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Vibrational studies of biomolecules. I. 2-Thiouracil

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Abstract. IR, far-IR and Raman spectra of 2-thiouracil are reported and interpreted. All the thirty normal modes could be assigned. The Raman spectrum and the vibrational assignments for all the thirty modes are reported for the first time. The ring breathing and Kekule stretching modes for 2-thiouracil are observed to have lower magnitudes when compared to those for uracil which could be due to the mass effect of the sulphur atom in place of the oxygen atom.

Keywords. Vibrational spectra; 2-thiouracil.

1. Introduction

The spectroscopic studies of pyrimidine and its derivatives have been motivated by their occurrence in natural biological systems. Although free molecules do not occur in biological systems, the understanding of the vibrational spectra of free base molecules would be very useful for the understanding of specific biological processes and in the analysis of relatively complex systems. The IR (Blout and Fields 1950; Short and Thompson 1952; Lacher *et al* 1955; Angell 1961; Bandekar and Zundel 1982, 1983), far-IR (Shimanouchi and Harada 1964; Beetz and Ascarelli 1980; Bandekar and Zundel 1982, 1983b) and Raman (Lord and Thomas 1967; Susi and Ard 1971) spectra of uracil have been reported earlier. Recently, gas-phase spectra (Nowak *et al* 1978; Bardi *et al* 1980; Nunziante-Cesaro, unpublished work) and spectra in solid matrices at low temperatures (Radchenko *et al* 1983; Szczesniak *et al* 1983; Barnes *et al* 1984; Chin *et al* 1984; Maltese *et al* 1984; Szczepaniak *et al* 1984) have also been reported. In addition, a number of workers (Bandekar and Zundel 1983; Bowman and Spiro 1980; Espinoza-Müller and Bravo 1982; and private communication**; Susi *et al* 1974; Shibata *et al* 1980; Nishimura *et al* 1981) have tried to interpret the vibrational spectrum of uracil on the basis of theoretical calculations of the fundamental frequencies by employing force field as well as molecular orbital methods. However, controversies still persist about the vibrational assignments proposed by different workers. In order to understand the

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vibrational spectrum of uracil the vibrational spectra of its thio-derivatives are of considerable importance. The IR (Short and Thompson 1952; Lacher *et al* 1955) and far-IR (Beetz and Ascarelli 1980; Bandekar and Zundel 1982, 1983b) spectra of 2-thiouracil have been studied by quite a small number of workers. However, to the best of the authors' information neither the Raman Spectrum nor the complete vibrational analysis for 2-thiouracil has been reported. Beetz and Ascarelli (1980) have assigned some of the lower frequency modes of 2-thiouracil and uracil whereas Bandekar and Zundel (1983b) have used infrared spectra of 2-thiouracil and 2,4-di-thiouracil to help assign the vibrational frequencies of uracil by the process of elimination. This paper presents the results of far-IR, IR and Raman spectroscopic studies and vibrational assignments of all the thirty modes of vibration of 2-thiouracil for the first time.

2. Experimental

The chemical 2-thiouracil was purchased from Sigma Chemical Co. (USA) and was used as such for recording the spectra. All the spectra were recorded at room temperature. The Raman spectrum (figure 1) of 2-thiouracil powder was recorded in the region 40–4000 cm^{-1} on a Carey-82 spectrometer with the following parametric adjustments–excitation: 4880 A° from Ar^+ laser with 100 mW power at sample; spectral slit-width : 2 cm^{-1}; constant scan-speed : 0.1 cm^{-1}/s. The infrared spectrum (figure 2) was recorded in the region 300–4000 cm^{-1} on a Beckman IR-12 Spectrometer using KBr optics. The far-IR spectrum (figure 3) was recorded in the region 95–600 cm^{-1} on a Beckman IR-11 Spectrometer using high density polyethylene optics. The frequencies reported here for all the spectra are accurate to within $\pm$ 3 cm^{-1}.

3. Results and discussion

The absorption frequencies observed in the Raman, IR and far-IR spectra along with their relative intensities and proposed vibrational assignments are given in table 1. Table 2 presents the intermolecular, overtone and combination frequencies and their assignments. The vibrational spectrum has been interpreted assuming planar geometry and C_s point group symmetry. The vibrational assignments proposed for 2-thiouracil are based on the vibrational assignments proposed for related molecules, especially for uracil by Susi and Ard (1971), Susi *et al* (1974), Bandekar and Zundel (1983b), Barnes *et al* (1984) and Harsányi *et al* (private commun.), for uracil and 2-thiouracil by Beetz and Ascarelli (1980), for 1-methyluracil by Colombo and Kirin (1986) and for related molecules by other workers (Spinner 1960; Shunmugam and Sathyanarayana 1984; Aruna and Shanmugam 1985; Sathyanarayana and Kasmir Raja 1985).

3.1 *C=O/C=S modes*

The C=O and C=S modes are some of the most interesting modes of 2-thiouracil. A number of workers (Beetz and Ascarelli 1980; Bandekar and Zundel 1983b;

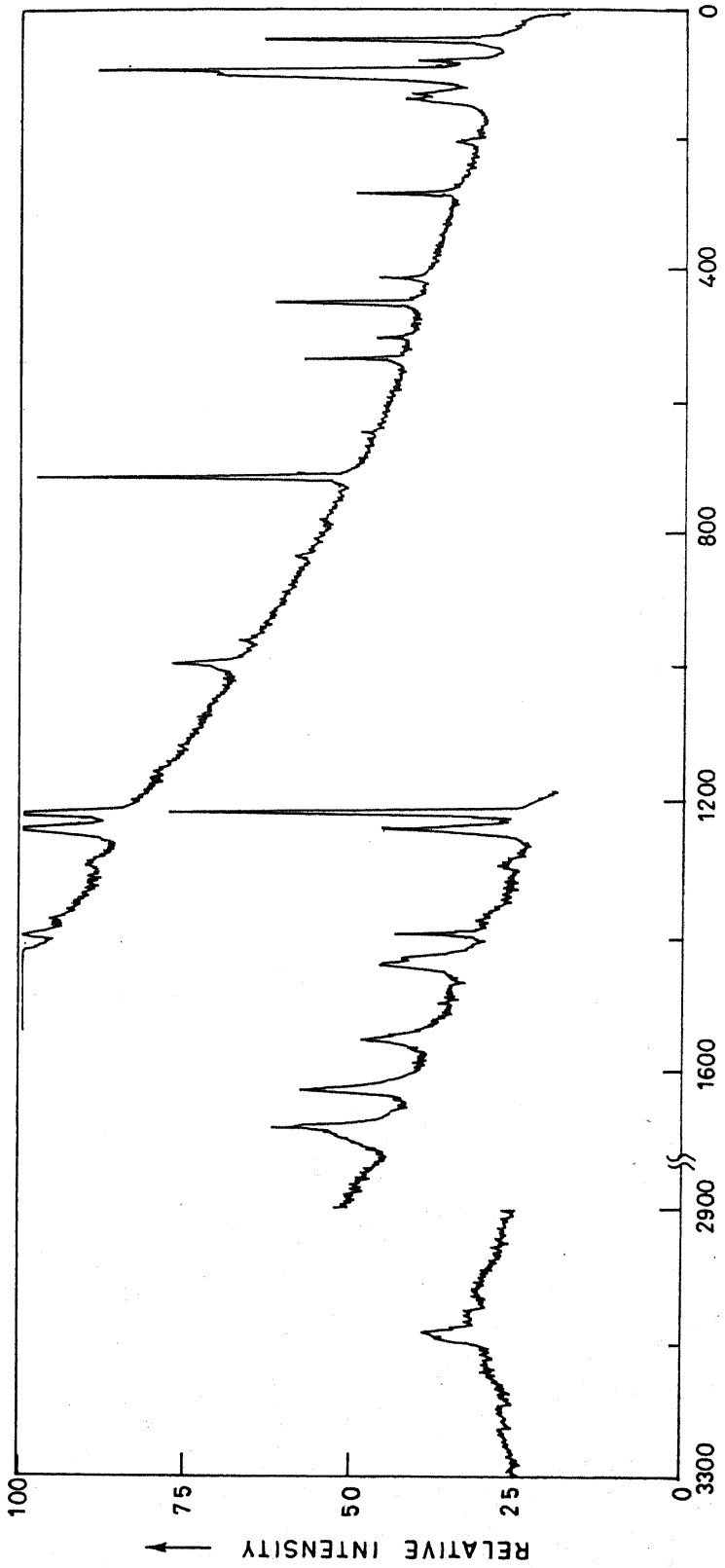

Figure 1. Raman spectrum of 2-thiouracil (powder).

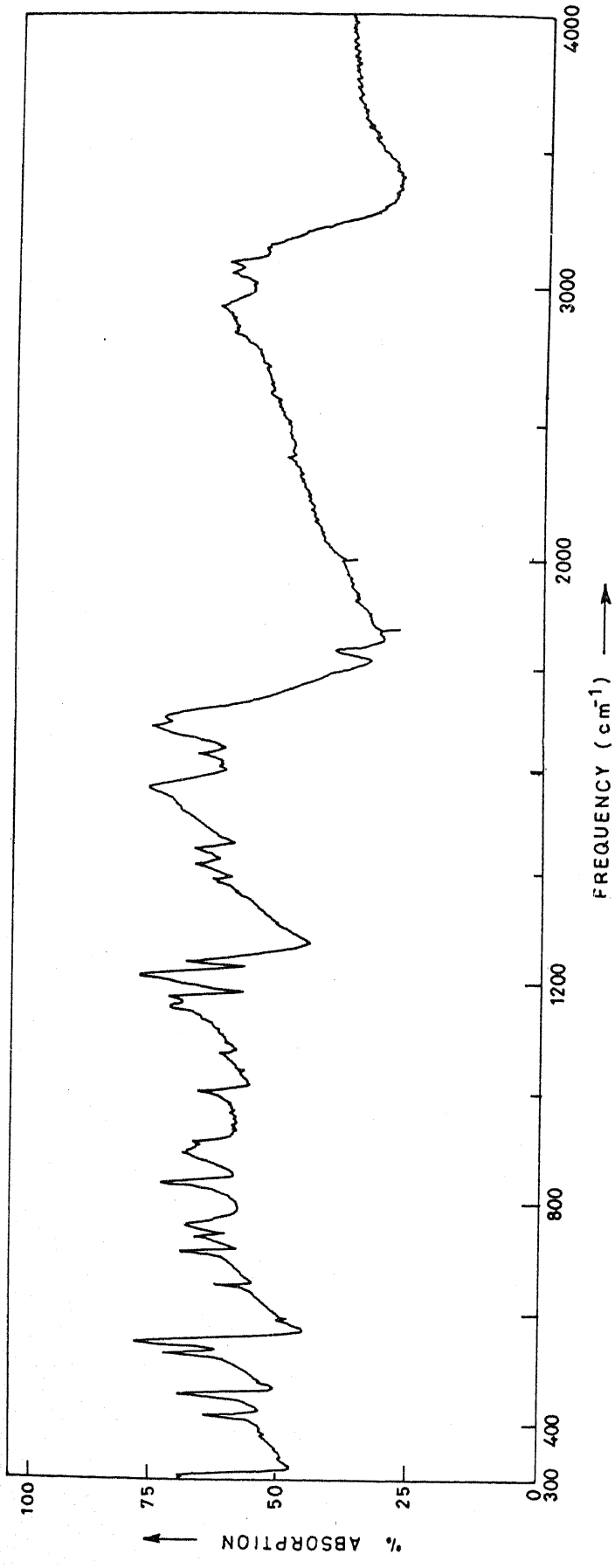

Figure 2. Near-IR spectrum of 2-thiouracil (KBr pellet).

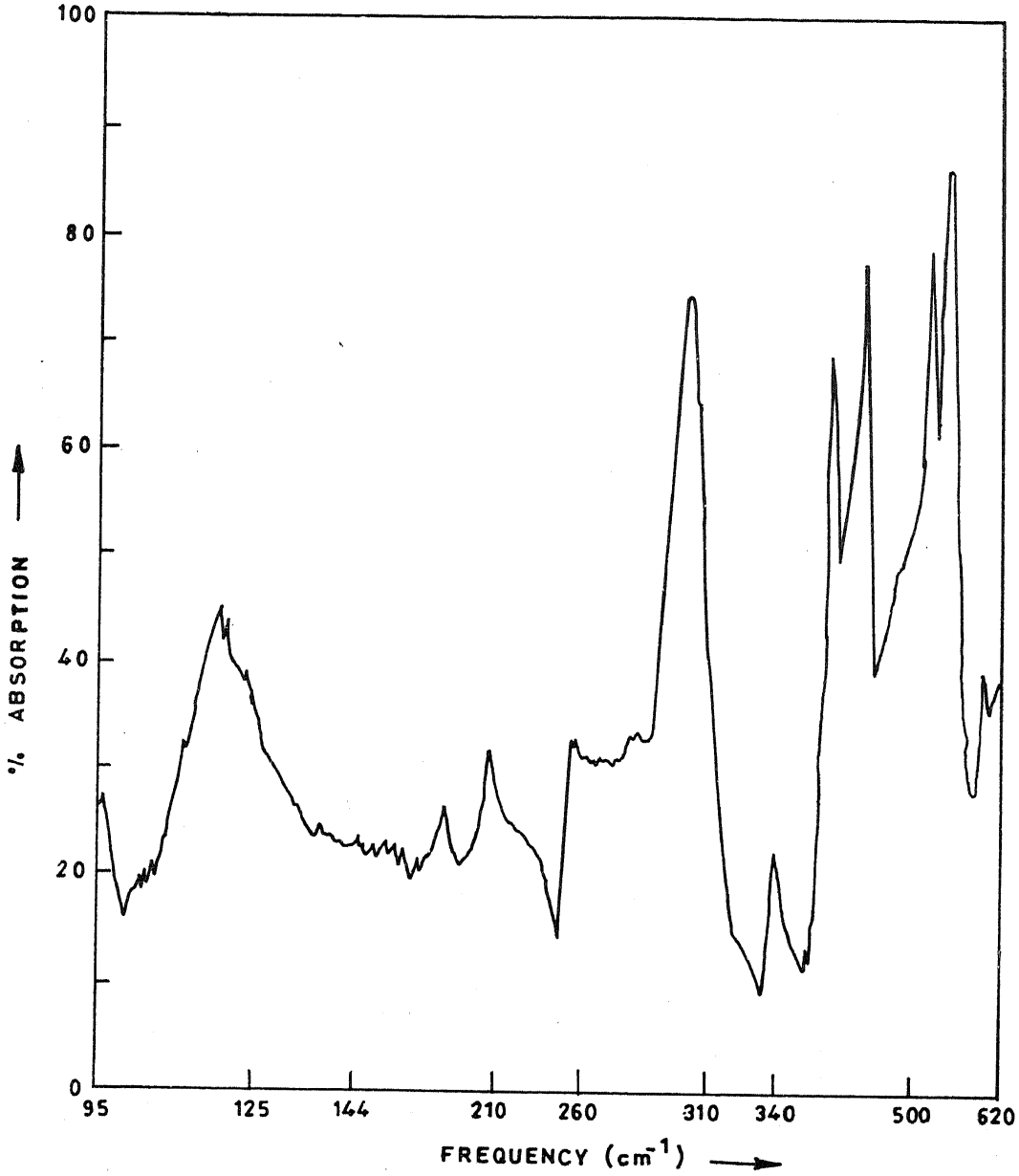

Figure 3. Far-IR spectrum of 2-thiouracil.

Barnes *et al* 1984) have argued that in uracil it is the oxygen atom attached to C_4 which takes part in the hydrogen-bonding whereas the oxygen atom attached to C_2 does not participate in it. Therefore, in 2-thiouracil the C=O modes would correspond to the hydrogen-bonded C=O modes of uracil. A very strong and broad IR band is observed with a peak at 1683 cm^{-1} corresponding to the medium Raman line at 1681 cm^{-1}. This band is completely absent in the spectra of 2-thiocytosine (present authors, unpublished work) suggesting its presence in the 2-thiouracil spectrum as being due to the C=O stretching mode. The C_4O in-plane bending mode has been assigned near 625 cm^{-1} by Susi and Ard (1971) and Susi *et al* (1974) for uracil, and by Colombo and Kirin (1986) for 1-methyl uracil, contrary to the assignment of a band near 565 cm^{-1} to this mode for uracil by Szczepaniak *et al* (1984), Harsānyi *et al* (private commun.) and Barnes *et al* (1984), and a band at

Table 1. Fundamental absorption frequencies of 2-thiouracil.

Observed frequencies*		Species	Proposed assignments†
Raman	Infrared		
285 (26)	285 (*vw*)	a″	δ (ring)
	304 (*vvs*, 82)	a″	γ (C=S)
417 (13)	413 (*vvs*, 71)	a″	δ (ring)
454 (36)	454 (*vvs*, 82)	a′	β (C=S)
	526 (*vvs*, 88)	a″	δ (ring)
539 (25)	546 (*vvs*, 99)	a′	α (ring)
	580 (*m*, 43)	a′	β (C=O)
649 (4)	652 (68)	a′	α (ring)
718 (78)	714 (82)	a′	ν (ring)
	739 (76)	a″	γ (C=O)
	758 (79)	a″	γ (N_1H)
840 (4)	839 (90)	a′	α (ring)
	893 (81)	a″	γ (N_3H)
	916 (76)	a″	γ (CH)
961 (5)	963 (*vw*)	a″	γ (CH)
999 (16)	1004 (74)	a′	ν (ring)
	1075 (66)	a′	ν (ring)
1157 (2?)	1157 (86)	a′	β (CH): Fermi resonance
	1177 (87)		between 1167 and
			2 × 580
1221 (92)	1216 (100)	a′	ν (C=S)
1243 (38)	1239 (79)	a′	ν (ring)
1397 (25)	1396 (69)	a′	β (N_3H)
1430 (19)	1420 (76)	a′	β (CH)
1441 (24)	1450 (76)	a′	ν (ring)
1553 (19)	1567 (97)	a′	β (N_1H)
1627 (28)	1630 (76)	a′	ν (ring)
1681 (31)	1683 (95)	a′	ν (C=O)
3055 (6)	3051 (66)	a′	ν (CH)
3083 (14)	3087 (67)	a′	ν (CH)
	3136 (s–sh)	a′	ν (N_3H)
	3200 (sh)	a′	ν (N_1H)

*Relative intensities of the frequencies (in cm^{-1}) observed in the Raman and IR spectra are given on a scale of 100 separately for each case and given in parentheses following every frequency. The relative intensities for the IR bands are given as, *w* = weak, *s* = strong, *m* = medium, *vw* = very weak, *ms* = medium strong, *vs* = very strong, *vvs* = very, very strong, *sh* = shoulder, *s-sh* = strong-shoulder. †ν: stretching, α: angle bending, β: in-plane bending, γ: out-of-plane bending and δ = out-of-plane ring deformation.

585 cm^{-1} by Beetz and Ascarelli (1980) and Bandekar and Zundel (1983b). In the spectra of 2-thiouracil we have observed absorption at 580 cm^{-1} and we have assigned the same to the C_4O in-plane bending mode.

There is a controversy among different workers in the assignments of CO out-of-plane bending modes of uracil. Some workers (Barnes *et al* 1984; Harsányi *et al* (private commun.); Szczepaniak *et al* 1984; Colombo and Kirin 1986) have

Table 2. Intermolecular combination and overtone bands* of 2-thiouracil.

Observed frequencies		Assignments
Raman	Infrared	
51 (63)		Intermolecular
82 (22)		Intermolecular
100 (100)	96 (*w*)	Intermolecular
106 (71)	105 (*sh*)	Intermolecular
	113 (*sh*)	Intermolecular
	119 (*vs*)	Intermolecular
131 (21)	133 (*sh*)	Intermolecular
140 (23)		Intermolecular
180 (3?)	187 (*m*)	Intermolecular
205 (7)	208 (*ms*)	2 × 105
	234 (*sh*)	2 × 119
	258 (*s*)	Intermolecular
	357 (*ms*)	Intermolecular
505 (9)	490 (*sh*)†	357 + 132
1700 (*sh*)	1708 (88)	413 + 1396, 1420 + 285
	1831 (21)	2 × 916, 1420 + 413, 1075 + 758
	2379 (42)	1630 + 739
	2840 (64)	1630 + 1216, 2 × 1420
	2926 (70)	1683 + 1239
3070 (*sh*)		1630 + 1450

*Intensities and abbreviations are the same as in table 1.
†Observed in IR and far-IR spectra as shoulder.

assigned these modes in the region 680–820 cm^{-1} while others (Beetz and Ascarelli 1980; Bandekar and Zundel 1982, 1983b) have assigned these modes in the region 425–440 cm^{-1}. Bandekar and Zundel (1982) and Beetz and Ascarelli (1980) have reported a weak IR band at 434 cm^{-1} for 2-thiouracil and have assigned this band to the C_4O out-of-plane bending mode. Surprisingly, this band is observed neither in the Raman nor in the infrared spectrum in the present case. For 2-thiouracil we have assigned the C_4O out-of-plane bending mode to 739 cm^{-1} based on the following arguments. In the region 600–825 cm^{-1} three absorptions are observed in the IR spectrum of 2-thiouracil at 714, 739 and 758 cm^{-1} whereas in the IR spectrum of 2-thiocytosine three bands are observed at 724, 752 and 804 cm^{-1} in the above region. The frequencies 714 and 758 cm^{-1} of 2-thiouracil seem to correspond to the frequencies 724 and 752 cm^{-1} of 2-thiocytosine. The vibrational assignments for these frequencies will be discussed later. Obviously the band at 739 cm^{-1} of 2-thiouracil disappears in the spectrum of 2-thiocytosine and a new band appears at 804 cm^{-1}. In going from 2-thiouracil to 2-thiocytosine the oxygen atom is replaced by the NH_2 group and therefore, it appears that the band at 739 cm^{-1} in the spectrum of 2-thiouracil is associated with the C_4O modes whereas the band 804 cm^{-1} of the 2-thiocytosine should be associated with the C_4-NH_2 or NH_2 modes. Out of the three C_4O modes the only mode left unassigned so far is the C_4O out-of-plane bending and hence we assign the frequency 739 cm^{-1} of 2-thiouracil to the C_4O out-of-plane bending mode.

As discussed earlier, 2-thiouracil is obtained by substituting a sulphur atom for the oxygen atom of uracil. The S-atom doesnot participate in the hydrogen-bonding. For pyridine-2-thione, Spinner (1960) has assigned the frequency 1140 cm^{-1} to the C=S stretching mode whereas for the same molecule Shunmugam and Sathyanarayana (1984) have argued that the bands at 1186 and 730 cm^{-1} arise from the mixing of the C=S stretching mode with other planar modes. In later work Sathyanarayana and Kasmir Raja (1985) have observed the absorptions at 1140 and 730 cm^{-1} as being due to the C=S stretching force constant for the same molecule, and in the pyrimidine-2-thione spectrum they have attributed the bands at 1210 and 750 cm^{-1} to the C=S stretching mode. For 6-amino-2-thiouracil, Aruna and Shanmugam (1985) have assigned the frequency 960 cm^{-1} to this mode. In the present case we assign the frequency 1219 cm^{-1} to the C=S stretching mode. For the in-plane and out-of-plane C=S bending modes we agree with the assignments proposed by Beetz and Ascarelli (1980).

3.2 *NH/CH modes*

The frequencies due to the NH/CH stretching modes are well separated from the frequencies due to other modes and the assignments for these modes are given in table 1. The choice of the higher frequency for the N_1H stretching is in accordance with the reported work (Barnes *et al* 1984; Sathyanarayana and Kasmir Raja 1985; Colombo and Kirin 1986). For uracil, Susi and Ard (1971) and Susi *et al* (1974) have assigned the frequencies 1507 and 1420 cm^{-1} to the N_1H and N_3H in-plane bending modes, respectively, based on N_1 and N_3 deuteration. Recent *ab initio* calculations by Harsányi *et al* (private commun.) place the N_3H in-plane bending mode at 1389 cm^{-1} whereas either of the two frequencies 1472 and 1399 cm^{-1} could be assigned to the N_1H in-plane bending mode. In the spectrum of 1-methyluracil (Colombo and Kirin 1986), in which N_1H modes are absent, no absorption could be observed in the region 1490–1590 cm^{-1} and the N_3H in-plane bending mode was observed at 1420 cm^{-1}. For 2-thiouracil we assign the frequencies 1396 and 1560 cm^{-1} to the N_3H and N_1H in-plane bending modes, respectively. Both of these frequencies indicate asymmetric structure in the lower wavenumber region which could be possibly due to the involvement of the N_1 and N_3 atoms in the hydrogen-bonding. For uracil Barnes *et al* (1984) have assigned the frequency 1360 cm^{-1} to the ring-stretching mode whereas Harsányi *et al* (private commun.) have assigned this frequency to mixed modes due to the C_5H and C_6H in-plane bending modes. Likewise, for the same molecule the frequency 1184 cm^{-1} was explained as arising due to ring stretching and NH in-plane bending modes by Barnes *et al* (1984), contrary to the assignment of this frequency to the CH in-plane bending modes by Harsányi *et al* (private commun.). In the IR spectrum of 2-thiouracil the region ~ 1360 cm^{-1} is masked by the 1396 cm^{-1} and, therefore, we assign frequency 1425 cm^{-1} to one of the two CH in-plane bending modes. In the region 1100–1200 cm^{-1} the IR spectrum of 2-thiouracil contains two absorptions at 1177 and 1157 cm^{-1} with comparable intensities. These are explained as the Fermi doublet arising due to the first overtone of 580 cm^{-1} and the fundamental at 1167 cm^{-1} (the average of the two frequencies 1177 and 1157 cm^{-1}). The absorption at 1167 cm^{-1} is assigned to the remaining CH in-plane bonding mode.

Harsányi *et al* (private commun.) and Barnes *et al* (1984) have assigned the absorptions 633 and 556 cm^{-1} observed in the gas phase spectrum of uracil to the

N_3H and N_1H out-of-plane bending modes respectively. For 6-amino-2-thiouracil these modes have been assigned to 925 and 830 cm^{-1} by Aruna and Shanmugam (1985) based on deuterated spectra and normal coordinate calculations. For 1-methyluracil Colombo and Kirin (1986) have assigned the N_3H out-of-plane bending mode to 865 cm^{-1}. The assignments of the 633 and 556 cm^{-1} absorption frequencies to the NH out-of-plane bending modes by Barnes *et al* (1984) and Harsányi *et al* (private commun.) seem rather low. In the present case we have assigned the N_1H and N_3H out-of-plane bending modes to 758 and 893 cm^{-1}, respectively. The absorptions arising from the NH out-of-plane bending modes are known as the amide V bands and have been widely discussed by Bandekar and Zundel (1982) and have been assigned to 805 and 853 cm^{-1}.

3.3 *Ring modes*

The breathing mode is one of the interesting ring modes and is characteristic of the Raman spectra of cyclic molecules. In the Raman spectrum of 2-thiouracil the line at 718 cm^{-1} observed with very strong intensity corresponds to the strong IR band at 714 cm^{-1} and we have assigned this frequency to the ring breathing mode. This mode has been assigned to 760 cm^{-1} for uracil by Barnes *et al* (1984) and Harsányi *et al* (private commun.) and to 760 cm^{-1} for 1-methyluracil by Colombo and Kirin (1986). For uracil Susi and Ard (1971) and Susi *et al* (1974) have assigned the frequency 1237 cm^{-1} to the ring stretch corresponding to the 1309 cm^{-1} frequency of benzene which is due to the Kekule stretching mode. *Ab initio* results of Harsányi *et al* (private commun.) show that this frequency is due to a mixing of the ring stretch with the CH in-plane bending mode. For 1-methyluracil Colombo and Kirin (1986) have assigned this mode to 1225 cm^{-1}. In the present case the frequency 1241 cm^{-1} observed with strong intensity in both the Raman and IR spectra is assigned to the Kekule ring stretch mode. For the assignment of the other ring stretch modes we agree with the assignments of Barnes *et al* (1984) and Harsányi *et al* (private commun.) proposed for uracil and of Colombo and Kirin (1986) for 1-methyluracil and these are given in table 1.

In benzene and its derivatives, out of the three in-plane ring deformation modes, the mode due to trigonal angle bending is one of the interesting and most widely discussed modes. The frequency of this mode is substantially reduced due to its strong mixing with other modes. Colombo and Kirin (1986) have assigned this mode to 807 cm^{-1} for 1-methyluracil whereas Sathyanarayana and Kasmir Raja (1985) have assigned the frequencies 730 and 750 cm^{-1} to this mode for pyridine-2-thione and pyrimidine-2-thione, respectively. For 2-thiouracil we assign the frequency 840 cm^{-1} to this mode. The assignments for the other two ring in-plane deformation modes are in accordance with those proposed by Colombo and Kirin (1986) and Sathyanarayana and Kasmir Raja (1985). The out-of-plane ring deformation modes have been assigned to below 415 cm^{-1} by a number of workers for uracil (Barnes *et al* 1984; Harsányi *et al*, (private commun.) and substituted uracil (Aruna and Shanmugam 1985; Sathyanarayana and Kasmir Raja 1985). Contrary to this, for 1-methyluracil Colombo and Kirin (1986) have assigned these modes to 525, 445 and 268 cm^{-1}. For 2-thiouracil we assign the frequencies 526, 415 and 285 cm^{-1} to the three ring out-of-plane deformation modes.

4. Conclusions

All the thirty normal modes of 2-thiouracil could be assigned. Compared to uracil, the breathing and Kekule ring stretching modes for 2-thiouracil are reduced in magnitude by about 50 and 20 cm^{-1} respectively. The asymmetric structures of the IR bands due to the in-plane NH bending modes indicates the involvement of the two N-atoms in the hydrogen-bonding. The assignment of the out-of-plane bending modes of C_4O to higher frequencies as compared to the in-plane bending mode suggests that the in-plane motion of the oxygen is easier as compared to the out-of-plane motion.

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