(C5-Cm)(17); W(N1-H)(13)				
			149	T(N1-C2)(38); W(N1-Cm)(33) W(C2=O)(13)		
					131	T(N1-C2)(53); W(N1-Cm)(22) T(C2=O)(10); W(C2=N3)(10)

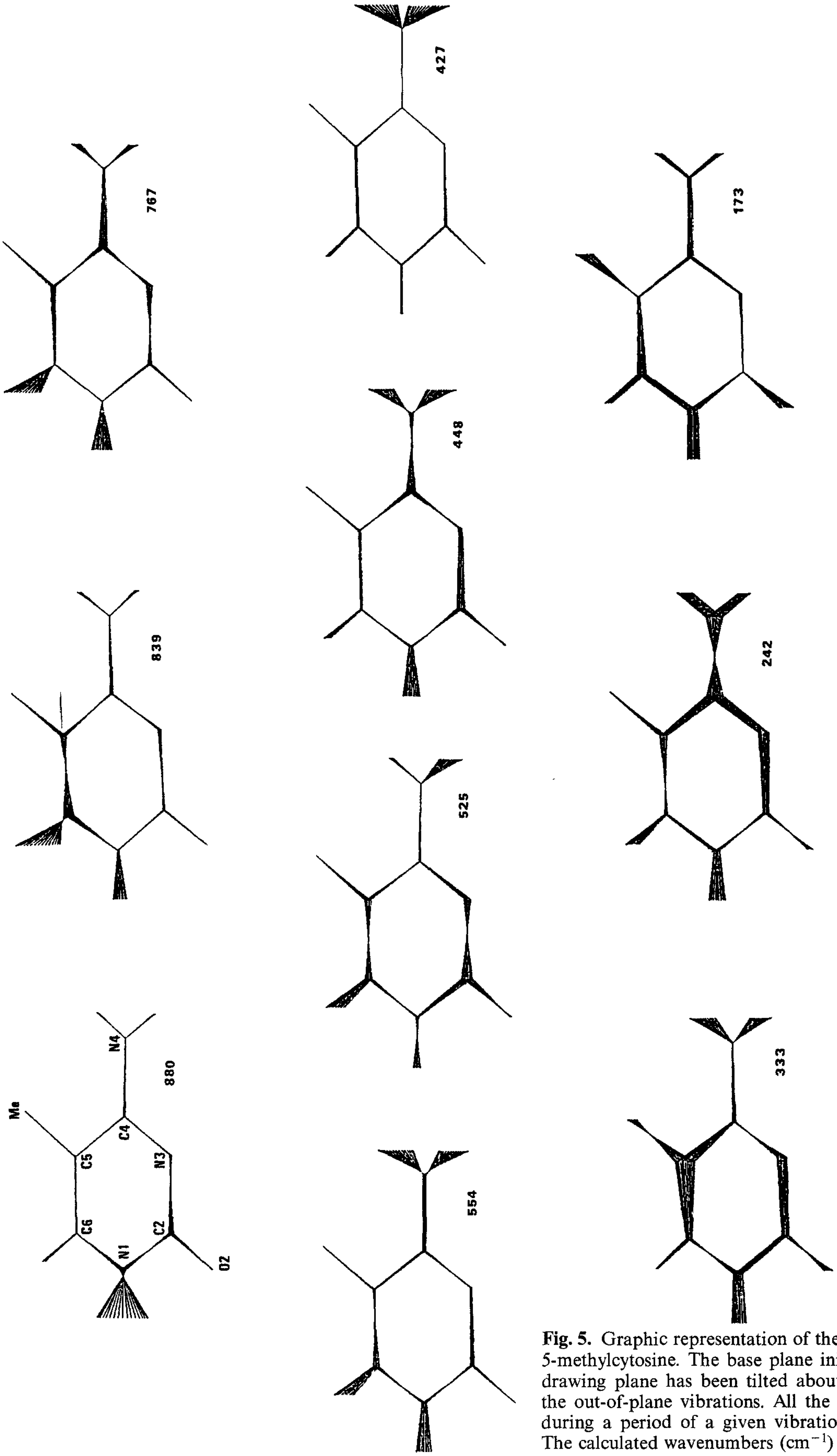

Fig. 5. Graphic representation of the 10 out-of-plane modes for 5-methylcytosine. The base plane initially perpendicular to the drawing plane has been tilted about 10° in order to visualize the out-of-plane vibrations. All the situations of the base ring during a period of a given vibration mode have been drawn. The calculated wavenumbers (cm^{-1}) of each mode is noted. See also Table 5

assign the non-planar vibration modes arising from the base residues observed experimentally in the 800–750 cm^{-1} spectral region of the polynucleotide infrared spectra. The non-planar character of these infrared bands had been confirmed previously by linear dichroism which clearly showed that the dipole moment orientation corresponds to a direction parallel to the double-helix axis (Ghomi et al. 1984; Chinsky et al. 1984).

Table 6a shows the position of a rather intense and narrow band observed around 780 cm^{-1} in infrared spectra of 2′-deoxycytidine. This mode is also observed in poly $(dG-dC)\cdot$ poly $(dG-dC)$ and poly $(dA-dC)\cdot$ poly $(dG-dT)$ (Taillandier et al. 1984, 1985) infrared spectra and is sometimes superimposed with an out-of-plane vibration mode of the guanine-residue which will be discussed elsewhere (Letellier et al. 1986a).

Moreover, this mode is not affected by N-deuteration as shown by the infrared spectrum of deuterated poly $(dG-dC)\cdot$ poly $(dG-dC)$ (Taboury et al. 1985). Although the methylation in the C5-site of cytosine residues does not cause a significant displacement of the 778 cm^{-1} band ($\sim$ 2 cm^{-1}), the infrared spectrum of poly $(dG-dm^5C)\cdot$ poly $(dG-dm^5C)$ in D_2O shows a considerable shift of this band ($\sim$ 10 cm^{-1}) towards the low frequency region (Taboury et al. 1985). As can be seen, all these spectral characteristics have been satisfactorily explained by calculations performed on pure or N-deuterated 1-methylcytosine and 1,5-dimethylcytosine considered as reasonable dynamic models for the out-of-plane vibration modes of the cytosine residue and its C5-methylated analog (Table 6a). However, we should avoid any undesirable confusion of this infrared band with the cytosine and cytosine residue Raman line located at about 785 cm^{-1} and assigned to the ring-breathing vibration mode by several theoretical investigations (Susi et al. 1973; Letellier et al. 1986b; Tsuboi et al. 1986).

As far as the thymine residue is concerned, the infrared spectra of 2′-deoxythymidine 5′-monophosphate (5′-dTMP), poly $(dA-dT)\cdot$ poly $(dA-dT)$ and poly $(dA-dC)\cdot$ poly$(dG-dT)$ present a common band located at about 765 cm^{-1} showing a high frequency shift ($\sim$ 8 cm^{-1}) upon N3-deuteration (Taillandier et al. 1984, 1985; Adam et al. 1986). As Table 6b shows. 1-methylthymine constitutes an acceptable model for interpreting the behaviour of this out-of-plane mode corresponding to the thymine residue involved in mononucleotide or polynucleotides. Figure 6a and b show the perspective representation of the out-of-plane modes explained above.

In conclusion, on the basis of the present calculations one can confirm the experimental assignment

Table 6a. Comparison between the experimental and calculated results for the characteristic infrared band located around 780 cm^{-1} and correlated to a cytosine residue out-of-plane mode.
5′-deoxycytidine (5′-dC) infrared spectra (Taillandier et al. 1985).
Poly $(dG-dC)\cdot$ poly $(dG-dC)$ infrared spectra (Taboury et al. 1985).
Poly $(dA-dC)\cdot$ poly $(dG-dT)$ infrared spectra (Taillandier et al. 1984).
(A): A form; (B): B form and (Z): Z form.
The calculated wavenumbers of 1-methylcytosine and 1,5-dimethylcytosine (this work) are also reported. For assignments see Table 5

Experimental						Calculated		Experimental		Calculated	
5′-dC		poly(dG-dC).poly(dG-dC)		poly(dA-dC).poly(dG-dT)		1-methylcytosine		poly(dG-dm5C).poly(dG-dm5C)		1,5-dimethylcytosine	
in H2O	in D2O	in H2O	in D2O	in H2O	in D2O	pure	N-deut.	in H2O	in D2O	pure	N-deuteraed
785	785	778 (B) 778 (Z)	778 (B) 778 (Z)	780 (A) 780 (B) 782 (Z)	782 (A) 787 (B) 784 (Z)	778	777	776 (Z)	768 (Z)	776	771

Table 6b. Comparison between the experimental and calculated results for the characteristic infrared band of the thymine residue located around 765 cm^{-1}.
5′-deoxythymidine monophosphate (5′-dTMP) infrared spectra (Taillandier et al. 1985).
Poly (*dA* – *dT*) · poly (*dA* – *dT*) infrared spectra (Adam et al. 1986).
Poly (*dA* – *dC*) · poly (*dG* – *dT*) infrared spectra (Taillandier et al. 1984).
(*A*): *A* form; (*B*): *B* form; (*D*): *D* form and (*Z*): *Z* form.
The calculated wavenumbers of 1-methylthymine and its N3-deuterated analogs are also reported. For assignments see Table 3

Experimental						Calculated	
5′-dTMP		poly(dA-dT).poly(dA-dT)		poly(dA-dC).poly(dG-dT)		1-methylthymine	
in H2O	in D2O	in H2O	in D2O	in H2O	in D2O	pure	N3-deut.
765	775	765 (A)	775 (A)	765 (A)	772 (A)	763	768
		765 (B)	774 (B)	764 (B)	775 (B)		
		765 (D)	774 (D)	765 (Z)	774 (Z)		

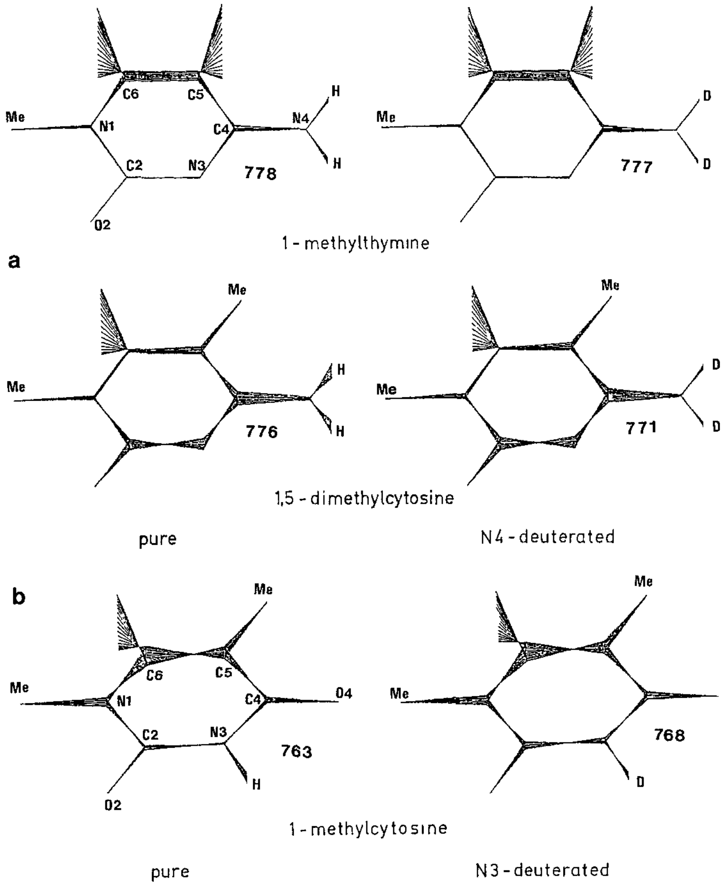

Fig. 6. Representation of the calculated vibration modes allowing us to assign the characteristic infrared bands observed in the 800–750 cm^{-1} spectral region correlated with the out-of-plane modes of cytosine and thymine residues involved in mononucleotides and polynucleotides. See footnote of Fig. 2. **a** Characteristic vibration modes calculated in 1-methylcytosine and 1,5-dimethylcytosine. The behaviour of these modes upon the N-deuteration has also been shown. See Table 6a. **b** Characteristic vibration modes calculated in 1-methylthymine and its N3-deuterated analogs. See also Table 6b

of the infrared bands observed in polynucleotide spectra around 780 cm^{-1} and 765 cm^{-1}, to the out-of-plane modes of cytosine and thymine residues respectively. At the same time, Raman spectra of these molecules present two intense lines at about 785 cm^{-1} (cytosine residue) and at 650 cm^{-1} (thymine residue) (Benevides et al. 1984) which are assignable to the pyrimidine base in-plane breathing vibration modes (Letellier et al. 1986b, c).

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