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F.T.-I.R. AND LASER-RAMAN SPECTRA OF ADENINE AND ADENOSINE*

MOHAMMED MATHLOUTHI, ANNE-MARIE SEUVRE,
Département "Biologie Appliquée", Institut Universitaire de Technologie, Université de Dijon, B.P. 510 – 21014 Dijon-cedex (France)

AND JACK L. KOENIG
Department of Macromolecular Science, Case Western Reserve University, Cleveland, OH 44106 (U.S.A.)

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ABSTRACT

F.t.-i.r. and laser-Raman spectra of adenine in the solid state and the Raman spectrum of its aqueous sodium salt were recorded, and assignments of the frequencies observed are proposed. The F.t.-i.r. spectrum of adenosine in the solid state is compared to the spectra of D-ribose and adenine. Analysis of the observed frequencies of adenosine permitted identification of a region of frequencies characteristic of the sugar on the one hand, and of the base on the other. Vibrational-spectroscopy results seem to be efficient in differentiating molecules of an increasing degree of complexity.

INTRODUCTION

The ring structure of the D-ribosyl group of adenosine was investigated in 1931, and proved[2] to be furanosyl. Adenine, and adenosine and its mono-, di-, and tri-phosphate derivatives, play a central role in energy transfer in biological systems. Their Raman[2a] and n.m.r. spectra[3] have been investigated, and the powerful, recent technique called Fourier-transform infrared (F.t.-i.r.) spectroscopy has been applied[4] to biological macromolecules.

I.r. and Raman spectroscopy have been utilized with success in the study of such complex molecules as carbohydrates[5–7], where several physical interactions take place. Vibrational spectra of the sugars of nucleic acids were discussed in the first part of this work[1], with particular reference to configurational and conformational changes in aqueous solution. As stated earlier, our approach in the study of nucleic acids consists in accumulating experimental data for simple molecules having an increasing degree of complexity (sugar, sugar + base, sugar + base + phosphate, and oligonucleotides). Our previous work on the ring-isomerism equilib-

*F.t.-i.r. and Laser-Raman Spectra of Constituents of Nucleic Acids, Part II. For Part I, see ref. 1.

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rium of D-fructose[5] was used as a basis in the study of the furanoid sugars of nucleic acids. However, when purine and pyrimidine bases are dealt with, the number of different atoms, the number of different bonds (C=C, C=N, C–N, N–H, *etc.*) and the number of different interactions (such as intra- and inter-molecular bonding, and base-stacking) increases, and makes solving of the vibrational problem almost impossible. Fortunately, the ring-isomerism vibrations do not interfere with those of the purine and pyrimidine bases. Numerous investigations[8–11] of the conventional i.r. and laser-Raman spectra of nucleic acids and nucleotides have been published, but, as far as we are aware, no F.t.-i.r. studies have been made on purine and pyrimidine bases and nucleosides, except a recent study by Tajmir-Riahi[12] on metal complexes of nucleotides. The F.t.-i.r. spectra are well resolved, and provide useful experimental data.

Study of the F.t.-i.r. and laser-Raman spectra of adenine and adenosine may be profitably conducted by comparison with those of methylated derivatives[10–13] and simple, model molecules containing similar groups. Although assignment of frequencies can be treated by comparing calculated and observed values, even the most precise calculations have value only if, at each calculated frequency, an observed frequency may be associated therewith. Some calculations of vibrational frequencies have been made[3,13,14] wherein the force-field models were those of melamine (2,4,6-triamino-*s*-triazine) and cyanuric acid. Extrapolation of features of such simple models to nucleosides, nucleotides, and nucleic acids remains only a rough simplification. In such an approximation, it happens that the number of frequencies observed exceeds the number of frequencies calculated, especially in the case of molecules having no elements of symmetry, such as adenine or adenosine. In a previous study[15] of the laser-Raman spectra of poly(adenylic acid), comparisons were made between adenosine, 2′-deoxyadenosine, 5′-AMP, and 3′-AMP, but only qualitative localizations of frequencies from the sugar, the adenin-9-yl moiety, or the phosphate group were proposed.

We now compare F.t.-i.r. and Raman spectra of adenine in the solid state, and propose assignments of observed frequencies for the crystal and for a basic solution. The association of adenine with D-ribose through a glycosylic linkage induces changes in the conformation of both the sugar and the purine base components. Therefore, a comparison of the i.r. results for D-ribose, adenine, and adenosine was conducted.

EXPERIMENTAL

The methods of obtaining the Raman and F.t.-i.r. spectra have been described[1]. The spectra were recorded in the 1700–200- and 3600–2600-cm^{-1} ranges. Adenine and adenosine were purchased from Sigma (St Louis, MO). Adenine was found to be insufficiently soluble in water to provide good spectra, and therefore 10% (w/w) solutions in 0.1M NaOH were investigated.

RESULTS AND DISCUSSION

A. *Adenine*

The laser-Raman spectrum of adenine in the solid state is shown in Fig. 1, and in sodium hydroxide solution (with an aqueous background), in Fig. 2. The F.t.-i.r. spectrum of solid adenine in a KBr pellet, in the region below 1800 cm^{-1}, is given in Fig. 3. Observed frequencies and relative intensities are listed in Table I.

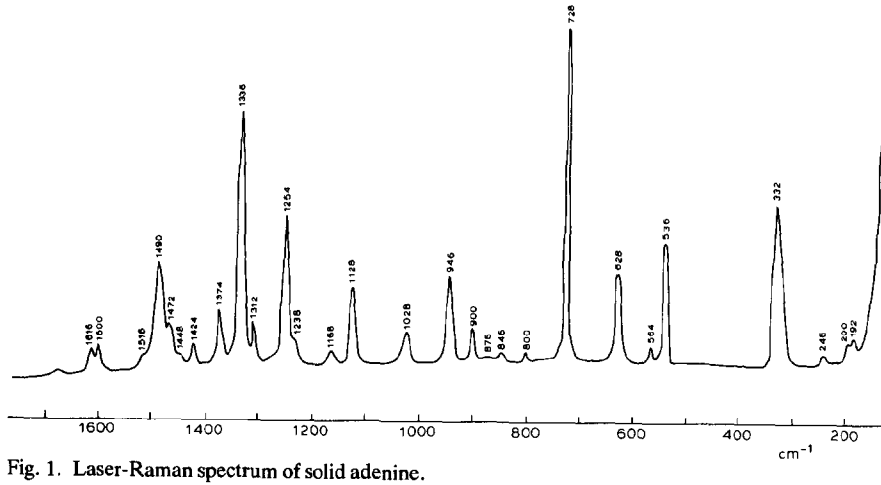


Fig. 1. Laser-Raman spectrum of solid adenine.

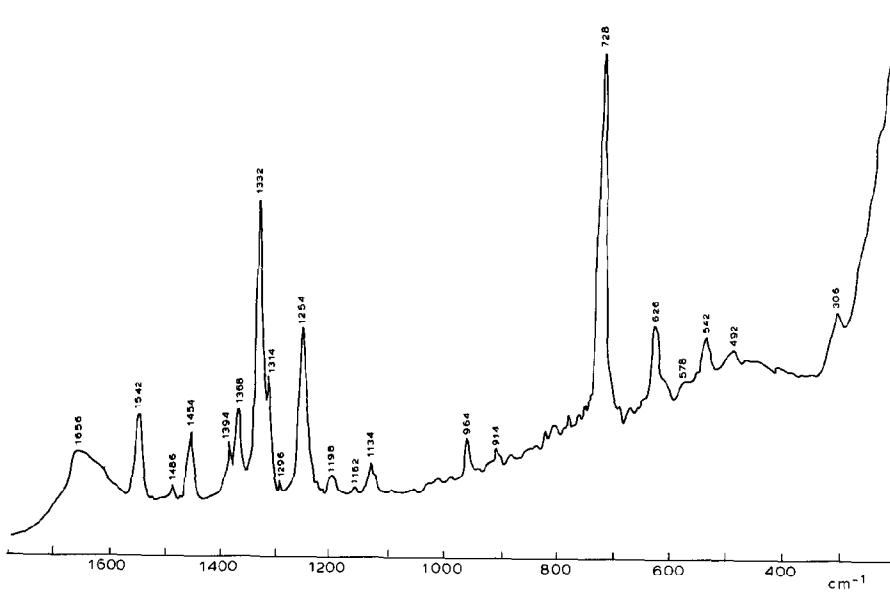


Fig. 2. Laser-Raman spectrum of solution of adenine in sodium hydroxide.

TABLE I

BANDS OBSERVED[a] IN THE LASER-RAMAN AND F.T.-I.R. SPECTRA OF ADENINE

Solid sample				*Aqueous solution*		*Assignments (modes)*
I.r.		*Raman*		ν (cm^{-1})	*I*	
ν (cm^{-1})	*I*	ν (cm^{-1})	*I*			
		192	4.7			
		200	4.0			
		246	3.3			
				306	12.0	
		332	48.7			hydrogen bonding
				492	12.7	
		536	36.7	542	15.8	δ(C–C=C)
		564	5.3			
				578	6.3	
622	24.8	628	28.0	626	22.2	δ(N–C–C)
642	28.4					
668	23.5					
725	36.5	728	100[b]	728	100[b]	ν(C–C), ν(C–N) in-phase breathing
800	16.2	800	2.7			ν(C–C)
850	15.7	846	2.0			skeletal mode, in-plane
874	13.7	876	1.0			δ(N-9–H) out-of-plane
		900	12.7			
913	31.4			914	5.7	r NH_2
940	43.1	946	25.3			δ(N–C=N)
				964	11.4	
1025	18.1	1028	9.3			δ(C–N–C)
1126	22.8	1128	23.3	1134	8.2	δ(C-2–N-1=C-6), ν(C-5–N-7=C-8)
1156	12.5	1168	4.0	1162	1.9	δ(CH) in-plane
				1198	5.1	
		1238	7.3			
1254	39.2	1254	43.3	1254	44.9	ν(C–NH_2)
				1296	2.5	
1310	50.0	1312	13.0	1314	30.4	ν(C–N), ν(C=N)
1336	41.2	1336	75.3	1332	79.1	ν(C–N), ν(C=N)
1370	25.7	1374	17.3	1368	23.4	δ(C-8–H), δ(C-2–H) out-of-plane
1420	46.3	1424	6.7			δ(N=CH)
1453	23.5			1454	19.6	ν imidazole ring
1470	17.6	1472	13.3			
		1490	32.0	1486	5.1	δ NH_2
1510	19.1	1516	4.7			δ(C–N-9–H)
				1542	24.7	ν(C=C)
1606	100[b]	1600	7.3			ν(C=N), ν(C=C)
		1616	6.7			
				1656	18.4	
1675	73.5					δ NH_2

[a]Key: I = relative intensity; δ = bending mode; r = rocking; and ν = stretching mode. [b]Taken as reference.

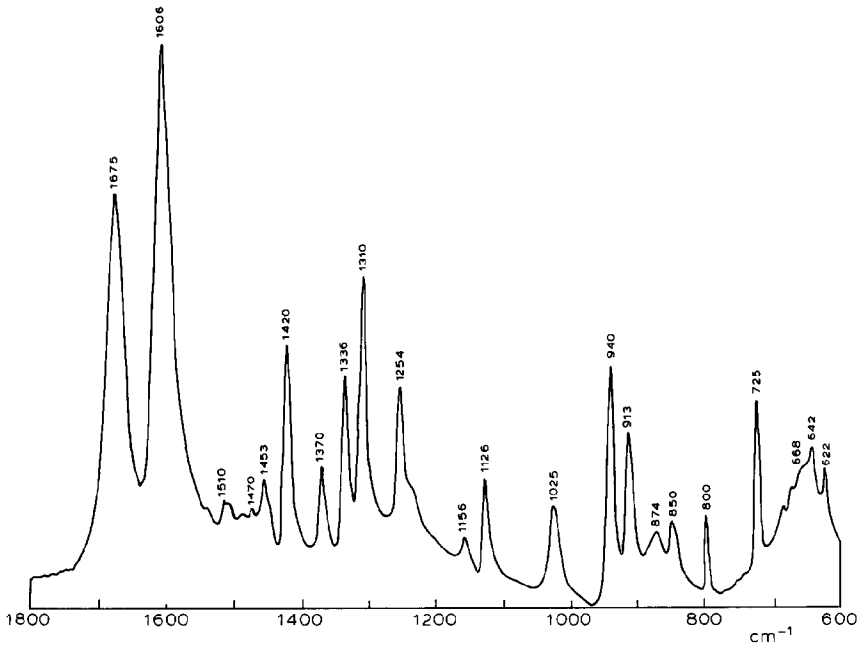
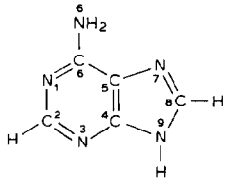


Fig. 3. F.t.-i.r. spectrum of solid adenine.

The general aspect of the solid and the solution Raman spectra shows differences due to the aqueous solvent. The band at 1650 cm^{-1} and the broad background below 900 cm^{-1} come from water. It may be seen that the prominent lines at 728 cm^{-1} and in the region of 1400–1200 cm^{-1} are not affected by the change in structure of the sample. The most perturbed frequencies lie in the regions sensitive to intermolecule interactions (below 350 cm^{-1}) and in the crystallinity region (1200–800 cm^{-1}). On comparing the F.t.-i.r. and Raman results (see Figs. 2 and 3), most of the vibrations are observed in both spectra, but with considerable discrepancies in the relative value of intensities. This arises from the selection rule applied to the adenine molecule (1).



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Analysis of the observed bands. — a. The 1800–1200-cm^{-1} region. Two sharp i.r. absorptions at 1675 and 1606 cm^{-1} are seen in Fig. 3. No equivalent Raman lines are present, except for two very weak vibrations at 1616 and 1600 cm^{-1}. The

1675 i.r. absorption arises from a prominent contribution of the NH_2 bending mode, which is in agreement with previous work[8,16,17]. The general charts of correlation[18–20] between spectral regions and structures localize NH_2 deformations in the 1660–1580 cm^{-1} range. The observed frequency at 1675 cm^{-1} shows a shift towards high frequencies that is attributable to the strong hydrogen-bonding of the NH_2 group in the crystal. The band at 1606 cm^{-1} is probably due to the combination of several groups. Its nonsymmetrical shape, the presence of a shoulder, and the broadness prove the complexity of its composition. Absorptions of the C=N and C=C double bonds are expected in this region of frequencies. Tsuboi *et al.*[13] assigned calculated frequencies at 1631 and 1582 cm^{-1} to ν(C=C) and ν(C=N), respectively, with a contribution to each of these frequencies of ν(C-5–C-6).

Crystallographic data for adenosine[21] show that the bond lengths are shorter than the average values given by Pauling[22]. The observed lengths are N-1–C-2, 134.0; N-3–C-4, 134.9; N-1=C-6, 135.1; N-3=C-2, 133.0; C-5–C-6, 141.5; and C-4–C-5, 138.1 pm.

It may be noted that the N-1=C-6 double bond is longer than the N-3–C-4 single bond, which is the opposite of the well known rule that single bonds are longer than double bonds. This feature is probably associated with a resonating structure in the six-membered ring of adenine. The group-frequency correlations[18–20] predict a strong Raman effect for C=C and C=N at ~1600 cm^{-1}. (Double-bond stretching-frequencies tend to be of weak to moderate strength in the i.r. spectrum, but tend to be very strong and polarized in the Raman spectrum.) However, the frequencies observed at 1616 and 1600 cm^{-1} (see Fig. 1) are weak; this is attributable to the resonance effect. Consequently, the bands at 1606 cm^{-1} in the i.r. spectrum and at 1616–1600 cm^{-1} in the Raman spectrum may be assigned to C=C, C=N, and a contribution of C–C and C–N in the 6-membered ring of adenine, which is in agreement with the calculated results of Tsuboi *et al.*[13]. In the spectrum of the solution (see Fig. 2), the nonsymmetrical band in the 1700–1600-cm^{-1} range contains C=N stretching, but the major contribution is that of water bending. The Raman line at 1542 cm^{-1} could have its origin in C=C stretching. The dissolution of adenine in NaOH solution certainly leads to sodium substitution on the ring, which affects the resonance effect. This behavior is comparable to that of the C=C vibrations in benzene. This vibration occurs at 1600 cm^{-1} and is lowered to 1597–1562 cm^{-1} for monosubstituted benzenes[19]. The fact that the vibration at 1542 cm^{-} does not appear in the i.r. and Raman spectra of solid adenine suggests that it is related to Na substitution.

Vibrations at 1514, 1491 and 1486–1483 have been reported to occur in the spectra of DNA[8]. poly AT and yeast RNA[9]. The study of i.r. and Raman spectra of adenine in the crystalline form permitted assignment of the bands at 1510 (i.r.) and 1490 cm^{-1} (Raman) to the CNH deformation modes, including the imidazole nitrogen atom. An early i.r. investigation[23] of 2-amino-2-deoxy-D-glucose showed the presence of an NH deformation at 1580–1510 cm^{-1}. The correlation charts[18,20] localize aromatic C=C, NH deformation in dilute solutions, and symmetrical NH_2

bending at ~1500 cm^{-1}. We propose that the frequencies observed at 1510 (i.r., see Fig. 3) and 1516 cm^{-1} (shoulder, Raman; see Fig. 1) arise from the asymmetrical bending modes of N-9–H. The relatively strong vibration at 1490 cm^{-1} (Raman), which is weak for the solution (1486 cm^{-1} ; see Fig. 2) and absent from the F.t.-i.r. spectrum, could be assigned to $\delta(NH_2)$. The i.r. absorption at 1453 cm^{-1}, which corresponds to a very weak, Raman vibration at 1448 cm^{-1} (see Fig. 1) and which is observed at 1454 cm^{-1} in the Raman spectrum of the solution, is absent from the spectra of the adenylic derivatives. Lautie and Novak[16] assigned this band to a ring vibration of the pyrimidine part. The facts that substitution of adenine occurs on N-9, and that this vibration seems to be more characteristic of the five-membered ring, leads us to assign it to a vibration sensitive to the substitution generally localized[18] in the 1500–1430-cm^{-1} region for a heterocyclic, five-membered ring, that is to say, the imidazole ring of adenine. The sharp i.r. absorption at 1420 cm^{-1} (see Fig. 3) is weak in the Raman spectrum of the solid sample (at 1424 cm^{-1} ; see Fig. 1) and is absent from that of the solution (see Fig. 2). This vibration was observed in the spectra of rRNA[11], calf thymus DNA[9,11], yeast RNA, polyA · polyU, and poly dAT[9], so that it seems to be characteristic of adenine. As for assignment of this frequency (1420), Hartman *et al.*[11] gave as its origin the ν vibration of the ring, whereas Lautie and Novak[16] assigned it to an in-plane vibration of the pyrimidine ring. The relatively high intensity of the i.r. absorption, as well as the absence of this vibration from the spectrum of the NaOH solution, led us to conclude that it could be a highly asymmetrical vibration taking place on a site of protonation, and we propose to assign it to δ(N=CH). In the region of 1380–1370 cm^{-1}, a band having a relatively weak intensity is observed in the three spectra (see Figs. 1–3) ; this vibration has been observed for a number of compounds[8,9,13,16]. Experiments on a Na-adenine crystal resulted in assignment of this frequency to a vibration of the pyrimidine ring, whereas Tsuboi *et al.*[13] concluded from their calculations that it comes from an in-plane deformation mode of C–H. No protonation takes place on a C–H site, as illustrated by the presence of this vibration with the same energy in the spectrum of the solid (see Fig. 1) and the NaOH solution (see Fig. 2). Because this frequency appears to be high for an in-plane, CH deformation, we propose the assignment of this band to an out-of-plane bending of C–H: δ(C-8–H) and δ(C-2–H), which have the same environment $\begin{matrix} N \\ \diagdown \\ N \end{matrix}\!\!>$C–H, the in-plane deformation δ(CH) being at 1156 cm^{-1}.

Most of the adenylic derivatives previously studied show a vibration at 1340–1330 cm^{-1} . Lord and Thomas[8] observed this frequency in the spectra of calf thymus DNA and rRNA, and Peticolas[9] observed it for yeast RNA, calf thymus DNA, polyA · polyU, and polyA. This vibration has a high intensity in the Raman spectra (see Figs. 1 and 2), and is a relatively intense band in the F.t.-i.r. spectrum (see Fig. 3). The frequency at ~1340 cm^{-1} was assigned to an in-plane deformation of imidazole. This assignment does not seem to be in agreement with the deuterium-substitution study of Tsuboi *et al.*[13]; indeed, the frequency at 1342 cm^{-1}

is not shifted by deuteration. The high intensity of the observed vibration at 1336–1332 cm^{-1} (see Table I) led us to assign it to ν(C–N), especially because of the relatively important number of C–N bonds. This frequency seems to be relatively high for a single-bond stretching. However, the proximity of each of the four C–N bonds to a C=N bond suggests that a resonance phenomenon may occur, and adds a contribution of C=N stretching to the C–N vibration. This is in agreement with the results of Tsuboi *et al.*[13,24] and of Chinsky *et al.*[14]. The observed frequency at 1310 cm^{-1} (F.t.-i.r.; see Fig. 3), 1312 cm^{-1} (Fig. 2), and 1314 cm^{-1} (Fig. 1) corresponds to the band observed by Lautie and Novak[16] at 1309 cm^{-1} and assigned to ν(C–NH_2). This frequency was assigned by Chinsky *et al.*[14] to ν(C-8=N-7). The calculations[13] show an important contribution of ν(C-8=N-7) to this frequency. We may invoke, for this frequency also, the resonance argument (between C–N and C=N), and assign this frequency to a combination of ν(C–N) and ν(C=N). The relative intensities of the i.r. bands observed at 1336 and 1310 cm^{-1} and the Raman lines at 1338 and 1312 cm^{-1} lead to an interpretation of the 1338-cm^{-1} line as due to ν(C–N) and ν(C=N) of the pyrimidine ring [higher contribution of the (C=N) vibration], and of that at 1312–1310 cm^{-1} to ν(C–N) and ν(C=N) of the imidazole ring.

A relatively intense vibration observed at the same frequency (1254 cm^{-1}) in the three spectra (see Figs. 1–3) was also reported previously[8,9,11,16], but its assignment seems to be controversial. It was assigned to δ(NH) imidazole[16], to ν(C–NH_2)[8], and to a ring vibration[11]. We propose the assignment of the 1254-cm^{-1} band to ν(C–NH_2), which is in agreement with the results of the deuteration study of Lord and Thomas[8]. It may be noted that all of the C–N stretching vibrations occur at frequencies shifted towards higher values, probably because of interference by the C=N contributions.

b. The 1200–800-cm^{-1} region. The vibrations at 1126 and 1025 cm^{-1} (i.r.), and at 1128–1028 cm^{-1} (Raman of the solid sample), vanish in the spectrum of the NaOH solution (see Fig. 2), or are weak, with a shift towards higher values (1134 cm^{-1} instead of 1128 cm^{-1}). Assignments in the previous work[8,16] were scarce, or given for the ring vibrations in the plane. The observed differences between the crystal and the sodium salt in solution are consistent with an assignment that takes into account the protonation sites of the purine base. Indeed, it was stated[25] that N-1 and N-7 are preferentially protonated, which leads to the assumption that vibrations including these atoms and the imidazole N-9 should be found in this region. We propose assignment of the band at 1128 cm^{-1} to δ(C–N=C), probably δ(C-2–N-1=C-6) and (C-5–N-7=C-8), and 1028 (Raman) and 1026 cm^{-1} (i.r.) to δ(C–N–C). The ratio of the Raman intensities I(1028):I(1128) is ~1:2, which is in agreement with the ratio of the number of (C–N=C) to (C–N–C). The frequencies at ~940–900 cm^{-1} are generally observed in the spectra of nucleic acids, and have been assigned to the phosphate groups, but in the present study, such an assignment is impossible. Frequencies at 946 cm^{-1} (i.r.) and 940 cm^{-1} (Raman) could be assigned to ring vibrations. The relatively high intensity of this vibration is probably due to a coupling of such vibrations as C–N with N=C–N deformations. Lautie

and Novak[16] observed a frequency at 939 cm^{-1} which was shifted to 931 cm^{-1} by deuteration, and a frequency at 912 cm^{-1} which was shifted to 895 cm^{-1} for the deuterated adenine crystal. The band observed at 913 cm^{-1} (i.r.; see Fig. 3), 914 cm^{-1} (Raman; solution; see Fig. 2) probably has as its origin the rocking mode of NH_2. The i.r. absorption at 874 cm^{-1} (see Fig. 3) is observed at 876 cm^{-1} in the Raman spectrum (see Fig. 1) with a very weak intensity, but is absent from the Raman spectrum of the solution (see Fig. 3). This vibration is not observed in the spectra of adenylic derivatives, and could be assigned to an out-of-plane deformation of (N-9–H), which is in agreement with the results of Lautie and Novak[16].

A Raman line at 846 cm^{-1} (see Fig. 1) was observed in the spectrum of a crystal of the Na salt of adenine[16]. The results of calculations permitted Tsuboi *et al.*[13] to assign this frequency to the combination of δ(N-1–C-2=N-3) and ν(C-5–N-7). The i.r. absorption corresponding to this vibration is found at 850 cm^{-1} (see Fig. 3). It could be assigned to an in-plane skeletal mode, as well as that at 800 cm^{-1}, which is probably due to a C–C stretching-mode. It may be noted that, whereas nucleosides exhibit strong vibrations in the region of 1000–800 cm^{-1}, readily attributable to the sugar moiety, the assignment of the frequencies observed in this region for the pure bases is controversial, and includes coupling of stretching and bending vibrations from the skeleton of the molecules.

c. *The observed bands below 800 cm^{-1}.* The most-intense vibration in the Raman spectra (see Figs. 1 and 2) at 728 cm^{-1}, and the relatively medium-strength i.r. band at 725 cm^{-1} (see Fig. 3), had been observed by different authors[8,9,11,16] in the vibrational spectra of adenine and its derivatives. Experimental, as well as theoretical[13], results assigned this frequency to the breathing mode of the skeleton. The facts that this vibration is not shifted by deuteration[8,13], and that it is generally admitted that stretching modes of C–C, C–N, *etc.*, are found in this region, make it reasonable to propose that an in-phase vibration of the single-bond modes [ν(C–C) and ν(C–N)] of the ring is the origin of this intense frequency.

Below 700 cm^{-1}, three well resolved Raman lines are observed in the spectrum of the crystal (see Fig. 1) at 628, 536, and 332 cm^{-1}. The vibration at 628 cm^{-1} is also found in the Raman spectrum of the solution (see Fig. 2) and in the F.t.-i.r. spectrum (see Fig. 3). The assignment proposed for this frequency is the bending mode of (N–C–C), which is in agreement with the results of Strobel and Scovell[10]. The second vibration observed in this region appears at 536 cm^{-1} in the Raman spectrum of solid adenine (see Fig. 1) and at 524 cm^{-1} in that of the solution of the sodium salt (see Fig. 2). Information from the literature[8,13] corresponding to the absence of a shift of this frequency on deuteration[8], and the probable skeleton-bending mode[13] as its origin, incline us to attribute this frequency to δ(C–C=C). In the Raman spectrum of the solid sample (see Fig. 1) is observed a relatively intense line at 332 cm^{-1} that is broadened in the spectrum of the solution. The important hydrogen bonding in the adenine crystal could be the origin of this frequency. Although it is impossible to achieve a one-to-one correspondence between observed frequencies and assigned modes, an attempt is made, in order to assign a maximum

of observed frequencies. As stated earlier[1], the aim of these assignments is to have reference marks in our study of nucleosides and nucleotides. The discussed vibrations of D-ribose[1] and adenine (see Table I) should make it easier to interpret the vibrational spectra of adenosine.

d. The 3600–2000-cm^{-1} region. The F.t.-i.r. spectrum of solid adenine is shown in Fig. 4. The observed frequencies and their assignments are given in Table II. As expected, NH and CH modes are observed. Symmetrical and asymmetrical stretching of NH_2 are well resolved, and occur at frequencies relatively higher than the average value for ν(NH), which is probably due to the hydrogen bonding. The ν(N-9–H) vibration is close to the generally admitted 3000-cm^{-1} value for NH stretching. Two bands are observed at 2800–2700 cm^{-1} for the ν(C–H) vibrations. The difference in environment of the C–H groups is probably the cause of the splitting of the observed frequency.

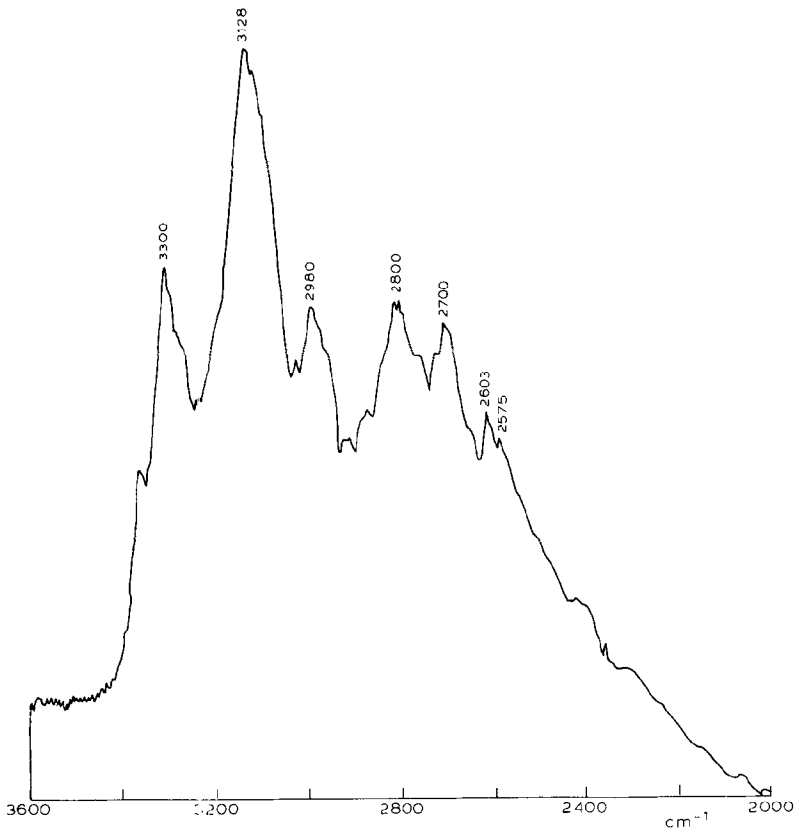


Fig. 4. F.t.-i.r. spectrum of solid adenine in the 3600–2000-cm^{-1} region of frequencies.

TABLE II

BANDS OBSERVED[a] IN THE F.T.-I.R. SPECTRUM OF ADENINE (THE 3600–2000-cm^{-1} REGION)

ν (cm^{-1})	I	*Assignments*
2575	47.8	
2603	51.0	
2700	63.5	
2800	65.7	ν(C–H)
2980	65.7	ν(N-9–H)
3128	100[b]	ν_a (NH_2)
3300	70.6	ν_s (NH_2)

[a]Key: I = relative intensity; ν = stretching mode (ν_a: asymmetrical stretching, and ν_s: symmetrical stretching). [b]Taken as reference.

B. *Adenosine*

Only the F.t.-i.r. spectrum of adenosine (**2**) was recorded. It is shown in Fig. 5, and appears to be richer in bands than that of adenine (see Fig. 3). It may be noted that the 1200–800-cm^{-1} region, which is the "fingerprint" region for sugars, and which has but few bands in the spectrum of the pure base, contains absorptions attributable to the D-ribosyl group.

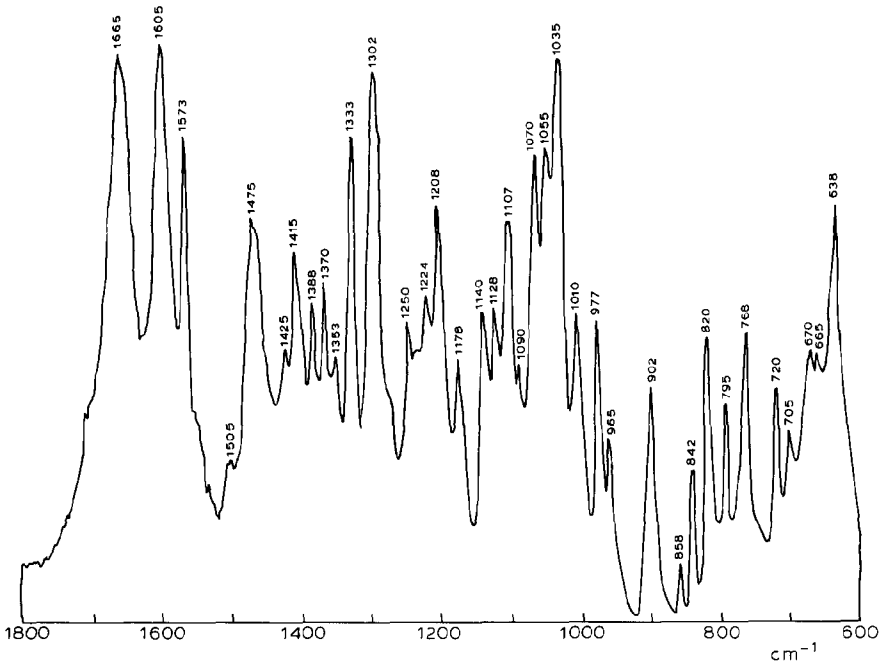
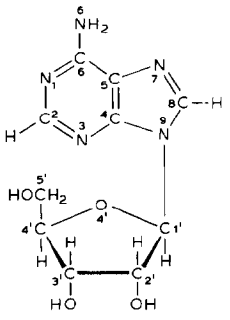


Fig. 5. F.t.-i.r. spectrum of solid adenosine.



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a. Observed bands identified from the spectra of adenine and D-*ribose.* The 1800–1200-cm^{-1} region appears to be characteristic of adenine. Indeed, the number of vibrations observed in the spectrum of adenine (see Fig. 3) which remain at the same frequencies, or only slightly shifted, in the F.t.-i.r. spectrum of adenosine (see Fig. 5) is relatively important. These frequencies are given in Table III. The lack of any shift of frequencies of adenine in the 1800–1200-cm^{-1} region is due to the absence of interactions with the sugar moiety. This range of frequencies mainly concerns the local symmetry vibrations of D-ribose (CH_2 deformations), which are not affected by hydrogen bonding. Thus, except for a few bands,

TABLE III

BANDS OBSERVED[a] IN F.T.-I.R. SPECTRUM OF ADENOSINE

ν (cm^{-1})	*I*	*Assignments*	ν (cm^{-1})	*I*	*Assignments*
638	72.0	A	1126	54.4	A
665	46.6	A	1140	53.4	R
670	47.0	A	1178	45.6	R
705	32.8	δ(C-2′–C-1′–N-9)	1208	72.0	ν(N-9–C-1′)
720	40.2	A + R	1224	56.6	R
768	50.5	δ(O–C–N-9)	1250	51.0	A + R
795	37.3	A	1302	94.4	A
820	48.5	δ(C–N-9–C)	1333	84.1	A
842	26.0	δ(C-2′–C-1′–O-4′)	1353	46.3	R
858	9.8	A	1370	58.8	A + R
902	40.2	R	1388	55.4	R
965	32.1	R	1415	63.7	R
977	52.0	δ(O-4′–C-1′–H-1′)	1425	47.5	A
1010	53.4	R	1475	69.6	A
1035	96.6	R	1505	28.4	A
1055	81.9	δ(N-9–C-1′–H-1′)	1573	84.3	ν(C=C)
1070	80.9	R	1605	100[b]	A
1090	45.1	R	1665	99.0	A
1107	69.1	R			

[a]Key: I = relative intensity; δ = bending mode; ν= stretching mode; A = adenine (detailed assignments are given in Table I); R = D-ribose (detailed assignments are given in ref. 1). [b]Taken as reference.

most of the frequencies observed in the spectrum of adenosine (see Fig. 5) will be assigned by comparison to the previous assignments (see ref. 1 and Table I).

b. Observed bands differentiating adenosine from adenine and D-*ribose.* The only vibrations to be discussed herein are the i.r. absorptions observed in the spectrum of adenosine (see Fig. 5) that are absent from the spectra of D-ribose and adenine. The relatively intense band at 1573 cm^{-1} could be assigned to the double-bond, C=C stretching. Among the arguments for assignment of the band observed at 1606 cm^{-1} in the spectrum of adenine, the resonance of the double bonds C=N and C=C with single bonds was invoked. The attachment of the D-ribosyl group to N-9 stabilizes the structure of the purine base, and the vibrations corresponding to the double bonds are well resolved.

An important number of vibrations are observed in the 1500–1300-cm^{-1} region. This part of the spectrum is similar to the local symmetry region of the sugar. Most of the vibrations correspond to the deformations of CH_2 (bending, twisting, and wagging). The frequency observed at 1353 cm^{-1} probably has as its origin the deformation of CH_2 displaced because of the hydrogen bonding.

Two other frequencies (1208 and 1055 cm^{-1}) which do not find homologous bands in the previously assigned spectra (see Table I and ref. 1) are situated in the "finger-print" region of D-ribose. The assignment of the band at 1208 cm^{-1} to ν(N-9–C-1′) is proposed; this is in good agreement with the charts of correlation[18–20] between frequencies and vibrations. The band at 1055 cm^{-1} occurs in a region of coupled vibrations corresponding to the CH deformation. The new CH deformation in the finger-print region of the sugar is N-9–C-1′–H-1′. Thus, the band at 1055 cm^{-1} is assigned to δ(N-9–C-1′–H-1′). According to the same argument, we propose the assignment of the band at 977 cm^{-1} to δ(O-4′–C-1′–H-1′). The modification of environment of C-1′ is probably responsible for the shift of this frequency from 1016 (see ref. 1) to 977 cm^{-1}.

The region most affected by the sugar–base association concerns the ring vibrations (below 900 cm^{-1}). The vibrations of the nucleoside skeleton are differ-

TABLE IV

BANDS OBSERVED[a] IN THE F.T -I.R. SPECTRUM OF ADENOSINE (THE 3600–2800-cm^{-1} REGION)

ν (cm^{-1})	*I*	*Assignments*
2842	54.9	$\nu(CH_2)$ from D-ribose
2918	64.7	
2933	62.3	ν(C–H)
2958	54.9	
3135	92.2	
3164	89.2	$\nu\,NH_2$
3315	92.2	
3340	100[b]	ν(OH) from D-ribose

[a]Key: I = relative intensity; ν = stretching mode (ν_a: asymmetrical stretching, and ν_s: symmetrical stretching). [b]Taken as reference.

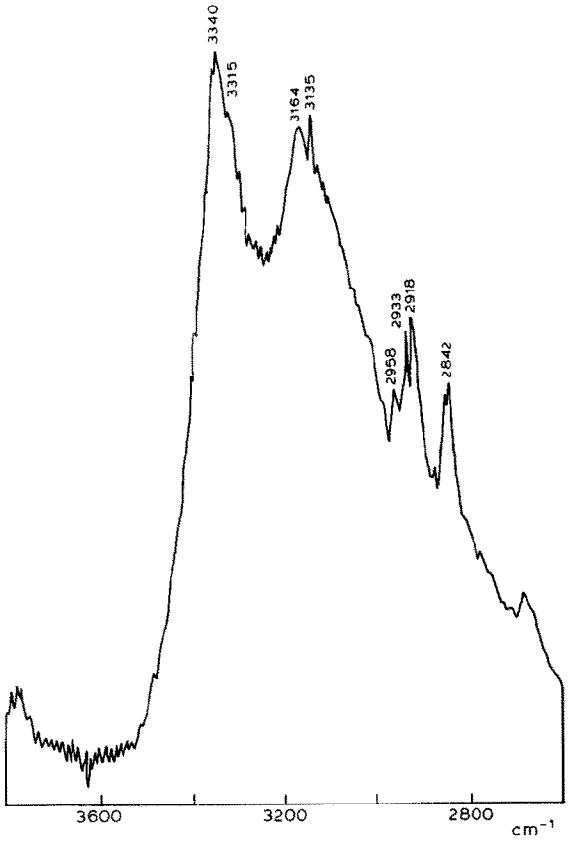

Fig. 6. F.t.-i.r. spectrum of solid adenosine in the 3600–2800-cm^{-1} region of frequencies.

ent from those of the D-ribose and adenine rings. The deformations around the N-9–C-1′ bond give rise to new frequencies. Hence, we may assign the band at 842 cm^{-1} to δ(C-2′–C-1′–O-4′), that at 820 cm^{-1} to δ(C–N-9–C), that at 768 cm^{-1} to δ(O–C–N-9), and that at 705 cm^{-1} to δ(C-2′–C-1′–N-9). These frequencies appear to be higher than the equivalent ring-vibrations which are expected, because D-ribose is subject to deformations around the C-1′–N-9 bond, and this gives these "quasi"-skeleton vibrations higher energies.

c. The 3600–2800-cm^{-1} region. The frequencies observed, and their assignments, are given in Table IV. By comparison of the spectra of adenine (see Fig. 4) and adenosine (see Fig. 6), it is evident that the N-9–H stretching is absent for adenosine; consequently, the vibrations at 3340–3315 cm^{-1} are assigned to ν(OH) from the sugar. The frequencies observed at 3164–3135 cm^{-1} correspond to the band of adenine at 3128 cm^{-1}, assigned to $\nu(NH_2)$. The third group of absorptions observed (see Fig. 6) is situated between 3000 and 2800 cm^{-1}, and corresponds to

the CH stretching. Two groups of bands are well separated: the first, at 2842 cm^{-1}, arises from $\nu(CH_2)$ (the CH_2OH of the D-ribose) and the other group, at 2958–2918 cm^{-1}, is attributable to $\nu(CH)$.

CONCLUSION

Interpretation of the Raman and i.r. spectra of nucleic acid constituents has led us to assign the observed frequencies to the most probable modes of vibration. Comparison of the spectrum of the nucleoside to those of the sugar and the base showed that the most sensitive region is the sugar–base linkage region. The increase of complexity in the molecule achieved by addition of a phosphate group, by dimerization, or by multiplying the number of nucleotide residues should leave, at each step, a "finger-print" of the preceding step, so that it should be possible, by this method of analysis, to identify the modification and the unchanged, molecular residues in a nucleic acid molecule. However, in aqueous medium, which is the biological medium, the hydrogen bonding (intra- and inter-molecular) influences the shape of the spectra (shifts of frequencies), and assignment of frequencies then becomes an almost insurmountable task.

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