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F.T.-I.R. AND LASER-RAMAN SPECTRA OF GUANINE AND GUANOSINE*

MOHAMED MATHLOUTHI, ANNE MARIE SEUVRE†,
Departement "Biologie Appliquée", Institut Universitaire de Technologie, Université de Dijon, B.P. 510–Dijon cedex (France)

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

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ABSTRACT

Fourier-transform infrared (F.t.-i.r.) and laser-Raman spectra have been obtained for solid guanine. The F.t.-i.r. spectrum of guanosine in the solid state was also recorded. Assignments are proposed for the i.r. bands. The molecular basis of the spectral differences between guanine and guanosine are discussed.

INTRODUCTION

Guanine, guanosine, and inosine and their phosphate derivatives play a major role in energy transfer and metabolic control. Our investigation of constituents of the nucleic acids using vibrational spectra started with the sugars[2], the pyrimidine bases[1,3], and the purine base adenine[4]. The conformation of the guanine residue seems to be essential in determining the B or Z form of DNA as deduced[5] from the Raman spectra of $d(CpGp)_3$ and poly(dG-dC).poly(dG-dC). Investigation of the vibrational spectra of guanine[6], guanosine[7], and their ^2H- and ^{15}N-substituted derivatives led to the conclusion[6,7] that guanosine displays the unique property of forming gels. A crystalline hemihydrate and a pseudo-polymeric helix were found[6,7]. The aggregate structures of guanosine and 3'GMP were described[7] as being very similar to that of polyG. In solution, at pH 7, each guanosine molecule is associated in a tetrameric unit by hydrogen bonding[7]. The stacking of the tetrameric units was found[8] to be regular, and a four-stranded helix was proposed for the polymer (polyG), based on Raman studies[7,8]. Vibrational spectroscopy was

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†Present address: Laboratoire de Chimie des Oligomères, Faculté des Sciences et Technique de Rouen, Université de Rouen, 76130 Mont Saint Aignan, France.

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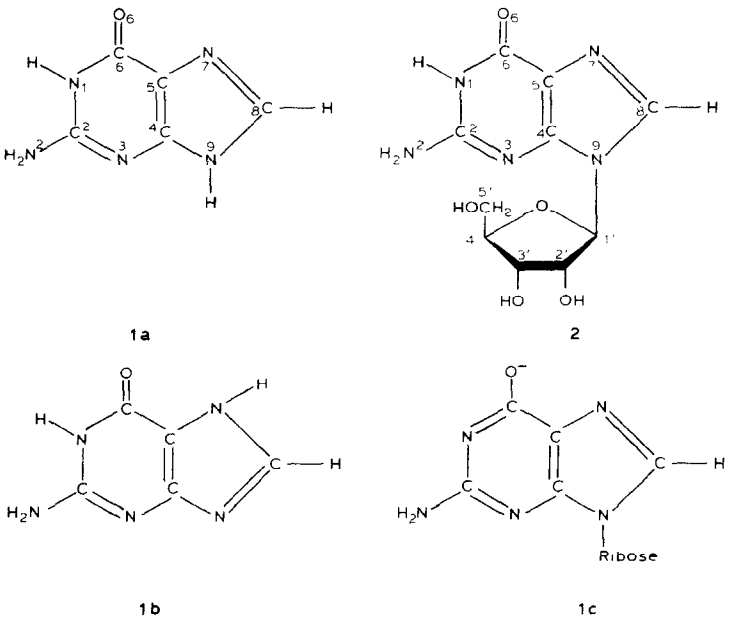


Scheme I. Tautomeric forms (**1a, 1b, 1c**) and numbering of atoms of guanine and guanosine (**2**).

also applied[9,10] to study the sites of complexation of metal ions and the conformation of metal complexes of guanine[9] and guanosine 5′-monophosphate[10].

Because of its special structure and the position of the double bonds in the five-membered ring, guanine is subjected to electron delocalization and may exist in different tautomeric forms. This consideration led Shapiro[11] to conclude that the guanine cation may exist in the N-7-H$^+$ or N-9-H$^+$ forms. It was deduced[11] from u.v. and i.r. studies that the deprotonation of guanosine gives rise to the C-6-O$^-$ anion. The preponderant structure of this anion is given in Scheme I.

The interpretation of F.t.-i.r. and laser-Raman spectral data was based[1–4] on the bond distances and angles obtained from crystallographic results. The molecular dimensions of the guanosine residue in 5′GMP · 3 H_2O (ref. 12), cyclic GMP[13], and guanosine dihydrate[14] derived from X-ray crystallography provided a basis for understanding the frequencies observed. The crystallographic data (bond lengths and angles) were averaged[15] from the structures of 17 neutral and 3 protonated guanine species.

The approach used for study of the F.t.-i.r. and Raman spectra of guanine and guanosine is similar to that adopted previously[1,2]. It consists of taking into account the resonant forms for assigning the observed frequencies of guanine, and then comparing the spectra of the nucleoside to that of the sugar on the one hand and to that of the purine base on the other. The assignment of frequencies could then be used in the investigation of molecules having an increased degree of complexity, such as nucleotides and nucleic acids.

EXPERIMENTAL

Materials and methods. — Guanine and guanosine (9-β-D-ribofuranosylguanine)[16] were Sigma products. The F.t.-i.r. spectra of their solid forms were recorded with a Digilab Fourier-transform, infrared spectrometer (FTS 20) in the 1800–600 and 3600–2400 cm^{-1} frequency regions. The Raman spectra were obtained according to the method described previously[4]. The ranges of Raman frequencies explored were 1700–200 cm^{-1} for both the purine base and the nucleoside.

RESULTS AND DISCUSSION

A. Guanine. — The F.t.-i.r. and laser-Raman spectra of guanine (**1a**) are shown in Figs. 1 and 2, respectively. The general profiles of these spectra are completely different. Noteworthy in the Raman spectrum (see Fig. 2) is a very sharp line at 642 cm^{-1}, whose high intensity contrasts with the many weak signals scarcely distinguished from the noise background. The Raman spectrum of guanine has a diffuse background because of fluorescence of the yellowish sample. This fluorescence is not observed for the other bases[1,3,4]. Cursory inspection of the i.r. spectrum shows very strong absorptions in the carbonyl region; the other observed frequencies have moderate to weak intensities.

Analysis of the observed bands. a. The 1800–1300 cm^{-1} region. Two strong i.r. bands are observed at 1700 and 1676 cm^{-1} (see Fig. 1). The basis of absorptions in this region is particularly well documented. When using isotopic ^{15}N substitution on the imidazole nitrogen, Delabar and Majoube[6] did not observe frequency shifts in this region. The unsubstituted guanine molecule shows[6] i.r. absorptions at 1702 and 1650 cm^{-1}, whereas the d_4 and d_5 deuterated analogues (see Scheme II) give

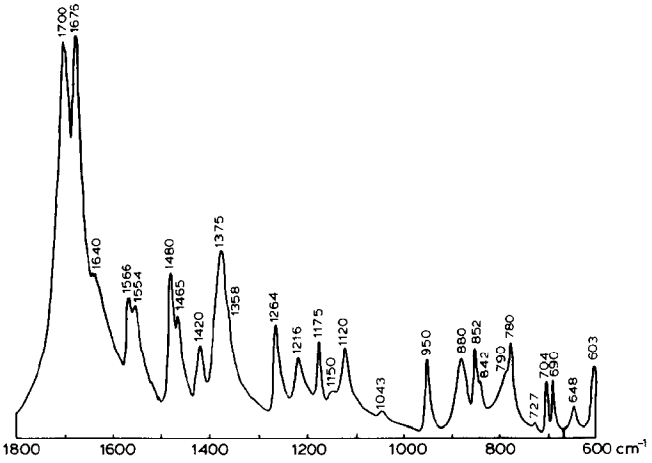


Fig. 1. F.t.-i.r. spectrum of guanine.

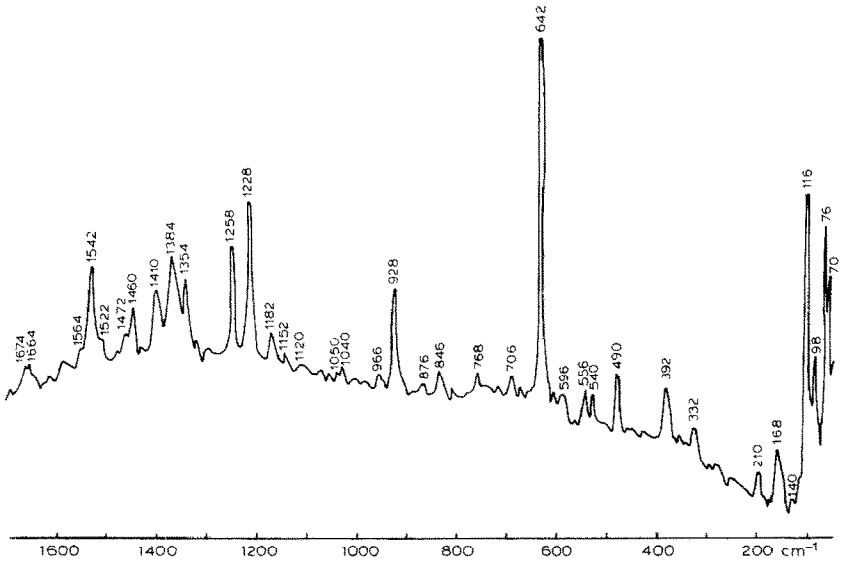
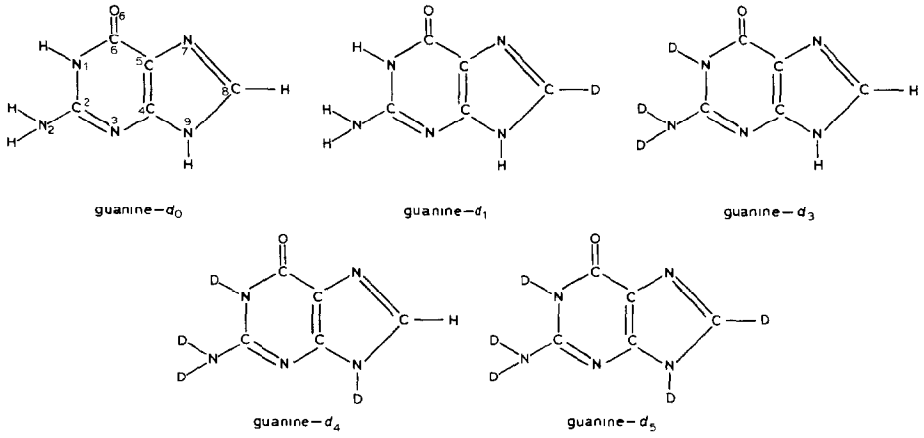


Fig 2 Laser-Raman spectrum of guanine.

rise the only one band, at 1677–1676 cm^{-1}. The carbonyl stretching-mode[17] is sensitive to hydrogen bonding in different nucleic acid derivatives. This frequency shifts towards higher values when the purine base is not hydrated[17]. Tsuboi *et al.*[18] derived from normal-coordinate analysis the value of 1692 cm^{-1} for the frequency corresponding to ν(C=O) in 9-methylguanine. A Raman investigation of 5'GMP at different temperatures permitted Savoie *et al.*[8] to localize ν(C=O) at 1670 cm^{-1} in hydrated guanine residues (at 80°); at lower temperatures two vibrations appear at 1658 and 1720 cm^{-1}, respectively, attributed to ν(C=O) in the solvated (by H_2O) guanine residue and the associated guanine in base pairs. Lord and Thomas[19] assigned the band, at 1680 cm^{-1} in the Raman spectra of guanine derivatives in aqueous solutions, to ν(C=O). Based on previous studies of vibrational spectra of guanine residues in different nucleotides, it seems reasonable to assign the observed vibration at 1676 cm^{-1} to ν(C=O). Interpretation of isotopic-substitution results[6] incline us to propose a contribution of $\delta(NH_2)$ to 1700 cm^{-1}, together with carbonyl stretching. Only weak bands at 1674–1664 cm^{-1} are observed in the Raman spectrum of guanine (see Fig. 2). The same observation was made by Delabar and Guschlbauer[7].

Two nonresolved i.r. absorptions are observed at 1566 and 1554 cm^{-1} (see Fig. 1). The corresponding Raman line is localized at 1542 cm^{-1} with a shoulder at 1564 cm^{-1} (see Fig. 2). In this frequency region, double-bond (C=C and C=N) stretching-modes are expected on the basis of a normal-coordinates treatment. Tsuboi *et al.*[18] considered that the carbonyl (C-6=O) is coupled to the ring stretching of the purine ring (C-4=C-5 and C-5–C-6). They assigned[18] the observed band



Scheme II. Guanines-d_0, -d_1, -d_3, -d_4, and -d_5.

at 1577 cm^{-1} to the C-4=C-5 and C-5–C-6 in-phase stretching mode, and the vibration at 1565 cm^{-1} to a combination of ν(C-6=O), ν(C-5–C-6), and ν(C-4=C-5). Delabar and Majoube[6] observed i.r. absorptions at 1565 and 1550 cm^{-1}. They deduced from ^{15}N isotopic substitution that these bands do not arise from a pure C-N stretching mode. A polarized Raman line was observed[10] at 1579 cm^{-1} in aqueous 5'GMP. Although, the shape of the observed bands shows that the vibrations at 1566–1554 cm^{-1} (i.r.) and 1564–1542 cm^{-1} (Raman) are not pure stretching-modes, we suggest that the major contribution to these vibrations comes from (C=N) and (C=C) stretching modes. This assignment is in good agreement with that proposed by Makrigiannis *et al.*[10]. It is likely that there is a contribution from ν(C-N) to these vibrations, especially for C-2–N-2, which has a bond length close to that of a double bond. Indeed, the C-N distances averaged[15] from 17 neutral guanine residues are: N-1–C-2 = 137.5; N-1–C-6 = 139.3; N-2–C-2 = 134.1; N-9–C-8 = 137.4; N-7–C-5 = 138.9; N-9–C-4 = 137.7; N-3–C-2 = 132.7; and N-7=C-8 = 130.4 pm.

The F.t.-i.r. spectrum (see Fig. 1) shows a relatively strong absorption at 1480 cm^{-1} coupled with a moderate band at 1465 cm^{-1}. This coupling is also observed in the Raman spectrum of guanine (see Fig. 2) with higher intensity for the 1460 cm^{-1} absorption compared with that at 1472 cm^{-1}. A vibration in the 1490–1480 cm^{-1} region was found to be characteristic of the guanine or adenine residues. It has been observed by various authors[6–10,18–21] in the vibrational spectra of guanine, guanosine, and nucleic acids. Various assignments have been proposed for this vibration, including double-bond stretching[19], δ(N-1-H)[6], ν(N-7=C-8)[8,10], or a wave-like mode[9,18]. Moreover, vibrations in this region are[8] sensitive to hydrogen bonding. Metal-complexation studies[10] indicated the possibility of fixation of cis-platinum at N-7 or N-9. Likewise, the guanine cation may exist in the N-7-H^+ or N-9-H^+ forms[11], which means that the N-7=C-8 double bond could be delocalized to the N-9=C-8 position. The vibration at 1490 cm^{-1} shifts after ^{15}N sub-

stitution[7] of N-7 and N-9. According to this information, it appears that the 1480–1465 cm^{-1} frequency-region involves vibrations that include N-7, N-9, and C-8. We propose assignment of 1480 cm^{-1} (i.r.) to ν(N-7=C-8), with a contribution from ν(C-8–N-9) and 1465 cm^{-1} (i.r.) to the deformation of CNH angles involving the C-8 atom (Schemes Ia, Ib). The contribution of C-8-H deformation to this frequency has been mentioned by Delabar and Majoube[6], while Tsuboi *et al.*[18] associate three modes of vibration, including the N-7, C-8, and N-9 atoms to the calculated frequency at 1459 cm^{-1}. The contribution of δ(C-8–N-9-H) to the vibration observed at 1465 cm^{-1} (i.r.) and 1460 cm^{-1} (Raman) is supported by the absence of such a frequency from the spectrum of the nucleoside (see later, Fig. 4).

An i.r. absorption is observed at 1420 cm^{-1} (see Fig. 1). The corresponding Raman line is localized at 1410 cm^{-1} (see Fig. 2). We previously[4] proposed assignment of 1420 cm^{-1} (i.r.) to δ(N=C-H) in adenine. A Raman study[8] of 3′5′GpG shows that this band is not affected by an increase of temperature, but is shifted after deuteration. This frequency did not change[19] when the pH of aqueous solutions of guanine and guanosine was varied. As this vibration concerns a group of atoms sensitive to deuteration but insensitive to hydrogen bonding and pH, we suggest assignment of 1420 cm^{-1} (i.r.) to δ (N-7–C-8-H).

The i.r. band at 1375 cm^{-1}, which is relatively broad (see Fig. 1) with a shoulder at 1358 cm^{-1}, corresponds to the doublet observed in the Raman spectrum of guanine (see Fig. 2) at 1384 and 1354 cm^{-1}. This absorption has been observed in the vibrational spectra of many polynucleotides and nucleic acids. It was found at 1378 cm^{-1} in the spectrum of calf-thymus DNA[20,21] and assigned to thymine, adenine, and guanine residues; at 1374 cm^{-1} in the spectrum of yeast RNA[20]; at 1380 cm^{-1} in rRNA[21]; and 1367 cm^{-1} in polyG[20]. Theophanides[9] observed a band at 1375 cm^{-1} in the spectra of guanine residues that he assigned to ν(C-8–N-9) and ν(C-8=N-7). An isotopic-substitution investigation[6,7] of guanine and its derivatives clearly indicates that the atoms involved in the vibration localized at 1375 cm^{-1} (i.r.) for guanine[6] and 1368 cm^{-1} (Raman) for guanosine[7] are C-8, N-7, N-9, and H (C-8). In particular, the i.r. band at 1375 cm^{-1} shifts to 1360 cm^{-1} for guanine-d_1 (see Scheme II). The modification of frequencies (1373–1350 cm^{-1}) observed[19] in the Raman spectra of guanine at different pH values shows that the N-7-H or N-9-H sites of protonation[11] are involved. Accordingly, it may be proposed that the observed frequencies 1375–1358 cm^{-1} (i.r.) and 1384–1354 (Raman) originate from a combination of the stretching and bending modes around C-8 (see Table I).

b. The 1300–1000 cm^{-1} region. An i.r. absorption is observed at 1264 cm^{-1} (see Fig. 1) that corresponds to the Raman line at 1258 cm^{-1} (see Fig. 2). Lord and Thomas[19] localized the external C-N stretching modes in the 1300–1250 cm^{-1} region. An ^{15}N-substitution study of guanine[6] and guanosine[7] shows that this frequency region contains ν(C-N) vibrations. Another intense, well-resolved Raman line is observed (Fig. 2) at 1228 cm^{-1} and probably has the same origin as the broad i.r. band at 1216 cm^{-1} (see Fig. 1). This frequency was also attributed[6] to ν(C-N). Observation of the Raman lines at 1258 and 1228 cm^{-1} (see Fig. 2)

TABLE I

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

I.r.		*Raman*		
$\nu(cm^{-1})$	*I*	$\nu(cm^{-1})$	*I*	*Assignments (modes)*
		70	64.4	
		76	78.8	
		98	43.2	
		118	85.6	
		140	4.5	
		168	16.7	
		210	8.3	
		332	15.1	H bonding
		392	15.1	δ(C=O)
		490	16.2	δ(N-9-C-4=C-5) and δ(N-7-C-=C-4)
		540	9.7	δ(N-3-C-4=C-5)
		556	15.1	δ(C-C=C)
603	17.6	596	7.6	
648	8.1	642	100[b]	breathing
690	14.2			
704	13.7	708	6.1	δ(ring)
727	3.9			
780	23.5	768	6.1	
790	16.4			δ(N-1-H)
852	22.0	846	7.6	ν(C-C)
880	19.4	876	3.8	δ(N-9-H) out-of-plane
		928	29.2	δ(N-C=N) and δ(N-C-N)
950	19.6	966	4.5	
1043	6.9	1040	4.5	r(NH_2)
		1050	3.0	
1120	22.0	1120	3.0	δ(C-N=C)
1150	11.5	1152	4 9	
1175	23.5	1182	9.1	δ(C-8-H) in plane
1216	19.6	1228	44.3	ν(C-2-NH_2)
1264	27.7	1258	31.4	ν(C-5-N-7) and (C-4-N-9)
1358	34.0	1354	21.6	δ(C-8-N-H), δ(C-8-H), ν(C-8-N)
1375	46.6	1384	27.6	
1420	29.5	1410	17.8	δ(N-7=C-8-H)
1465	30.4	1460	13.5	δ(C-N-H)
1480	40.7	1472	7.0	ν(N-7=C-8) and ν(C-8-N-9)
		1522	6.1	
1554	32.4	1542	26.5	ν(C=C)
1566	35.0	1564	5.4	ν(C-2=N-3)
1676	100[b]	1664	15.1	ν(C=O)
1700	98.0	1674	15.1	ν(C=O), δ(NH_2)

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

shows a difference in intensity that could be due to a vibration of the same kind ν(C-N), but differing in number. Indeed, it may be observed that there are 3 endocyclic C-N bonds and an exocyclic one in guanine (**1a**). Moreover, the C-2–N-2 distance is shorter (134.1 pm) than the other C-N bonds. Accordingly, we propose assignment of 1258 cm^{-1} (Raman) and 1264 cm^{-1} (i.r.) to ν(C-2–N-2) and 1228 cm^{-1} (Raman) and 1216 cm^{-1} (i.r.) to other C-N stretchings.

A relatively weak i.r. absorption is observed at 1175 cm^{-1} (see Fig. 1). The corresponding Raman frequency is localized at 1182 cm^{-1} (see Fig. 2). A vibration was observed[20] in the Raman spectrum of polyG and assigned to ν(C-N). The same assignment was proposed[21] for 1180 and 1185 cm^{-1} in spectra of rRNA and calf-thymus DNA. An observed band at 1181 cm^{-1} in the spectrum of 3',5'GpG was attributed to ν(C-N). Tsuboi *et al.*[18] determined from normal-coordinate calculations that the 1172 cm^{-1} frequency is attributable to a combination of ν(C-8=N-7), ν(N-9-R), and ν(N-3–C-4) in 9-methylguanine. From a deuteration study[6], it was concluded that the observed i.r. absorption at 1174 cm^{-1} and the Raman line at 1188 cm^{-1} in the spectra of guanine could correspond to a major contribution from an in-plane deformation of C-H. Thus we suggest that 1175 cm^{-1} (i.r.) and 1182 cm^{-1} (Raman) originate from a combination of ν(C-8=N-7) and ν(C-8-H).

The observed band at 1120 cm^{-1} is relatively weak in the F.t.-i.r. spectrum (see Fig. 1), and very weak in the Raman (see Fig. 2) spectrum. It was not always reported in the previous[8–10,18–21] studies. Only Delabar and Majoube[6] observed this frequency in the i.r. spectrum of guanine, and they assigned it to an in-plane deformation of imidazole. As proposed previously for the 1126 cm^{-1} absorption in the spectrum of adenine[4], the observed absorption at 1120 cm^{-1} could be due to a (C-N=C) deformation. The weak band at 1043 cm^{-1} (i.r.) and 1040 cm^{-1} (Raman) is assigned to r(NH_2). This assignment is in agreement with that proposed by Delabar and Majoube[6]. However, contributions from ν(C-N) to this vibration have also been reported[6,18].

c. The region below 1000 cm^{-1}. The i.r. spectrum of guanine (see Fig. 1) shows moderate to weak intensity bands, and it may be observed in the Raman spectrum (see Fig. 2) that, apart from the sharp lines at 928 and 642 cm^{-1}, most of the vibrations are very weak. The i.r. absorption at 950 cm^{-1} was reported[6] to be sensitive to both ^{15}N and ^{2}H isotopic substitution. An observed i.r. band at 940 cm^{-1} in the spectrum of adenine has been assigned to δ(N–C=N). Accordingly, we propose assignment of 950 cm^{-1} to δ(N–C=N), with particular reference to the ^{15}N- and ^{2}H-sensitive region, namely, δ(N-3=C-2–N-2) and δ(N-3=C-2–N-1). The frequencies observed in this region in the Raman spectrum (see Fig. 2) are very weak, except for a band at 966 cm^{-1} and a sharp line at 928 cm^{-1}. The Raman band at 966 cm^{-1} probably corresponds to the i.r. absorption at 950 cm^{-1}. Assignment of 927 cm^{-1} to ν(N-9-R) and δ(N-7=C-8–N-9) in the guanine residue was deduced from normal-coordinates analysis[18]. The form of the observed Raman line at 928 cm^{-1} in Fig. 2 probably arises from the symmetrical character of the vibration. We propose assignment of 928 cm^{-1} to δ(N-3–C-4–N-9).

The observed i.r. band at 880 cm^{-1} (see Fig. 1) corresponding to the Raman frequency of 876 cm^{-1} (see Fig. 2) probably originates from different vibrations, as indicated by the broadness of the i.r. band. It was found[6] to be affected by N-D substitution, and consequently, it is attributed to out-of-plane δ(N-H), which is in agreement with previous results[4,6]. The i.r. spectrum (see Fig. 1) shows an absorption at 852 cm^{-1}, corresponding to the Raman vibration at 846 cm^{-1} (see Fig. 2). The C-C stretching mode is generally localized in this frequency region. An observed[6] i.r. band at 850 cm^{-1} has been assigned to a pyrimidine ring vibration. As proposed for adenine[4], we suggest assignment of 852 cm^{-1} (i.r.) and 846 cm^{-1} (Raman) to a ring vibration involving ν(C-C). The i.r. band at 780 cm^{-1}, with a shoulder at 790 cm^{-1} (see Fig. 1), could originate from an imidazole ring vibration, whereas absorptions at 704 and 690 cm^{-1} could arise from skeletal modes from the pyrmidine ring.

The Raman spectrum (see Fig. 2) exhibits a very strong line at 642 cm^{-1}, whereas only a weak absorption is present at 646 cm^{-1} in the F.t.-i.r. spectrum (see Fig. 1). It has been reported[5] that B and Z forms of DNA may be differentiated by the Raman lines at 675 and 625 cm^{-1}, assigned to the guanine residue in B and Z DNA, respectively. This frequency seems characteristic of guanine. A Raman line at 680 cm^{-1}, corresponding to a calculated vibration at the same frequency, was found similar to a ring-breathing motion. This line has also been observed by Hartman *et al.*[21] at 670 cm^{-1} for guanosine in neutral solution. Such a vibration in guanine may be shifted in guanine derivatives because of hydrogen bonding between the base and the sugar[22]. The strong intensity of the observed Raman frequency 642 cm^{-1} is very probably attributable to an in-phase stretching of all of the bonds in the purine ring (breathing mode).

Only the Raman spectrum is recorded below 600 cm^{-1}. Five weak bands are seen (see Fig. 2), at 556, 540, 490, 390, and 332 cm^{-1}. As previous discussed[2], we suggest assignment of 556 cm^{-1} to δ(C-C=C) and 540 cm^{-1} to δ(N-C=C) in the pyrimidine ring. The Raman band at 540 cm^{-1} could also originate from another (N-C–C) deformation, namely, the δ(N-9–C-4=C-5) and δ(N-7–C-4=C-5) modes. The C=O bending-mode in thymine[2] was localized at 432 cm^{-1}. It has also been found[21] from a Raman study that the carbonyl deformation is located in this frequency region. Accordingly, we assign 392 cm^{-1} to δ(C=O). The observed frequencies at and below 332 cm^{-1} probably originate from hydrogen bonding and intercrystalline forces.

d. Frequencies in the region 3600–2400 cm^{-1}. The F.t.-i.r. spectrum of guanine in the solid form is shown in Fig. 3. The observed frequencies and the proposed assignments are listed in Table II. The C-H and N-H stretching modes are localized at 2700 and 2912–2852 cm^{-1}, respectively. The N-H vibrations give rise to two bands corresponding to a differentiation of N-1-H and N-7-H stretchings by their environment. Observed frequencies 3122 and 3334 cm^{-1} are assigned to symmetrical and antisymmetrical NH_2 stretching-modes, respectively. These vibrations occur at relatively high frequencies because of the hydrogen bonding in crystalline guanine.

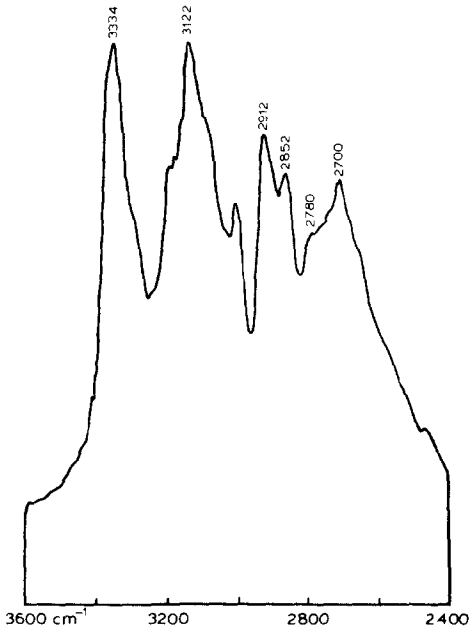


Fig. 3 F.t.-i.r. spectrum of guanine in the frequency region 3600–2400 cm^{-1}.

TABLE II

BANDS OBSERVED[a] IN THE F T -I R SPECTRUM OF GUANINE (THE 3600–2400 cm^{-1} REGION)

$\nu(cm^{-1})$	*I*	*Assignments (modes)*
3334	100[b]	$\nu_a(NH_2)$
3122	100[b]	$\nu_s(NH_2)$
2912	82 8	ν(N-H)
2852	76 0	
2780	65.7	ν(C-H)
2700	75.0	

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

B. Guanosine. — The F.t.-i.r. spectrum of solid guanosine (**2**) is shown in Fig. 4. The general profile of the spectrum of the nucleoside is completely different from that (Fig. 1) of guanine. It may be noted that frequency and intensity shifts occur across the whole range of frequencies, including the carbonyl region, whereas major modifications were observed only in the 1200–800 cm^{-1} region for adenosine[4], the other purine nucleoside.

a. Observed bands identified from the spectra of guanine and D-*ribose.* The strongest bands in the spectrum of guanosine (see Fig. 4) are localized in the same region (1800–1600 cm^{-1}) as for guanine (see Fig. 1). However, whereas two

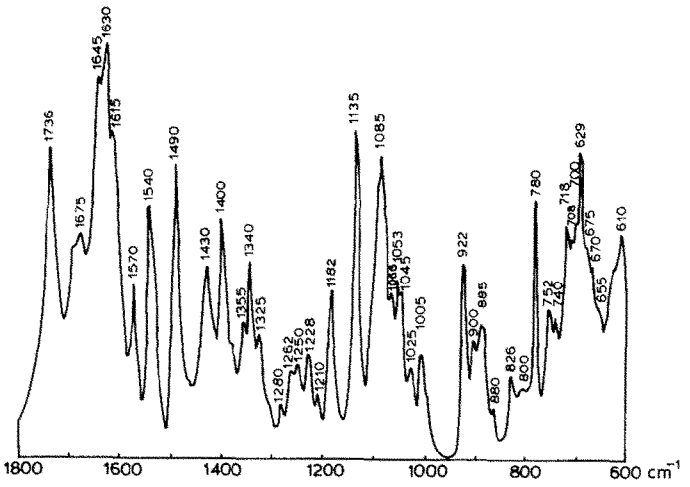


Fig. 4. F.t.-i.r. spectrum of guanosine.

TABLE III

BANDS OBSERVED[a] IN THE F T -I R SPECTRUM OF GUANOSINE

$\nu(cm^{-1})$	*I*	*Assignment (modes)*	$\nu(cm^{-1})$	*I*	*Assignments (modes)*
610	53.9	G	1085	73.0	R
655	36.8	G + R	1135	78.4	R
670	46.3		1182	40.7	ν(C-1′–N-9)
675	50.7		1210	16.7	G
692	73.0	G	1228	25.7	R
700	56.4	G	1250	23.5	R
708	52.0	δ(C-2′–C-1′–N-9)	1262	21.6	G
718	56.4	R	1280	13.7	R
740	34.0	δ(O-4′–C-1′–N-9)	1325	30.4	R
752	35.8	R	1340	47.0	R
780	61.8	G	1355	32.8	R
800	17.2	R	1400	57.6	R
826	20.1	δ(C-N-9-C)	1430	46.6	G + R
866	12.7	R	1490	70.6	G
885	32.4	R + G	1540	61.0	G
900	28.9	R	1570	41.7	G
922	46.1	R	1615	78.9	G
1005	25.0	δ(O-4′–C-1′–H)	1630	100[b]	G
1025	22.8	R	1645	91.7	G
1045	40.7	G	1675	53.4	G
1053	43.1	δ(N-9–C-1′–H)	1736	74.5	G
1065	39.5	R			

[a]Key: I = relative intensity; δ = bending mode; ν = stretching mode. G = guanine (detailed assignments are given in Table I); R = D-ribose (detailed assignments are given in ref. 2). [b]Taken as reference.

maxima at 1700 and 1676 cm^{-1} are seen in Fig. 1 and are assigned to carbonyl stretching with contributions from NH_2 bending, five vibrations are observed in the spectrum of guanosine. The origin of these vibrations is also the shift of C=O stretching to 1736 cm^{-1} and the contribution from the group of atoms C-3, C-2, and N-2, namely, $\delta(NH_2)$, ν(C-2–N-2) and ν(C-2=N-3). It is probable that the carbonyl stretching is shifted towards higher frequency because of hydrogen bonding in the solid nucleoside. Assignments of the observed i.r. bands to the ribosyl (R) or the guanylyl (G) moieties of guanosine are listed in Table III. Apart from the change in the carbonyl region discussed here, detailed assignment may be found in the previous lists (ref. 3 and Table I).

b. Observed bands differentiating guanosine from guanine and D-*ribose.* The only frequencies discussed are the i.r. absorptions observed in Fig. 4 that are absent from the spectra of D-ribose[3] and guanine (see Fig. 1). These frequencies certainly arise from stretching and bending modes around the glycosylic (N-9–C-1′) linkage. A new i.r. absorption observed at 1182 cm^{-1} may be assigned to ν(N-9–C-1′) according to the correlation charts[23] between frequencies and vibrations. The band at 1053 cm^{-1} is a part of a group of i.r. bands (see Fig. 4) in the "fingerprint" region of the sugar. It may originate from a C-H bending mode around carbon atom C-1′: δ(O-4′–C-1′–H). The frequency region most affected by the association of the sugar and the purine base through a glycosylic bond is the region of ring vibrations below 850 cm^{-1}. This is probably due to inter-residue hydrogen bonding, which leads to a quasi-ring structure around C-4, N-9, and C-1′. The deformations of the quasi-ring structure take place at higher frequencies than those associated with ring vibrations. Accordingly, we propose assignment of the bands at 826, 740, and 709 cm^{-1} to δ(C-N-9-C), δ(O-4′–C-1′–N-9), and δ (C-2′–C-1′–N-9), respectively.

CONCLUSION

F.t.-i.r. and laser-Raman spectra of guanine were analyzed by reference to previous results and by taking into account its crystalline structure. Among the four bases of DNA, guanine (**1a**) seems[5] to have a particular influence on the B or Z conformation of the nucleic acid. In consequence, all structural information, including the assignments of frequencies proposed here, may be helpful for elucidating the role of guanine in the regular or zig-zag helical structure of nucleic acids. While D-ribose was found[4] to induce less perturbation on the structure of the nucleosides than 2-deoxy-D-*erythro*-pentose for the nucleosides studied[1,3,4], many intensity and frequency shifts have been observed for the ribosylnucleoside guanosine.

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