In-Plane Vibrational Modes of Guanine from an *ab Initio* MO Calculation

Yoshifumi Nishimura, Masamichi Tsuboi,* Shigeki Kato,†,†† and Keiji Morokuma†
Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113
†*Institute for Molecular Science, Myodaiji, Okazaki 444*

(Received October 12, 1984)

Harmonic force constants, in-plane vibrational frequencies, and in-plane vibrational modes of guanine have been calculated by an *ab initio* MO method. The force constants were calculated by the use of an energy gradient method with STO-3G basis set, and then they were corrected into "4-31G force constants" by the scaling factors given by us previously for the case of uracil. The corrected set of force constants could reproduce the observed frequencies of guanine, guanine-7, 9-$^{15}N_2$, guanine-1,3,*amino*-$^{15}N_3$ (at positions 1, 3 and amino), guanine-8-*d* (at position 8), guanine-1,9, *amino*-d_4 (at amino and iminos), and guanine-d_5. The calculated vibrational modes could also account for the results of earlier experiments on infrared and Raman spectra of guanine residues in 5′-GMP and other nucleotides.

This is our second paper in a series of *ab initio* MO studies of the internal force field of nucleic acid. The previous one[1] dealt with uracil, and the present paper deals with guanine.

The vibrational spectroscopic studies of the guanine residue have now begun to provide a good insight into the conformation of nucleic acids. This residue has been found to cause many of the Raman lines of DNA and RNA,[2–12] and some of them are found to be useful to diagnose the polynucleotide conformation.[2,3,8,12] The location of a strong guanine Raman line around 650 cm^{-1}, for example, is sensitive to the internal rotation angle around the glycosidic bond and to the pseudo-rotation phase angle of the five-membered ribose ring directly linked to the guanine residue.[2,3] To establish such a spectrum-conformation correlation, it is necessary to clarify the normal mode of vibration for each Raman line of the guanine residue.

For a normal coordinate treatment, it is essential to obtain a reliable set of force constants of the molecule. In the previous paper,[1] we showed that it is practical to calculate the force constants of a big molecule, namely uracil, by the use of an *ab initio* MO method with STO-3G basis set, and it was possible to predict to a significant extent the vibrational modes by the use of "4-31G" force constants which were reached by a correction from the STO-3G force constants. Thus, the calculation could reproduce the observed frequencies, not only of uracil itself, but also of *C, C*-dideuterouracil, *N, N*-dideuterouracil, perdeuterouracil, and a uracil-$^{15}N_2$ residue.

In this paper, we report a similar study on guanine. Delabar and Majoube[13] reported the experimental frequencies, not only for guanine itself, but also for its three deuterated derivatives and two ^{15}N-substituted derivatives. All the normal frequencies and the corresponding vibrational modes of these guanine derivatives have been calculated in the present study and we can predict satisfactorily the observed frequencies.

Computational Details

The geometry and the harmonic force constants were calculated by the use of IMSPACK program.[14] We optimized the geometry of guanine without any constraint starting from a geometry which had been optimized under the constraint that the molecule is planar. This initial geometry was kindly provided by Mrs. Aida.[15] Our new optimization did not cause any significant change. The internal coordinates adopted here are shown in Fig. 1. Here, the redundancy of the angular coordinates caused by the ring system has been removed by the method of Pulay *et al.*[16]

The vibrational frequencies and the normal modes of vibration were calculated by a program written by us, which is a modified version of NCTB program written by Shimanouchi *et al.*[17] The force constants calculated from the STO-3G basis set were corrected into "4-31G" force constants by the use of the scaling factors, which are given in the previous paper (Table 1 and Eq. (4)) on uracil.[1] These were obtained by a direct comparison of the actually calculated force constants both with the STO-3G and 4-31G basis sets for urea, acrylaldehyde, and formamide. It will be shown below that they are useful for guanine as well as for uracil. Let us also mention here of our trial calculations on imidazole. We have calculated the force constants of imidazole with the STO-3G and the 3-21G basis sets. The STO-3G force constants were then corrected by the use of our scaling factors, and the results were found to be in a good agreement with the 3-21G force constants. The off-diagonal elements of the STO-3G force constant matrix of guanine, smaller than 0.1 mdyn/Å for the stretching-stretching, 0.03 mdyn for the stretching-bending, and 0.01 mdyn · Å for the bending-bending are taken to be zero, so that the number of non-zero force constants turned out to be 171.

Results and Discussion

Equilibrium Geometry. The optimized geometry calculated using the STO-3G basis set is given in Table 1. This should correspond to the geometry of the free guanine molecule, but it showed a good coincidence

---

[††] Present address: Department of Chemistry, College of General Education, Nagoya University, Chikusa-ku, Nagoya 464.

$S_1 = \Delta r_1$	νN_1C_2 str.
$S_2 = \Delta r_2$	νC_2N_3 str.
$S_3 = \Delta r_3$	νN_3C_4 str.
$S_4 = \Delta r_4$	νC_4C_5 str.
$S_5 = \Delta r_5$	νC_5C_6 str.
$S_6 = \Delta r_6$	νC_6N_1 str.
$S_7 = \Delta r_7$	νC_6O str.
$S_8 = \Delta r_8$	νN_1H str.
$S_9 = \Delta r_9$	νC_2NH_2 str.
$S_{10} = \Delta r_{10}$	νNH str.
$S_{11} = \Delta r_{11}$	νNH str.
$S_{12} = \Delta r_{12}$	νC_5N_7 str.
$S_{13} = \Delta r_{13}$	νN_7C_8 str.
$S_{14} = \Delta r_{14}$	νC_8N_9 str.
$S_{15} = \Delta r_{15}$	νN_9C_4 str.
$S_{16} = \Delta r_{16}$	νC_8H str.
$S_{17} = \Delta r_{17}$	νN_9H str.
$S_{18} = \beta_3-\beta_4+\beta_5-\beta_6+\beta_1-\beta_2$	δR_6 I
$S_{19} = 2\beta_3-\beta_4-\beta_5+2\beta_6-\beta_1-\beta_2$	δR_6 II
$S_{20} = \beta_4-\beta_5+\beta_1-\beta_2$	δR_6 III
$S_{21} = \beta_{18}-0.8090169\beta_{19}+0.309017\beta_{20}+0.309017\beta_{16}-0.8090169\beta_{17}$	δR_5 I
$S_{22} = -1.1180339\beta_{19}+1.8090169\beta_{20}-1.8090169\beta_{16}+1.1180339\beta_{17}$	δR_5 II
$S_{23} = \beta_7-\beta_8$	δCO bend.
$S_{24} = \beta_9-\beta_{10}$	δN_1H bend.
$S_{25} = \beta_{11}-\beta_{12}$	δC_2N bend.
$S_{26} = 2\beta_{15}-\beta_{13}-\beta_{14}$	δNH_2 scis.
$S_{27} = \beta_{13}-\beta_{14}$	δNH_2 rock.
$S_{28} = \beta_{23}-\beta_{24}$	δN_9H bend.
$S_{29} = \beta_{21}-\beta_{22}$	δC_8H bend.

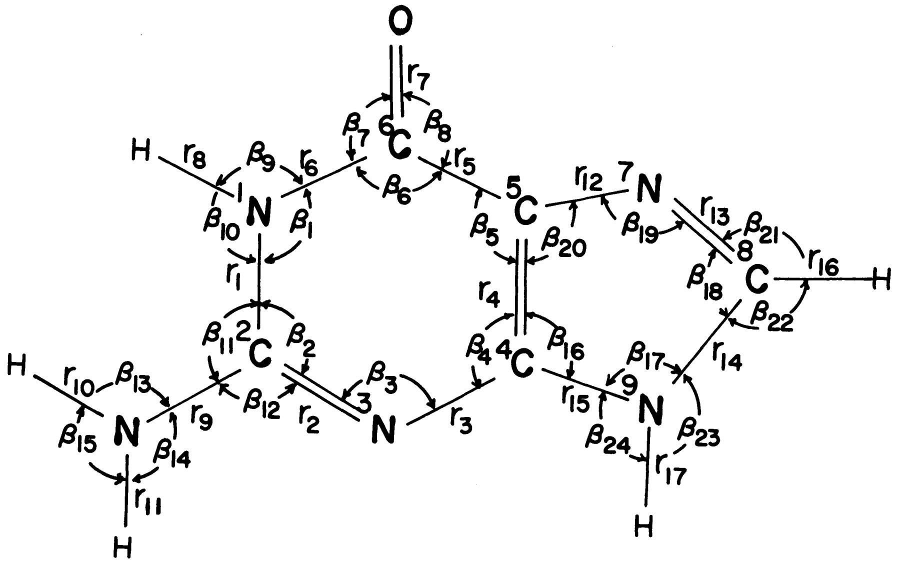

Fig. 1. Internal coordinates of the guanine molecule.

with the averaged values of the bond lengths and bond angles found in the X-ray crystallographical analyses of many guanine derivatives.[18] The averaging may cause a cancelling one another of various effects of intermolecular forces in the crystals to result in an experimental geometry of a guanine molecule, somewhat free from intermolecular force field. It is an interesting finding that the skeletal geometry (involving C, N, and O) of the guanine molecule calculated with the STO-3G basis set reproduced such an experimental geometry.

Force Constants (corrected). The "4-31G" force constants after the correction from the STO-3G force constants, are shown in Tables 2-A—E. In Table 2-A, the diagonal elements of the force constants matrix are shown. The stretching force constants of the ring system varies from 6.427 mdyn/Å for the C_6N_1 to 9.636 mdyn/Å for the N_7C_8 bond, and it is found that the greater force constant corresponds to the shorter bond length. The off-diagonal elements among the stretching coordinates of the six-membered ring system are nearly equal to the corresponding elements of the uracil molecule[1] as shown in Table 2-B. The off-diagonal elements for the "ortho" positions are

TABLE 1. BOND LENGTHS IN THE GUANINE MOLECULE (unit: Å, 1Å=0.1 nm)

	Calcd on STO-3G	Obsd[a]
N1C2[b]	1.400	1.375
C2N3	1.318	1.327
N3C4	1.403	1.355
C4C5	1.376	1.377
C5C6	1.477	1.415
C6N1	1.455	1.393
C6O	1.218	1.239
N1H	1.021	
C2NH$_2$	1.390	1.341
NH	1.013	
C5N7	1.414	1.389
N7C8	1.312	1.304
C8N9	1.397	1.374
N7C4	1.390	1.377
C8H	1.083	
N9H	1.020	

a) Reference 18. b) see Fig. 1.

TABLE 2. FORCE CONSTANTS OF THE GUANINE MOLECULE, CALCULATED ON THE STO-3G BASIS SET, AND THEN CORRECTED AS DESCRIBED IN THE TEXT

A. Diagonal Elements

Numbering of internal coord.[a]	Force const.[b]	Classification
1.	7.980	S_{6r}
2.	8.967	
3.	7.174	
4.	8.524	
5.	6.458	
6.	6.427	
7.	13.482	S_{6p}
8.	8.098	
9.	8.518	
10.	8.428	
11.	8.445	
12.	7.129	S_{5r}
13.	9.636	
14.	8.249	
15.	8.817	
16.	6.296	S_{5p}
17.	8.213	
18.	2.005	Δ_r
19.	2.012	
20.	1.782	
21.	2.581	
22.	2.721	
23.	1.203	Δ_p
24.	0.616	
25.	1.392	
26.	0.572	
27.	0.698	
28.	0.512	
29.	0.586	

a) See Fig. 1. b) Units: mdyn/Å for S's and mdyn.Å for Δ's where 1Å=0.1 nm.

B. Off-Diagonal Elements between One of the Ring Stretching Coordinates (S_{6r} and S_{5r}) and Another

Internal coord.[a]	Force const.[c]	Classification
1- 2.	0.852	S_{6r}–S_{6r}
1- 3.	−0.455	
1- 5.	−0.376	
1- 6.	0.535	
2- 3.	0.328	
2- 4.	−0.352	
2- 5.	0.337	
2- 6.	−0.199	
3- 4.	0.650	
3- 5.	−0.236	
3- 6.	0.204	
4- 5.	0.524	
4- 6.	−0.236	
5- 6.	0.415	
1-14.	0.202	S_{6r}–S_{5r}
1-15.	−0.112	
2-12.	−0.145	
2-13.	0.144	
2-14.	−0.169	
3-13.	−0.231	
3-15.	0.470	
5-12.	0.367	
5-13.	−0.176	
5-15.	−0.137	
6-13.	0.117	
4-12.	0.804	S_{5r}–S_{5r}
4-13.	−0.230	
4-14.	−0.292	
4-15.	0.826	
12-13.	0.476	
12-14.	−0.305	
12-15.	−0.317	
13-14.	0.976	
13-15.	0.182	
14-15.	0.378	

a) See Fig. 1. c) Unit: mdyn/Å, where Å=0.1 nm.

C. Off-Diagonal Elements between Ring-Stretching and Ring-Deformation Coordinates

Internal coord.[a]	Force const.[d]	Classification
1-19.	−0.298	Δ_r–S_{6r}
1-21.	0.116	
2-18.	0.441	
2-19.	0.704	
2-20.	−0.138	
3-18.	0.411	
3-19.	0.610	
3-20.	0.204	
3-21.	−0.136	
3-22.	0.314	
4-20.	−0.186	
4-21.	0.403	
5-20.	−0.229	
5-21.	−0.091	
5-22.	−0.196	
6-19.	0.142	
6-20.	0.230	
6-22.	−0.193	

12-18.	−0.223	Δ_r–S_{5r}
12-19.	0.175	
12-20.	0.190	
12-21.	−0.821	
12-22.	0.164	
13-19.	−0.173	
13-22.	−0.812	
14-19.	−0.162	
14-21.	0.267	
14-22.	0.525	
15-18.	0.248	
15-19.	0.155	
15-20.	−0.284	
15-21.	−0.567	
15-22.	−0.306	

a) See Fig. 1. d) Unit: mdyn.

D. Off-Diagonal Elements between the Ring-Stretching and Peripheral-Stretching (or Deformation) Coordinates

Internal coord.[a]	Force const.[e]	Classification
1- 9.	0.472	S_{6r}–S_{6p}, or Δ_p
1-23.	0.119	
1-24.	−0.205	
1-25.	0.477	
2- 8.	−0.098	
2- 9.	0.937	
2-23.	−0.048	
2-25.	−0.441	
2-27.	0.054	
3- 9.	−0.169	
3-23.	0.054	
3-24.	0.055	
3-28.	0.032	
4- 7.	−0.161	
4-23.	−0.085	
4-25.	−0.032	
4-28.	0.061	
5- 7.	0.632	
5- 9.	−0.168	
5-23.	0.225	
6- 7.	1.074	
6-23.	−0.489	
6-25.	−0.087	
14- 9.	0.090	S_{5r}–S_{5p}, or Δ_p
12-23.	−0.056	
13-23.	0.046	
13-29.	0.157	
14-28.	0.041	
14-29.	−0.190	
15-23.	0.037	
15-28.	−0.051	
15-29.	0.060	

a) See Fig. 1. e) Units: mdyn/Å for S–S's and mdyn for S–Δ's (Å=0.1 nm).

E. Some Other Off-Diagonal Elements

Internal coord.[a]	Force const.[f]	Classification
7-18.	0.258	S_{6r}–Δ_r, or Δ_p
7-19.	−0.362	
7-23.	−0.111	
7-25.	0.033	
8-18.	−0.093	
8-25.	−0.057	
9-18.	0.187	
9-19.	0.133	
9-20.	0.271	
9-26.	−0.188	
9-27.	0.048	
10-11.	−0.184	
10-25.	−0.076	
10-26.	0.050	
10-27.	0.059	
11-25.	0.044	
11-27.	−0.072	
16-21.	−0.108	S_{5r}–Δ_r
18-22.	0.481	
18-23.	−0.081	Δ_r–Δ_r, or Δ_p
18-29.	−0.031	
19-21.	−0.242	
19-23.	0.124	
19-24.	0.118	
19-25.	−0.082	
19-27.	0.032	
20-22.	−0.441	
20-23.	−0.165	
20-24.	0.080	
20-25.	0.078	
21-22.	−0.106	
21-28.	0.048	
22-25.	−0.045	
22-28.	0.101	
22-29.	−0.088	
23-24.	0.070	
23-25.	−0.046	
23-27.	0.011	
24-25.	−0.012	
24-27.	−0.012	
25-26.	−0.044	
25-27.	−0.180	
26-27.	−0.012	

a) See Fig. 1. f) Units: mdyn for S–Δ's, and mdyn.Å for Δ–Δ's(Å=0.1 nm).

positive, those for the "meta" positions are negative, and those for the "para" positions are positive. Their absolute values are all greater than 0.2 mdyn/Å except for the off-diagonal element between the N_1C_2 stretching and the C_4C_5 stretching coordinates, which is nearly zero. The anomalously small value of this element is considered to be caused by a perturbation by the five-membered ring system, which shares the C_4C_5 bond with the six-membered ring. The off-diagonal elements on the five-membered ring system are nearly equal to those of imidazole molecule (the data are not shown here)[19] except for those involving the C_4C_5 stretching coordinate. It is rather surprising that some of the off-diagonal elements of the stretching coordinates of two bonds located far apart from each other, such as r_1 & r_{14} and r_3 & r_{13}, are as great as 0.2 mdyne/Å as shown in Table 2-B. We could never predict such effectual values of them without the present *ab initio* MO calculation. Also in the calculation, many non-zero off-diagonal elements between the ring stretching and the ring deformation coordinates are found as shown in Table 2-C. The off-diagonal elements among the ring vibrations and the peripheral vibrations are given in Table 2-D and Table 2-E.

Vibrational Frequencies. For the uracil molecule, we showed that the calculated vibrational frequencies using the corrected force constants ("4-31G"force constants) are almost uniformly greater by 8% than the observed fundamental frequencies. Therefore, in our present study on guanine we scaled down the vibrational frequencies by the factor of 0.92. The calculated vibrational frequencies, thus scaled, and the calculated modes are given in Table 3. The calculated frequencies of deuterated guanines are shown in Table 4. In parentheses in these tables, calculated ^{15}N isotope shifts are also given: The first numerical value here indicates the shift (cm^{-1}) for the product in which N_1, N_3, and amino-*N* are substituted by ^{15}N's, namely for pyrimidinic substitution, and the second one the shift for the product, in which N_7 and N_9 are substituted by ^{15}N's, namely for imidazolic substitution.

The largest isotope shift for the pyrimidinic substitution is calculated to be -24 cm^{-1} for ν_{21} (at 1026 cm^{-1}), and this corresponds to the observed isotope shift (-26 cm^{-1}) for the 1052 cm^{-1} Raman line. For imidazolic substitution, the largest shift is calculated to be -21 cm^{-1} for ν_{22} (985 cm^{-1}), and this is actually observed (-20 cm^{-1}) for the 950 cm^{-1} line.

In this way, it was attempted to correlate every calculated frequency with an observed frequency by taking an agreement in the isotope effect into account. This attempt was mostly successful except for those in the frequency range higher than 2000 cm^{-1}. The difficulty in the higher frequency region is probably caused by the hydrogen bonds. In our calculation, no hydrogen bonding effect was taken into account, whereas the observed frequencies are taken from the solid state guanine whose N_1H and N_9H groups are involved in intermolecular NH···N hydrogen bonds.

The difficulty due to the hydrogen bonds seems to extend into the NH in-plane bending range (1350—1420 cm^{-1}) in a less prominent manner. The amount of couplings of the N_1–H bending modes with some skeletal stretching modes may be sensitive to the diagonal and off-diagonal force constants involving these NH bending coordinates. Therefore only a slightly improper prediction of the force constants may fail in explaining observed ^{15}N-shifts (for 1420 cm^{-1} band, for example). In this frequency region, therefore the calculated vibrational modes should be taken with some reservation, even if the vibrational frequencies predicted for ν_{13}, ν_{14}, and ν_{15} are in good agreement with the observed ones (Table 3).

For the whole other spectral range, however, our present calculation has provided a very good set of vibrational frequencies, which fits the experimental results in a nealy perfect manner.

Vibrational Modes. *I. 4000—2000 cm^{-1}*: In this frequency region, special care must be taken in correlating the calculated and observed vibrations, because many of the infrared and Raman bands here are under severe perturbation by hydrogen bonds. Our present calculation shows that the vibration at 3686 cm^{-1} is the amino antisymmetric stretching and that at 3496 cm^{-1} is the amino symmetric stretching.

In the infrared spectrum on the other hand, 2′,3′-benzylidene-5′-tritylguanosine dissolved in deuteriochloroform shows two bands due to the molecule free from any hydrogen bond at 3515 and 3407 cm^{-1}.[20] These two may correspond to the above two. In that solution, however, other bands due to some guanine-guanine association complexes are also found at 3480, 3355, 3305, and 3230 cm^{-1}. The vibrational modes of the hydrogen-bonded NH_2 and those of NH are not the subject of our present study.

In the case of C_8–H stretching vibration, the situation is simpler. The calculation shows that the vibration at 3132 cm^{-1} is the C_8–H stretching and that at 2338 cm^{-1} is the C_8–D stretching. On the other hand, a strong Raman line is observed of 5′GMP (guanosine 5′-monophosphate) in D_2O solution at 3125 cm^{-1}, and of the C_8-deuterated derivative in H_2O solution at 2325 cm^{-1}.[21] The hydrogen bondings at the N-H groups do not seem to affect the C_8–H vibration in the same molecule.

II. 2000—1100 cm^{-1}: A strong infrared band and a medium intensity Raman line around 1700 cm^{-1} of guanine and guanine derivatives have been assigned to the C=O stretching vibration by a number of researchers, and this mode is reproduced in our calculation (ν_6 in Table 3).

In the Raman spectra of guanine, deuterated guanines, and 5′GMP in H_2O and D_2O solutions in the 1620—1500 cm^{-1} region, there are always three Raman lines, at 1610, 1580, and 1530 cm^{-1}.[22—24] These Raman lines are considered to correspond to the ring stretching vibrations, ν_8, ν_9, and ν_{10}, whose vibrational modes are shown in Table 3 as well as in Fig. 2. The calculated vibration ν_7 is the NH_2 scissoring, but this has never been identified in the Raman spectra of guanine and guanine derivatives. The calculated vibrations, ν_9 and ν_{11}, must correspond to the observed Raman lines at 1580 cm^{-1} and 1480 cm^{-1} of 5′GMP in H_2O solution, respectively. Those two Raman scatterings show marked resonance effect with the absorption bands at 278 and/or 253 nm.[25] In these excited states, the geometry of the guanine molecule is supposed to be deformed along these two normal coordinates which are shown in Fig. 2 (ν_9 and ν_{11}).

The Raman line at 1480 cm^{-1} shows a large frequency shift as great as -23 cm^{-1} upon the C_8–H deuteration,[21] and this was used as a monitor line of the C_8–H deuterium exchange kinetics.[21] In our calculation, this large shifts is well reproduced (-27 cm^{-1}) as shown in Table 5 ($k=11$). This 1480 cm^{-1} Raman line also shows a marked frequency shift and/or intensity change on the coordination at N_7 of heavy metal ions and on N_7 protonation.[22,24,25] This is also understandable because the calculated normal modes (as shown in Fig. 2) involves a big movement of the N_7 atom, and the

TABLE 3. VIBRATIONAL FREQUENCIES (cm^{-1}) OF THE GUANINE MOLECULE (CALCULATED NORMAL FREQUENCIES ω AND OBSERVED FUNDAMENTAL FREQUENCIES ν)

Numbering of nomal coord (k)	Calcd ω (isotope shifts)[a]	Vibrational modes[b]	Obsd ν[c] (isotope shifts) IR	Raman
1	3686(−12, 0)	NH_2 anti sym. str(97)	3515[d]	
2	3554(0, −9)	N_9H str(99)		
3	3522(−8, 0)	N_1H str(100)		
4	3496(−5, 0)	NH_2 sym. str(100)	3407[d]	
5	3132(0, 0)	C_8H str(98)		3125[e]
6	1802(−2, 0)	C_6O str(71), C_5C_6 str(17)	1702(−6, 0)	1678(−2,)
7	1661(−10, −1)	NH_2 sci(37), C_2NH_2 str(28)	1675(−9, 0)	
8	1630(−6, −8)	ring str(75)	1638(−7, 0)	1604(−4,)
9	1601(−7, 0)	NH_2 sci(44), ring str(34)	1565(−5,)	1552(−3, −5)
10	1581(−6, −1)	ring str(56)	1550(0,)	1535(,)
11	1526(−4, −10)	ring str(81), C_8H bend(22)	1477(−3, −2)	1480(2,)
12	1464(−4, −6)	ring str(85)	1464(−2, −9)	1468(−4, −8)
13	1415(−2, −15)	ring str(67), N_9H bend(22)	1420(−5, −2)	1422(−5, −2)
14	1350(−1, −3)	ring str(55), N_1H bend(13)	1375(−4, −3)	1392(−4, −4)
15	1343(−8, −2)	N_1H bend(37), ring str(27)	1362(−6, −4)	1361(−6, −4)
16	1273(−5, −14)	ring str(76), C_8H bend(13)	1261(−1, −10)	1266(0, −14)
17	1174(−3, −4)	ring str(38), C_8H bend(32)	1174(−4, −7)	1188(−4, −8)
18	1146(−12, −3)	ring str(78), CO bend(13)	1216(−8, 0)	1232(−12, −5)
19	1102(−6, −3)	ring str(36), NH_2 rock(28)	1150(−12, −1)	1161(−11,)
20	1079(−7, −5)	ring str(30), NH_2 rock(25)	1118(−7, −3)	
21	1026(−24, −1)	ring str(51), ring def(13)	1042(−23, 0)	1052(−26,)
22	985(0, −21)	ring def(81)	950(−1, −20)	940(−2, −19)
23	833(−17, −7)	ring def(62), ring str(33)	850(−15, −4)	850(−17,)
24	661(−8, 0)	CO bend(27), ring def(28)	703(−6, 1)	712(−4,)
25	619(−6, −2)	ring breathing (50)	645(−5, −3)	651(−5, −3)
26	533(−5, −4)	ring def(55)	557(−6, −2)	568(−10,)
27	481(−6, −3)	ring def(69)	501(1, 1)	496(−3, −4)
28	332(−4, 0)	C_2NH_2 bend(49), CO bend(23)	404(−5, 1)	400(−6, −1)
29	311(−2, −2)	CO bend(20), ring def(31)	348(−1, −2)	343(0,)

a) The first numerical value (cm^{-1}) in each pair of parentheses indicates the shift for the $^{15}N_3$ substitution at positions 1, 3 and amino, and the second one for the $^{15}N_2$ substitution at positions 7 and 9. b) Potential energy distribution (%) calculated is given in parentheses. c) Ref. 13. d) Ref. 20. e) Ref. 21.

TABLE 4. CALCULATED NORMAL FRQUENCIES AND ISOTOPE SHIFTS OF GUANINE DEUTERATED DERIVATIVES

	Guanine	Guanine-d_1	Guanine-d_4	Guanine-d_5
1	3686(−12, 0)[a]	3686(−12, 0)	3132(0, 0)[b]	2736(−17, 0)
2	3554(0, −9)	3554(0, −9)	2736(−17, 0)	2622(0, −15)
3	3522(−8, 0)	3522(−9, 0)	2622(0, −15)	2588(−13, 0)
4	3496(−5, 0)	3496(−5, 0)	2588(−13, 0)	2530(−8, 0)
5	3132(0, 0)[b]	2338(0, 0)[b]	2530(−8, 0)	2337(0, 0)[b]
6	1802(−2, 0)	1802(−2, 0)	1794(−1, 0)	1794(−1, 0)
7	1661(−10, −1)[c]	1660(−11, −1)[c]	1619(−3, −6)	1617(−3, −6)
8	1630(−6, −8)	1627(−6, −8)	1594(−13, −1)	1594(−14, −1)
9	1601(−7, 0)	1601(−7, 0)	1569(−6, −1)	1566(−7, −1)
10	1581(−6, −1)	1578(−7, −1)	1515(−3, −10)	1486(−3, −11)
11	1526(−4, −10)	1499(−4, −9)	1449(−6, −5)	1448(−6, −7)
12	1464(−4, −6)	1461(−3, −10)	1385(−2, −15)	1371(−2, −14)
13	1415(−2, −15)	1394(−1, −15)	1327(−1, −9)	1286(−2, −11)
14	1350(−1, −3)	1345(−6, 0)	1279(−14, −6)	1274(−17, −5)
15	1343(−8, −2)	1327(−5, −5)	1233(−12, −8)	1220(−10, −9)
16	1273(−5, −14)	1248(−7, −14)	1166(−8, −2)[c]	1159(−9, −1)[c]
17	1174(−3, −4)[d]	1145(−11, −2)	1146(−4, −4)[d]	1066(−9, −2)
18	1146(−12, −3)	1120(−1, −7)	1063(−8, −2)	980(−6, −1)
19	1102(−6, −3)	1088(−12, −1)	980(−1, −8)	974(−4, −23)
20	1079(−7, −5)	1026(−24, −1)	971(−14, −16)	930(−7, −4)[d]
21	1026(−24, −1)	983(−1, −22)	873(−13, −2)	872(−13, −1)
22	985(0, −21)	885(−5, −4)[d]	852(−2, −1)	813(−4, 0)
23	833(−17, −7)	814(−13, −5)	788(−11, −5)	772(−8, −4)
24	661(−8, 0)	660(−9, −1)	613(−6, −1)	613(−6, −1)
25	619(−6, −2)	616(−6, −2)	603(−6, −1)	603(−6, −1)
26	533(−5, −4)	529(−5, −4)	508(−3, −3)	506(−3, −3)
27	481(−6, −3)	479(−6, −3)	469(−5, −3)	468(−6, −3)
28	332(−4, 0)	332(−4, 0)	317(−1, −1)	314(−1, −1)
29	311(−2, −2)	305(−2, −2)	289(−3, −1)	286(−3, −1)

a) In each pair of parentheses, the first numerical value indicates the shift for the $^{15}N_3$ sunstitution at positions 1, 3, and amino, and the second one for the $^{15}N_2$ substitution at positions 7 and 9. b) C_8H or C_8D stretching vibration. c) NH_2 or ND_2 scissoring vibration. d) C_8H or C_8D bending vibration.

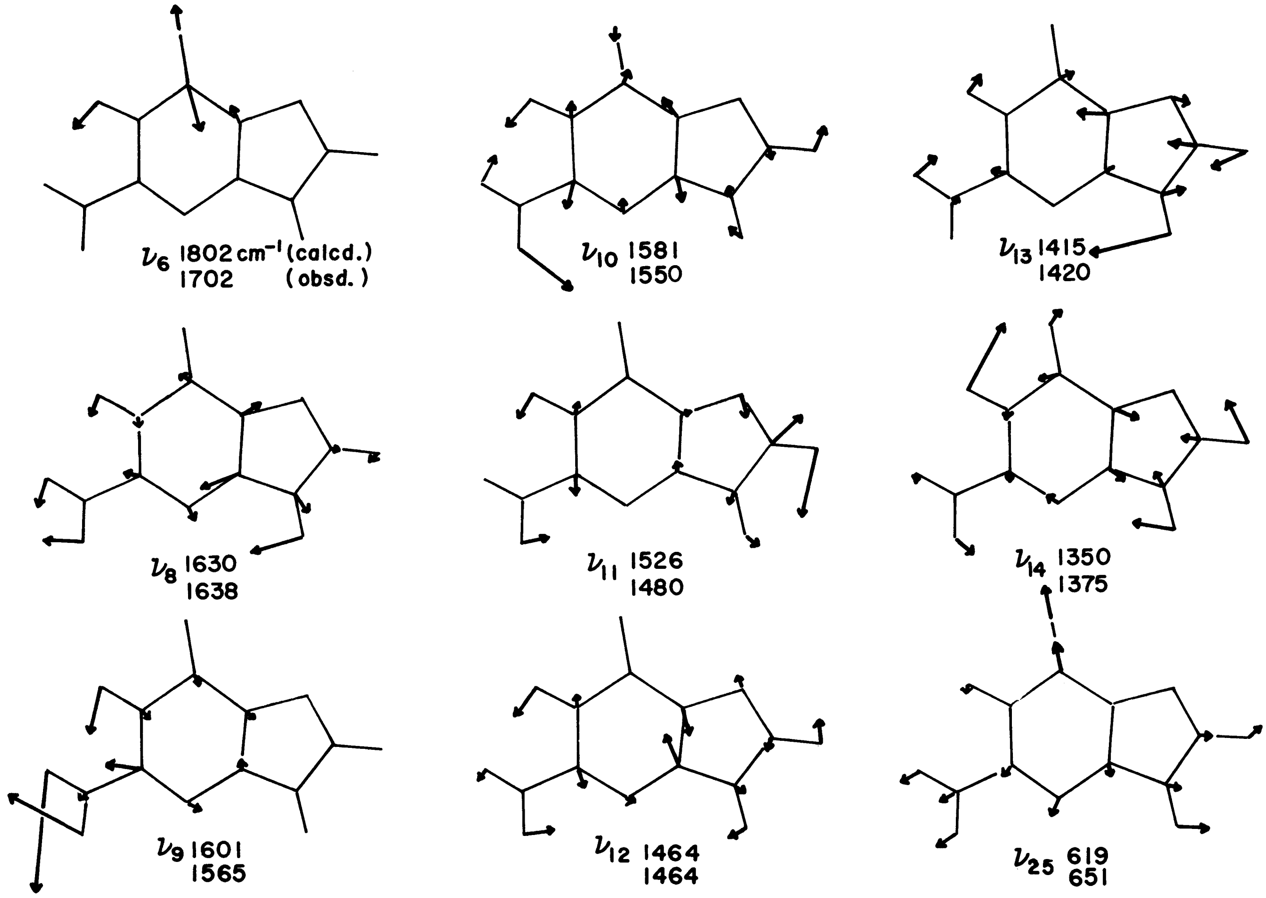

Fig. 2. Normal modes of vibration, calculated with the "4-31G force constants" which were reached by a correction from the STO-3G force constants of guanine. Calculated normal frequency (upper numerical value) and observed fundamental frequency (lower) are also given in cm^{-1} for each mode.

Table 5. Calculated and observed frequencies (in cm^{-1}) of some of the guanine vibrations with the isotope shifts due to the C_8-deuteration

Numbering of normal coord. (k)	Calcd	Obsd (5′GMP)[a]
6	1802 (0)[b]	1680 (−5)
7	1661 (−1)	—
8	1630 (−3)	1605 (+3)
9	1601 (0)	1576 (0)
10	1581 (−3)	1538 (−11)
11	1526 (−27)	1487 (−23)
12	1464 (−3)	—
13	1415 (−21)	1415 (−7)
14	1350 (−5)	1365 (−8)
15	1343 (−16)	1324 (−19)
25	619 (−3)	671 (−10)
26	533 (−4)	586 (−2)
27	481 (−2)	501 (−1)

a) From Ref. 21. b) The amount of shift (in cm^{-1}) by the C_8-deuteration is given in each pair of parentheses.

calculated potential energy distribution of C_8N_7 stretching in this vibration is as high as 42%. Therefore, this Raman scattering is naturally sensitive to a perturbation at N_7.

As has already been mentioned, the contribution of the N_1H, N_9H, and NH_2 bending modes in the vibrations in the 1450—1100 cm^{-1} region are not clearly predicted by the present calculation. However, the contribution of the C_8–H deformation mode has been satisfactorily predicted. The calculated vibrations at 1174 cm^{-1} for guanine, at 885 cm^{-1} for guanine-d_1, at 1146 cm^{-1} for guanine-d_4, and at 930 cm^{-1} for guanine-d_5 should involve a great amount of C_8–H or C_8–D in-plane bending mode (see Table 4). These vibrations are correlated with observed IR bands at 1174 cm^{-1}, 926 cm^{-1}, 1168 cm^{-1}, and 897 cm^{-1}, respectively.[13] Thus, the C_8-deuteration effect in *N*-deuterated guanine as well as undeuterated guanine spectra have been clearly reproduced and explained by our calculation.

III. Vibrations with Frequencies below 1100 cm^{-1}: In this frequency region, no complexity coming from the NH bending modes is involved any more. The calculated frequencies and their ^{15}N isotope effects of the ν_{20}, ν_{21}, ν_{22}, ν_{23}, ν_{24}, ν_{25}, and ν_{26} vibrations can be clearly correlated with what were actually observed. The calculated vibrational modes given in Table 3 are considered to be quite useful.

In particular, the ring breathing vibration should be mentioned. Raman spectroscopic studies of nucleic acids have so far showed that a strong and sharp Raman lines at 780, 790, and 725 cm^{-1} of uracil, cytosine, and adenine, respectively, are assignable to the ring breathing vibrations.[1,22–24] For guanine residues, in 5′GMP for example, no strong Raman line has been found in the frequency region higher than 700 cm^{-1}; instead a much broader and weaker line was observed around 680 cm^{-1}.[22,25] This line has been considered to correspond to some kind of ring breathing vibration perturbed by some ring deformations.[23] However, this Raman line at 650 cm^{-1} is strong enough in the spectrum of solid state guanine.[13] Our present calculation has now predicted that this line is nearly pure ring breathing vibration. Our recent study on guanosine residues indicates that this ring breathing frequency is appreciably affected by the sugar conformation and by the torsion angle around the glycosidic bond connecting the guanine and ribose moieties.[2,3] The broadness and weakness of the 670 cm^{-1} line of 5′GMP in H_2O, therefore, would mean that various conformations are involved in the solution whose frequencies are slightly different from one another.[27] Such a flexibility of the 5′GMP conformation in solution would be removed in the solid state or in a regular-form polynucleotide. On the basis of this argument, a strong, sharp Raman line should be observed if the conformation of 5′GMP is fixed. This is what is actually found as has been detailed elsewhere.[2,3,27]

As shown in Fig. 2, the calculated vibrational mode (ν_{25}) involves a big movement of the N_9 atom and the position-9 hydrogen atom. In the guanosine molecule, this hydrogen is replaced by the sugar, so that the ring breathing vibration should cause a coupling with a vibration in the sugar part. This is a qualitative explanation of the observed dependence of the ring breathing frequency upon the ribose conformation. More detailed description as for how it depends is given in a previous article.[3]

This work was supported by a grant (No. 57060004) from the Ministry of Education, Science, and Culture of Japan. The numerical calculations were carried out at the Computer Center of IMS.

References

1) Y. Nishimura, M. Tsuboi, S. Kato, and K. Morokuma *J. Am. Chem. Soc.*, **103**, 1354 (1981).

2) Y. Nishimura, M. Tsuboi, T. Nakano, S. Higuchi, T. Sato, T. Shida, S. Uesugi, E. Ohtsuka, and M. Ikehara, *Nucleic Acids Res.*, **11**, 1579 (1983).

3) Y. Nishimura, M. Tsuboi, and T. Sato, *Nucleic Acids Res.*, **12**, 6901 (1984).

4) E. W. Small and W. L. Peticolas, *Biopolymers*, **10**, 1377 (1971).

5) S. Mansy and W. L. Peticolas, *Biochemistry*, **12**, 2650 (1976).

6) L. Lafleur, J. Rice, and G. J. Thomas, Jr., *Biopolymers*, **11**, 2423 (1972).

7) B. Prescoff, R. Gamache, J. LIvermento, and G. J. Thomas, Jr., *Biopolymers*, **13**, 1821 (1974).

8) F. M. Pohl, A. Ranade, and M. Stockburger, *Biochim. Biophys. Acta*, **335**, 85 (1973).

9) J. Rice, L. Lafleur, G. C. Medeiros, and G. J. Thomas, Jr., *J. Raman Spectrosc.*, **1**, 207 (1973).

10) R. Savoie, H. Klump, and W. L. Peticolas, *Biopolymers*, **17**, 1335 (1978).

11) S. Brahms, J. Vergne, J. G. Brahms, E. D. Gupta, Ph. Bucher, and Th. Koller, *J. Mol. Biol.* **162**, 473 (1982).

12) T. J. Thaman, R. Lord, H. J. Wang, and A. Rich, *Nucleic Acids Res.*, **9**, 5443 (1981).

13) J.-M. Delabar and M. Majoube, *Spectrochim. Acta*, **34A**, 129 (1978).

14) K. Morokuma, S. Kato, K. Kitaura, I. Ohmine, S. Sakai, and S. Obara, IMS Computer Center Library Program, No. 0372 (1980).

15) M. Aida and C. Nagata, to be published.

16) P. Pulay, G. Fogarasi, F. Pang, and J. E. Boggs, *J. Am. Chem. Soc.*, **101**, 2550 (1979).

17) T. Shimanouchi, Computer Programs for Normal Coordinate Treatment of Polyatomic Molecules, Department of Chemistry, University of Tokyo.

18) R. Taylor and O. Kennard, J. Mol. Struct., **78**, 1 (1982).

19) Y. Nishimura, M. Tsuboi, S. Kato, and K. Morokuma, to be published.

20) Y. Kyogoku, R. C. Lord, and A. Rich, *Science* **154**, 518 (1966).

21) M. J. Lane and G. J. Thomas,Jr., *Biochemistry*, **18**, 3839 (1979).

22) R. C. Lord and G. J. Thomas,Jr., *Spectrochim. Acta*, **23A**, 2551 (1967).

23) M. Tsuboi, S. Takahashi, and I. Harada, "Physico-Chemical Properties of Nucleic Acids," ed by J. Duchesne, Academic Press, London, New York, 1973, Vol. 2, p. 91.

24) K. A. Hartman, R. C. Lord, and G. J. Thomas,Jr., "Physico-Chemical Properties of Nucleic Acids," ed by J. Duchesne, Academic Press, Lomdon, New York, 1973, Vol. 2, p. 1.

25) Y. Nishimura, A. Y. Hirakawa, and M. Tsuboi, "Advances in Infrared and Raman Spectroscopy," Vol. 5, R. J. H. Clark and R. E. Hester, Eds., Heyden, London, 1978, Chap. 4.

26) G. Y. H. Chu, S. Mansy, R. E. Duncan, and T. S. Tobias, *J. Am. Chem. Soc.*, **100**, 593 (1978).

27) Y. Nishimura, I. Sakuma, and M. Tsuboi, *Nucleic Acids Ras.*, Symp. Ser. **12**, 177 (1983).