On the Normal Modes of Vibration in the Uracil Residue — The Use of ^{15}N-Isotope Effects

Yoshifumi NISHIMURA, Hideyuki HARUYAMA, Kimiko NOMURA, Akiko Y. HIRAKAWA, and Masamichi TSUBOI*

Faculty of Pharmaceutical Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113

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β-Uridine-5′-phosphoric acid-$^{15}N_2$ has been prepared from ^{15}N-RNA of baker's yeast which was grown in $(^{15}NH_4)_2SO_4$ as the sole nitrogen source. Its Raman spectrum has been observed both in ^1H_2O and ^2H_2O solutions. On the basis of the observed ^{15}N isotope effects on the Raman spectra and on the basis of other data previously obtained, normal modes of vibration have been discussed for six Raman bands of the uracil residue that are considered to be in resonance with its electronic transition at 260 nm. The result has led to a conclusion that $C^5=C^6$ is longer and C^4–C^5 is shorter in the excited electronic state $\tilde{A}$ (260 nm) than those in the ground electronic state $\tilde{X}$.

Resonance Raman spectroscopy may sometimes be useful as a tool for exploring the excited state geometry of a molecule.[1,2] Let us suppose that our present aim is to ellucidate the geometrical structure of the electronic excited state of the uracil residue corresponding to its 260 nm absorption band. For this aim we first need to examine which Raman lines of the uracil residue are in resonance with the 260 nm electronic transition ($\tilde{A}$-$\tilde{X}$),[3] and secondly we need to find the normal modes of vibration that are to be assigned to these particular Raman lines. We can then predict that, on going from the ground ($\tilde{X}$) to the excited ($\tilde{A}$) state, a distortion of the molecular conformation takes place along such a normal coordinate or along a linear combination of such normal coordinates. This prediction is based upon a theory which is valid only in a certain approximation.[1,2] In combination with other data, however, this approach often leads to a useful piece of information on the excited state geometry.

In our present study we have used a set of ^{15}N isotope shifts in the vibrational frequencies of the uracil residue, as a data for fixing normal modes, in addition to the usual data obtained by an examination of deuterated products. In our normal coordinate treatment, it has been found that a fixing of the force constant values corresponding to interactions of two bond-stretching motions in the six-membered ring is critical. In this respect, a recent work on the force field in benzene[5] was found to be helpful. On the basis of these data, the normal modes of vibration of the six Raman bands, which were previously found[3,4] to be in resonance with the electronic transition at 260 nm, have now been determined with a considerable reliability.

Experimental

Preparation of ^{15}N RNA. Baker's yeast was grown in a medium in which $(^{15}NH_4)_2SO_4$ is the sole nitrogen source. This part of our experiment was made in collabotation with Dr. Masatsune Kainosho in the Central Research Laboratory, Ajinomoto Company, Ltd. 400 g of wet cells of such yeast were placed in 1 litre of 0.0125 M phosphate buffer (pH 6.8) with 2% sodium dodecyl sulfate and 4.5% ethanol. The suspension was incubated at 95 °C for 5 min, rapidly cooled with dry ice plus methyl cellosolve, and then centrifuged at 4000 rpm for 5 min. From the supernatant, crude RNA was precipitated by adding 2 litre ethanol. The precipitate was washed with 70% ethanol and then with 80% ethanol overnight. The precipitate was dissolved again in 500 ml of 5 mM phosphate buffer, and centrifuged at 4000 rpm for 5 min. RNA was precipitated from the supernatant with 1 M NaCl plus ethanol. This was dissolved in 500 ml of 2.5 mM phosphate buffer and dialyzed against 1 mM phosphate buffer. By an ethanol precipitation, 3.3 g purified ^{15}N RNA was obtained.

Preparation of β-Uridine-5′-phosphoric acid-$^{15}N_2$ (5′-UMP-$^{15}N_2$). ^{15}N-RNA was dissolved in phosphate buffer (pH 5.2) and incubated with nuclease P_1 (kindly provided by Dr. A. Kuninaka, The Research Laboratory of Yamasa Shoyu Co., Ltd., Chyoshi Japan) at 37 °C overnight. After the pH of the digested solution was adjusted to 7.2, the solution was subjected to a Dowex 1X2 column chromatography, where the formic acid form of 200—400 mesh was placed in a 20×1000 mm tube. For elusion, HCOOH gradient (from 0 to 3 M) was used, and 5′CMP, 5′AMP, 5′GMP, and finally 5′UMP came out. 5′UMP-$^{15}N_2$ was recrystallized with ethanol and water. This gave only one spot in a thin-layer chromatography. Its isotopic purity was judged from the isotopic purities determined of 5′AMP-$^{15}N_5$ and 5′-CMP-$^{15}N_3$, prepared from the same batch of ^{15}N-RNA with that for 5′-UMP-$^{15}N_2$. The isotopic purity of each of these two nucleotides was examined by mass-spectrometry of ammonia produced by a micro-Kjeldahl procedure from the nucleotide sample. The ^{15}N content was found to be 89% of the total nitrogen both for 5′-AMP and for 5′-CMP. Because the biosynthesis process is similar for cytosine residue to that for uracil residue, the ^{15}N content of our 5′UMP is considered to be also 89% of its total nitrogen.

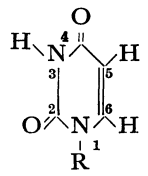
Raman Spectroscopic Measurement. Raman spectra of 5′-UMP-$^{14}N_2$ and 5′-UMP-$^{15}N_2$ were examined in neutral ^1H_2O and ^2H_2O solutions. The measurements were made by the use of a JEOL (Japan Electron Optics Laboratory Co.) JRS-Ul spectrophotometer with 514.5 nm line of a Coherent Radiation model 52 GA argon ion laser. The ^{15}N isotope shift was examined for each Raman line by repeating a short-range scan for 5′-UMP-$^{14}N_2$ and for 5′-UMP-$^{15}N_2$ alternately many times by keeping the conditions of the instrument as steady as possible.

Results and Discussions

^{15}N Isotope Effects on the Raman Spectra. In Figs. 1 and 2 are given the observed Raman spectra, observed Raman frequencies (in cm^{-1}), and the amounts of ^{15}N-shifts determined for 5′-UMP, both in ^1H_2O and in ^2H_2O (pH 7.2). In

TABLE 1. THE SET OF FORCE CONSTANTS FOR THE URACIL RESIDUE (md·A^{-1} for K, md·A for H, md·A^{-1} for stretch/stretch interaction constants and md for bend/stretch interaction constants)

1. K(N-C) 6.380; 2. K(C-C) 6.202; 3. K(C=C) 8.900; 4. K(N-R) 5.000;
5. K(C_2=O_8) 11.000; 6. K(N-H) 5.397; 7. K(C_4=O_{10}) 10.800; 8. K(C-H) 5.204;
9. H($C_6N_1C_2$) 1.163; 10. H($C_2N_3C_4$) 1.168; 11. H($N_1C_2N_3$) 1.587;
12. H($N_3C_4C_5$) 1.654; 13. H($C_4C_5C_6$) 0.522; 14. H($C_5C_6N_1$) 0.528;
15. H(RN_1C_2) 1.060; 16. H(RN_1C_6) 1.056; 17. H(OC_2N_3) 1.019;
18. H(OC_2N_1) 1.019; 19. H(OC_4C_5) 1.071; 20. H(OC_4N_3) 1.028;
21. H(HN_3C_4) 0.459; 22. H(HN_3C_2) 0.459; 23. H(HC_5C_6) 0.344;
24. H(HC_5C_4) 0.371; 25. H(HC_6N_1) 0.352; 26. H(HC_6C_5) 0.342;



27. f(ν_{ring}, ν_{ring})$_{ortho}$ 0.807; 28. f(ν_{ring}, ν_{ring})$_{meta}$ −0.522; 29. f(ν_{ring}, ν_{ring})$_{para}$ 0.161;
30. f($\nu_{C=O}$, ν_{ring}) 1.207; 31. f($\nu_{C_4=O}$, $\nu_{C=C}$) −0.187; 32. f(δ_{ring}, ν_{ring}) 0.401; 33. f($\delta_{C=O}$, ν_{ring}) 0.279;
34. f(δ_{N-H}, ν_{ring}) 0.072; 35. f(δ_{C-H}, ν_{ring}) 0.197; 36. f($\delta_{C=O}$, $\nu_{C=O}$) 0.980; 37. f(ν_{N-R}, ν_{ring}) 0.471.

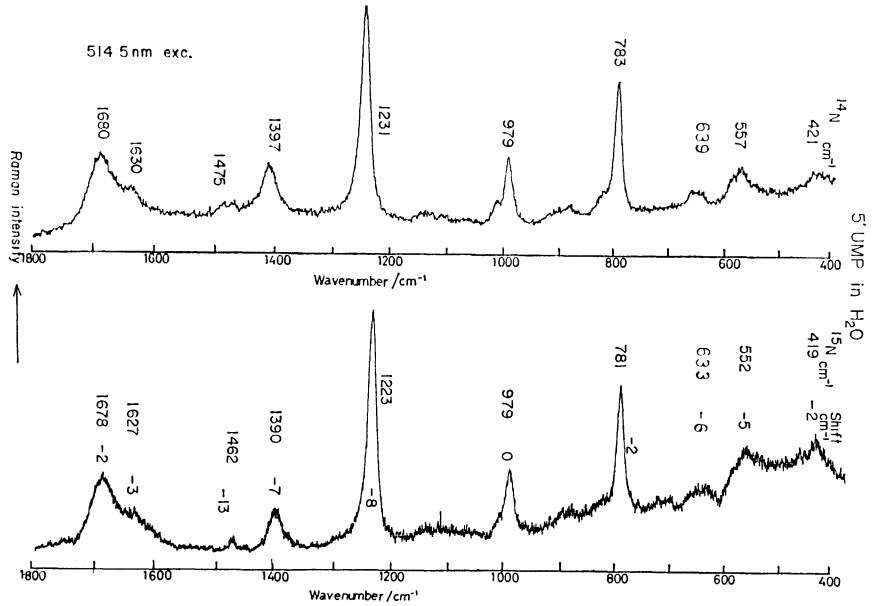


Fig. 1. Raman spectra of β-uridine-5′-phosphoric acid (5′-UMP-$^{14}N_2$ and 5′-UMP-$^{15}N_2$) in ^1H_2O, pH 7.2 at room temperature, concentation 10%. Excited by 514.5 nm line of an Ar^+ laser.

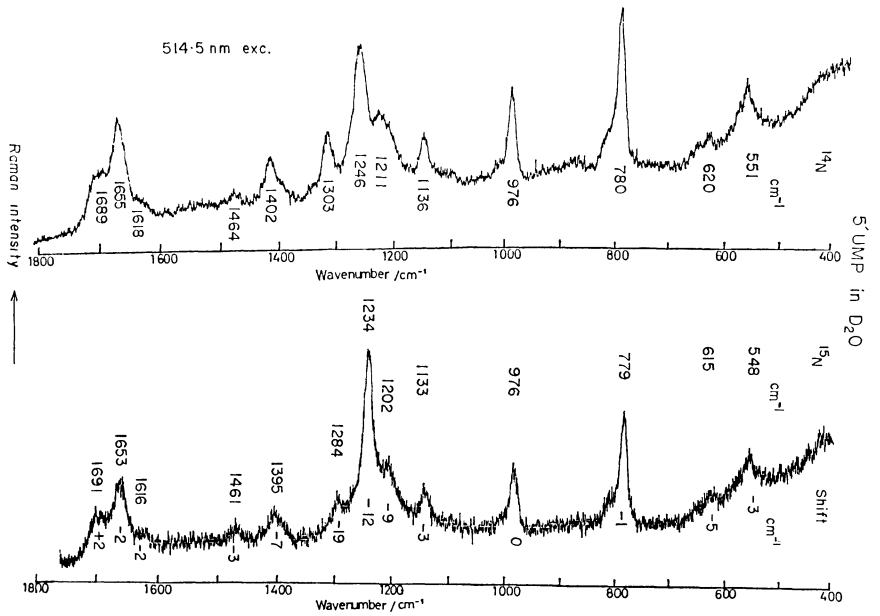


Fig. 2. Raman spectra of β-uridine-5′-phosphoric acid (5′-UMP-$^{14}N_2$ and 5′-UMP-$^{15}N_2$) in ^2H_2O, pH 7.2 at room temperature, concentration 10%. Excited by 514.5 nm line of an Ar^+ laser.

^2H_2O, the NH group at position 3 is deuterated (ND) (for numbering of the positions, see Table 1). As may be seen in Figs. 1 and 2, most of the Raman lines assignable to the uracil residue show appreciable ^{15}N-shifts, while the line at 979 cm^{-1} assignable to the phosphate group[6] shows no shift at all. The observed amounts of ^{15}N-shifts form a useful set of data for determining the force constants and normal modes of vibration of the uracil residue. These are discussed later in this paper. Here, a comment is added as for an interesting isotope effect on the Raman scattering intensity. As may be seen in Fig. 2, the relative intensity of the 1246 cm^{-1} line of 5′-UMP-d_3 is appreciably raised on ^{15}N substitution. This Raman line (let us call its mode "V") is one of the characteristic lines of pyrimidine bases. This is isolated and strong when it is located at about 1235 cm^{-1} or lower frequency (*e.g.*, in uracil residue, and uracil residue-d_1, $^{15}N_2$). On the other hand, it is weaker and has a few satellite lines when it is located at a frequency higher than 1240 cm^{-1} (*e.g.*, in deprotonated uracil residue, deuterated uracil residue, and cytosine residue). This is the strongest Raman line in the uracil residue and shows the greatest intensity enhancement on bringing the exciting wavelength from 514.5 nm to 351.1 nm. The ^{15}N-isotope effect on the intensity of this line may be ascribed to a change in the vibrational mode "V" in the electronic excited state $\tilde{A}$ (260 nm) rather than that in the electronic ground state $\tilde{X}$. It may be explained, in other words, by taking a Duschinsky effect into account.

Thus, it is not improbable that, in the first excited state $\tilde{A}$ (260 nm) of the uracil residue, the $C^5=C^6$ bond order is very low so that its intrinsic stretching frequency is as low as 1234 cm^{-1}. If so, "mode II" (mainly $C^5=C^6$ stretching) and "mode V" have nearly equal frequencies to each other in the upper $\tilde{A}$ state; while, in the ground electronic state, "mode II" (mainly $C^5=C^6$ stretching, at about 1600 cm^{-1}) and "mode V" (1234 cm^{-1}) are orthogonal to each other. In such a situation, the Franck-Condon overlap integrals that cause the Raman scattering of the "V-vibration" may have an appreciable value. If the "V-frequency" is exactly 1234 cm^{-1} (as that for 5′-UMP-$^{15}N_2$, d_3), then the vibrational coupling in $\tilde{A}$ would be very strong and the "V-Raman line" would be strong. If, on the other hand, the "V-frequency" is slightly higher than 1234 cm^{-1} (as that for 5′-UMP-$^{14}N_2$, d_3) the vibrational coupling in $\tilde{A}$ would be weaker and the "V-Raman line" would be weaker.

The Raman Lines That are in Resonance with the 260 nm Electronic Transition. Raman spectra of β-uridine-5′-phosphoric acid (5′-UMP) in neutral aqueous solution were examined with excitation at 647.1, 514.5, 488.0, 457.9, 363.8, and 351.1 nm (Fig. 3). By the use of the 979 cm^{-1} Raman line (PO_3^{2-} symmetric stretching) as an internal intensity standard, the Raman intensity *versus* excitation frequency relation (excitation profile) has been examined for each of the Raman lines of the uracil residue. From a comparison of such an observed excitation profile with theoretical ones, it was shown[4] (i) that the Raman lines at 1231, 1394, and 1475 cm^{-1} are primarily associated with the absorption band at 260 nm, (ii) that, to the 1680, 1630, and 783 cm^{-1} Raman lines, there are appreciable amounts of contribution from the 260 nm absorption band, but contribution from the 210 nm band or from other bands of shorter wavelengths should also be taken into account, and (iii) that the contribution of the 260 nm transition to the 560 cm^{-1} Raman line is rather small.

A rigorous resonance Raman effect of 5′-UMP was examined with 257 nm excitation[3] (see Fig. 3(e)). It became

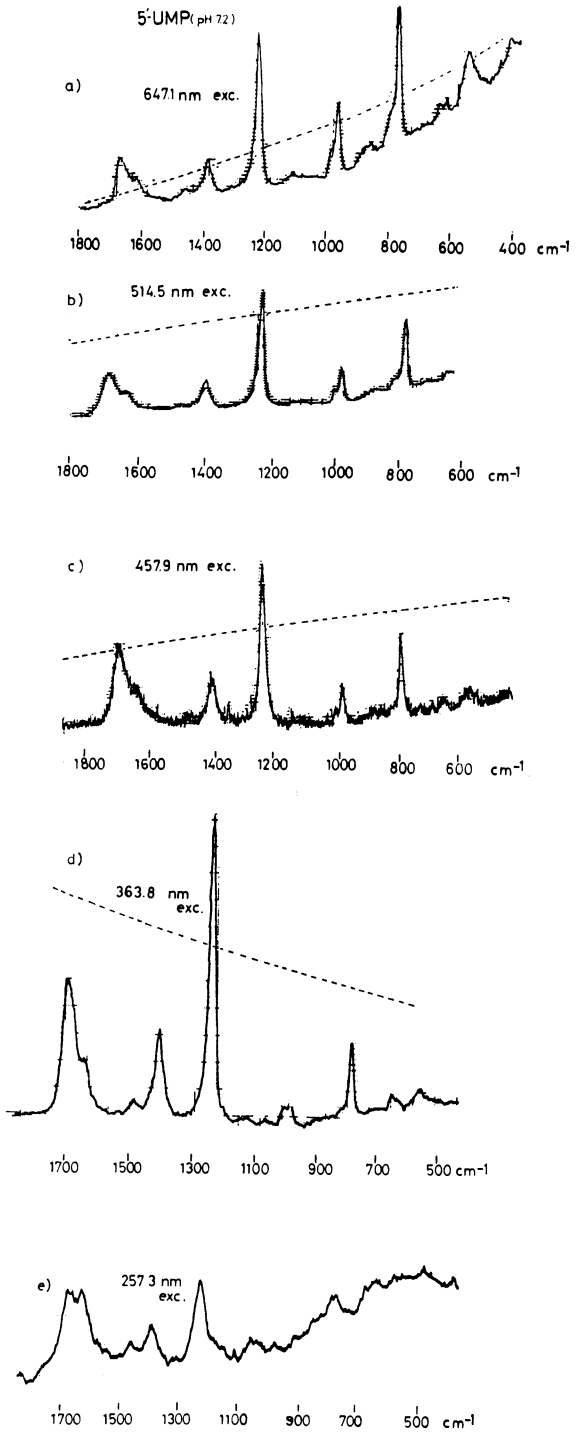


Fig. 3. Raman spectra of 5′-UMP in neutral aqueous solution. Excited by (a) 647.1 nm (Kr), (b) 514.5 nm (Ar), (c) 457.9 nm (Ar), (d) 363.8 nm (Ar), and (e) 257.3 nm (Ar ion laser plus a frequency doubler) lines. Broken lines indicate the spectral sensitivity of the monochromator and detector system.

evident that the six Raman lines at 1680, 1630, 1475, 1395, 1231, and 787 cm^{-1} of the uracil residue are caused by the electronic excited state at 260 nm. Let us call these six Raman lines UrI, UrII, UrIII, UrIV, UrV, and UrVI, respectively.

Characterization of the On-resonance Raman Lines. Pre-resonance and resonance Raman spectra were observed[4] of various related compounds which are considered to be

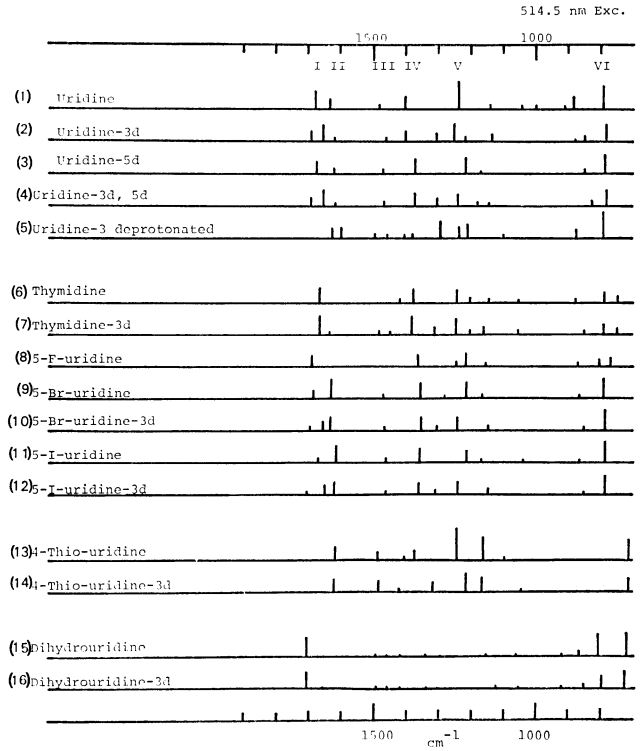


Fig. 4. Raman lines observed for uridine and its derivatives. ^1H is replaced by ^2H at the position indicated.

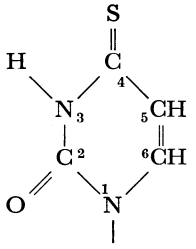
relevant to the interpretation of the Raman spectrum of the uracil residue. Observed Raman lines of uridine and some uridine derivatives are shown in Fig. 4. On the basis of these data, a detailed discussion were given on the Raman lines of the uracil residue in our previous article.[4] Some of the results and conclusions are briefly summerized below:

a) All of the six Raman lines, UrI to UrVI, are in the skeletal-stretching frequency region. All of these Raman lines, except UrIII, are strong in off-resonance condition. In addition, neither position-3 deuteration nor position-5 deuteration causes a wavenumber shift greater than 100 cm^{-1} for any of these Raman lines. Therefore, all of these are assignable primarily to in-plane skeletal bond-stretching vibrations of the neutral uracil residue.

b) The 787 cm^{-1} line is assignable to a vibration in which all eight skeletal stretching motions take place in-phase (ring breathing).[7,8] It is understandable that, while the relative intensity of this line is very strong in an off-resonance Raman spectrum (see Fig. 3(a)), it is very weak in a rigorous resonance Raman spectrum (Fig. 3(e)) excited in the longest wavelength (260 nm) absorption band ($\tilde{A}\leftarrow\tilde{X}$). The distortion of the uracil residue does not take place along such a ring breathing coordinate on going from $\tilde{X}$ to $\tilde{A}$ states.

(c) In the 1600 to 1700 cm^{-1} region there are three skeletal stretching vibrations expected, because of the three double bonds, $C^2{=}O$, $C^4{=}O$, and $C^5{=}C^6$. Actually there are observed three Raman lines (and three infrared bands) for the position-3 deuterated uracil residues (see Fig. 4; (2), (4), (10), and (12)). In each of the Raman spectra of undeuterated uracil residues (Fig. 4; (1), (3), (9), and (11)), however, one of these three is missing. The missing vibration here must be at 1710 cm^{-1}, because the undeuterated uracil residue gives a strong infrared absorption band at 1710 cm^{-1}.[8] This is assignable to the $C^2{=}O$ stretching vibration.[9] Thus, the UrI and UrII are probably assignable to a coupled pair of the $C^4{=}O$ stretching and the $C^5{=}C^6$ stretching vibrations.

(d) An additional support to the assignments of the UrI and UrII Raman lines is obtained from an examination of the Raman spectrum of 4-thiouridine. This is a derivative of uridine in which the position-4 carbonyl is replaced by thiocarbonyl, so that it has residue. In a resonance condition,



this shows six strong Raman lines at 1619, 1482, 1372, 1243, 1160, and 709 cm^{-1}. In the 1600—1700 cm^{-1} region only one Raman line (assignable to UrII) is observed as is expected. The $C^2{=}O$ stretching line is missing as it is for the uracil residue, and $C^4{=}O$ is absent here. Instead, there are two strong Raman lines observed at 1167 and 709 cm^{-1}, which are assignable to the two vibrations caused by a strong coupling of the $C^4{=}S$ stretching and the ring breathing motions. The three lines at 1482, 1372, and 1243 cm^{-1} are considered to correspond to the UrIII, UrIV, and UrV lines of the uracil residue, respectively.

(e) The Raman lines corresponding to the UrIII and UrIV are found at about 1470 and 1400 cm^{-1}, respectively, for all of the uridine derivatives examined (see Fig. 4) except for dihydrouridine. UrV Raman line is also found for all of the uridine derivatives with high intensities. Deuteration at position-3 causes a slight higher frequency shift of this line and at the same time an appearance of a few satellite lines (see Fig. 4; (2), (4), (7), (10), and (12)). A similar effect is caused also by the position-3 deprotonation (Fig. 4, (5)). Position-5 deuteration, on the other hand, causes a slight lower frequency shift of UrV line (Fig. 4, (3)). This is caused also by the position-5 substitution with bromine or iodine (Fig. 4, (9) and (11)).

(f) Dihydrouridine shows a completely different Raman spectrum (Fig. 4, (15) and (16)) from those of the uridine derivatives with $C^5{=}C^6$ double bond. Here, no strong Raman lines are found in the 900 to 1500 cm^{-1} region. It is clear that the $C^5{=}C^6$ double bond, which makes the whole six-membered ring nearly conjugated, is essential for giving rise to the UrI—UrV Raman lines (the $C^5{=}C^6$ double bond is also essential for the 260 nm absorption band).

Normal Modes of Vibration for the On-resonance Raman Lines. A normal coordinate treatment was made for in-plane vibrations of 1-methyl uracil. Here, every hydrogen atom (except that of the methyl group) was taken as a dynamical unit, while the methyl group was assumed as one dynamical unit. The bond lengths and bond angles in the uracil residue were assumed to be equal to those given by a crystallographic study of 1-cyclohexyluracil (Dr. T. Katsura, personal communication). The calculation was carried out using a HITAC 8800/8700 in the Computer Center at the University of Tokyo, and the programs, BGLZ, and LSMA, written by Shimanouchi and his collaborators.[10] In the calculation, a general valence force field was assumed, where all the bond-stretch bond-stretch interaction terms were taken into consideration. The force constants were determined by a trial and error method so that the calculated frequencies were in the best agreement with the observed frequencies. The adjustment of the force constant values was guided by the Jacobian matrix which was

calculated for each trial set of force constants. The adjustment was made by taking the following items into account:

(a) The calculated ^{15}N-shifts should be in the best agreement with the observed shifts (see Table 2).

(b) The observed effects of the position-3 deuteration and position-5 deuteration on the vibrational frequencies should be well reproduced by the calculation. This was achieved by a proper adjustment of the bending force constant values, so as to bring the N^3H in-plane bending vibration to about 1416 cm^{-1},[11] and the C^5H in-plane bending vibration to about 1100 cm^{-1}.

(c) Not only the Raman frequencies of the uracil residue observed in the aqueous solutions of uridine and its derivatives, but also Raman[12,13] and infrared[11] frequencies observed for powder 1-methyluracil are to be taken as a set of data.

(d) Not only the bond-stretch bond-stretch cross terms for adjacent two bonds (let us call "ortho"), but also those for two bonds "next door but one" from each other (meta) and for two bonds two doors away from each other (para) should have appreciable values. Such force constant values were not found to be negligible in a conjugated double-bond system such as benzene.[5]

The final set of force constant values are given in Table 1, and the calculated vibrational frequencies for the undeuterated and deuterated (at position-3) uracil residues are given in Table 2. The agreement between the observed frequencies (also given in Table 2) and the calculated ones are satisfactory. The agreement between the observed and calculated ^{15}N-isotope shifts are also good except for the 1397 (UrIV) line of the undeuterated uracil residue and for the 1689 (C^2=O), 1464 (UrIII), and 1402 cm^{-1} (UrIV) lines of the deuterated uracil residue . In the actually observed ^{15}N shifts, there must be some factors involved, such as anharmonicity, Fermi resonance, and vibrational couplings with the solvent molecules, which were not taken into consideration in our present calculation. It may also be pointed out here that the amount of the ^{15}N-shift of each vibration is extremely sensitive to the amount of the contribution of the N–H in-plane deformation motion to the vibration now in question. This is, however, not always be properly estimated in our present calculation on the basis of a simple harmonic potential function.

In Fig. 5, are depicted the normal modes of vibration calculated on the basis of the set of force constants given in Table 1. This forms one of the main conclusions of our present study. The modes of the UrI—UrVI vibrations now in question may be characterized as follows:

UrI(1680 cm^{-1}). C^4=O stretching and C^5=C^6 stretching vibrations take place in-phase.

UrII (1630 cm^{-1}). C^4=O stretching and C^5=C^6 stretching vibrations take place with 180° phase difference.

UrIII (1475 cm^{-1}). A skeletal stretching vibration which has a similarity to one of the e_{2g} stretching vibrations of benzene at 1584 cm^{-1}.

UrIV (1397 cm^{-1}). A skeletal stretcing vibration which has a similarity to one of the e_{1u} stretching vibrations of benzene at 1485 cm^{-1}.

UrV (1231 cm^{-1}). A skeletal stretching vibration which has a similarity to the b_{2u} vibration of benzene at 1309 cm^{-1} (Kekulé vibration).

TABLE 2. OBSEVED AND CALCULATED FREQUENCIES OF THE URACIL RESIDUE

Frequency, cm^{-1}		^{15}N-shift, cm^{-1}		PED[b]		Frequency, cm^{-1}		^{15}N-shift, cm^{-1}		PED[b]	
Obsd[a]	Calcd	Obsd	Calcd			Obsd[a]	Calcd	Obsd	Calcd		
Undeuterated						Deuterated (position-3)					
3131*	3132	—	−9	ν N-H (99)		3087*	3100	—	0	ν C-H (99)	
3079*	3100	—	0	ν C-H (98)		3087*	3091	—	0	ν C-H (99)	
3079*	3091	—	0	ν C-H (99)		2260*	2311	—	−12	ν N-D (96)	
1720*	1707	—	−8	ν C_2=O (64)		1689	1698	+2	−5	ν C_2=O (71)	
1680	1673	−2	−4	ν C_4=O, C=C (70)	UrI	1655	1666	−2	−4	ν C_4=O, C=C (75)	UrI
1630	1625	−3	−3	ν C=C, C_4=O (76)	UrII	1618	1622	−2	−2	ν C_4=O, C=C (78)	UrII
1475	1476	−13	−14	ν ring (72)	UrIII	1464	1465	−3	−13	ν ring (81)	UrIII
1423*	1415	—	−9	δ NH (55)		1402	1395	−7	−15	ν ring (51)	UrIV
1397	1397	−7	−15	ν ring (52)	UrIV	1303	1306	−19	−20	ν ring (47)	
1231	1245	−8	−9	ν ring (100)	UrV	1246	1242	−12	−15	ν ring (100)	UrV
1201*	1240	—	−17	δ C-H (56)		1211	1206	−9	−17	ν N-R (22), ν ring (34)	
1156*	1182	—	−18	ν ring (64), ν N-R (32)		1136	1115	−3	−1	δ C-H (83)	
1084*	1115	—	−3	δ C-H (84)		1043*	1102	—	−9	δ N-D (21), ν N-R (15)	
1008*	1001	—	−10	ν ring (47)		914*	886	—	−3	δ N-D (44)	
806*	811	—	−9	δ ring (38)		812*	793	—	−3	δ ring (42)	
783	780	−2	−1	ν ring (52)	UrVI	780	778	−1	−3	ν ring (60)	UrVI
639	622	−6	−4	δ C=O (57)		620	610	−5	−5	δ C=O (56)	
557	546	−5	−3	δ ring (61)		551	543	−3	−4	δ ring (61)	
482	485	—	−3	δ ring (48)		478*	485	—	−3	δ ring (48)	
421	385	−2	−3	δ C=O (65)		395*	384	—	−1	δ ring C=O (64)	
268*	336	—	−1	δ N-R (60)		266*	336	—	0	δ N-R (60)	

a) Raman or infrared frequencies observed[11–13] for powder 1-methyluracil are shown by *. b) Potential energy distribution is given in parenthesis. ν, stretching; δ, bending.

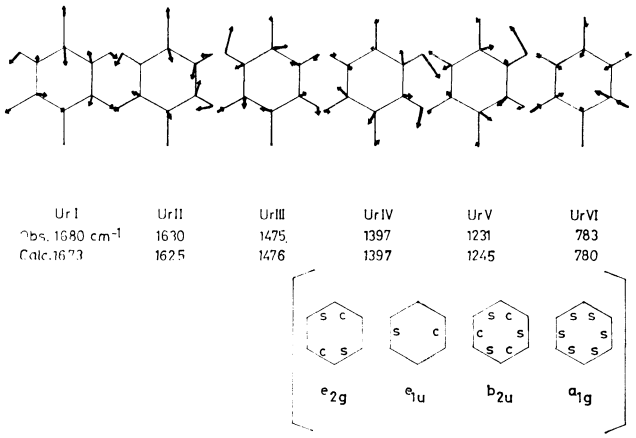


Fig. 5. Normal modes of vibration of the uracil residue, calculated using the set of force constants given in Table 1. Some of the normal modes of the skeletal stretching vibrations of benzene are shown in parentheses for reference. s: stretch, c: contract.

UrVI (783 cm^{-1}). All of the eight bonds, C=C, C–C, C–N, and C=O, stretch and contract in-phase (ring breathing vibration).

Excited State Geometry for the 260 nm Band. Among the six on-resonance Raman lines of the uracil residue, three at 1475 (UrIII), 1397 (UrIV), and 1231 cm^{-1} (UrV) are considered to be most intimately associated to the excited electronic state $\tilde{A}$ corresponding to the 260 nm absorption band. Among the vibrational modes for these three Raman lines, a motion in which the C^4–C^5 stretching and C^5=C^6 stretching take place with 180° phase difference is commonly predominant (see Fig. 5). Therefore, it is probable that, on going from the electronic ground state $\tilde{X}$ to the excited state $\tilde{A}$, a distortion of the molecualr conformation takes place along this coordinate; as shown in Fig. 6, the C^5=C^6 bond is probably longer in $\tilde{A}$ than in $\tilde{X}$, while C^4–C^5 is shorter in $\tilde{A}$ than in $\tilde{X}$. This is another main conclusion of our present study.

The conclusion just reached by our Raman spectroscopic investigation is in an agreement with an expectation from

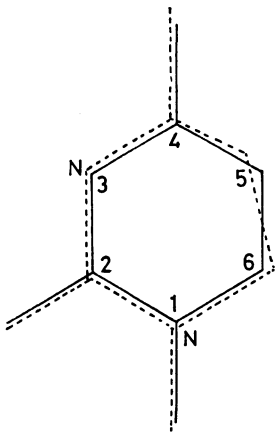


Fig. 6. A schematic drawing to show a possible change in the molecular conformation of the uracil residue on going from the electronic ground state $\tilde{X}$ (shown by full line) to the electronic excited state $\tilde{A}$ (shown by broken line).

theoretical studies on the electronic structure of the uracil residue. Thus, Nagata *et al.*[14] made a semiempirical Pariser-Parr-Pople calculation for the π-electron system of this base residue. This calculation shows that, on going from the highest occupied to the lowest vacant π-orbital, C^5=C^6 bond should change dramatically from *bonding* to *antibonding*, and C^4–C^5 bond should change from *antibonding* to *bonding*, while all other bonds should change only very slightly from *bonding* to *antibonding*. This means that, on going from $\tilde{X}$ to $\tilde{A}$, the bond order of the C^5=C^6 should appreciably decrease, that of the C^4–C^5 should appreciably increase, and those of other bonds should only slightly decrease (see also reference[4]).

Hug and Tinoco[15] made an all-valence electron MO-CI calculation for uracil. It was shown that a marked localization of the monopoles of transitions $\tilde{A}$←$\tilde{X}$ and $\tilde{B}$←$\tilde{X}$ along the acrolein-like fragment O=C^4–C^5=C^6 is expected to take place. For the $\tilde{A}$←$\tilde{X}$ transition, the above view based upon the result of the calculation of Nagata *et al.*[14] is supported by this more elaborate treatment.

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