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Nucleic acid bases studied by matrix isolation vibrational spectroscopy: uracil and deuterated uracils

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Abstract—A vibrational assignment of the isolated uracil molecule is presented, based on i.r. and Raman spectra of uracil trapped in argon and nitrogen matrices and i.r. spectra of seven deuterated derivatives of uracil isolated in argon matrices. The observed spectra agree well with previous quantum mechanical calculations. The relationships observed between the monomer bands, bands due to associated uracil in argon matrices and the spectrum of the pure solid lead to a revised vibrational assignment for uracil in the solid phase. As well as large shifts in the NH stretching and out-of-plane bending modes between the monomer and the hydrogen-bonded solid, many other bands show appreciable shifts as a consequence of the extensive mixing of the NH in-plane bending modes with the CH in-plane bending and ring stretching modes. The spectrum of the associated uracil species in matrices suggests that it is a cyclic dimer, linked by $C_2{=}O \ldots H{-}N_1$ hydrogen bonds.

INTRODUCTION

Infrared, Raman and resonance Raman spectroscopies are widely used in biophysical research on nucleic acids and related compounds. A full understanding of the vibrational spectra of nucleic acid bases is thus of great importance if such work is to be soundly based. The i.r. and Raman spectra of the simplest pyrimidine base, uracil, and its derivatives have been extensively studied, both experimentally (mainly as polycrystalline solids or in aqueous solution) and through force field calculations [1–22]. One of the most widely quoted studies is that of SUSI and ARD [5, 10], who assigned the spectra of uracil and its 1,3-, 5,6- and *per*-deuterated derivatives from the observed isotopic shifts with the aid of a normal coordinate calculation.

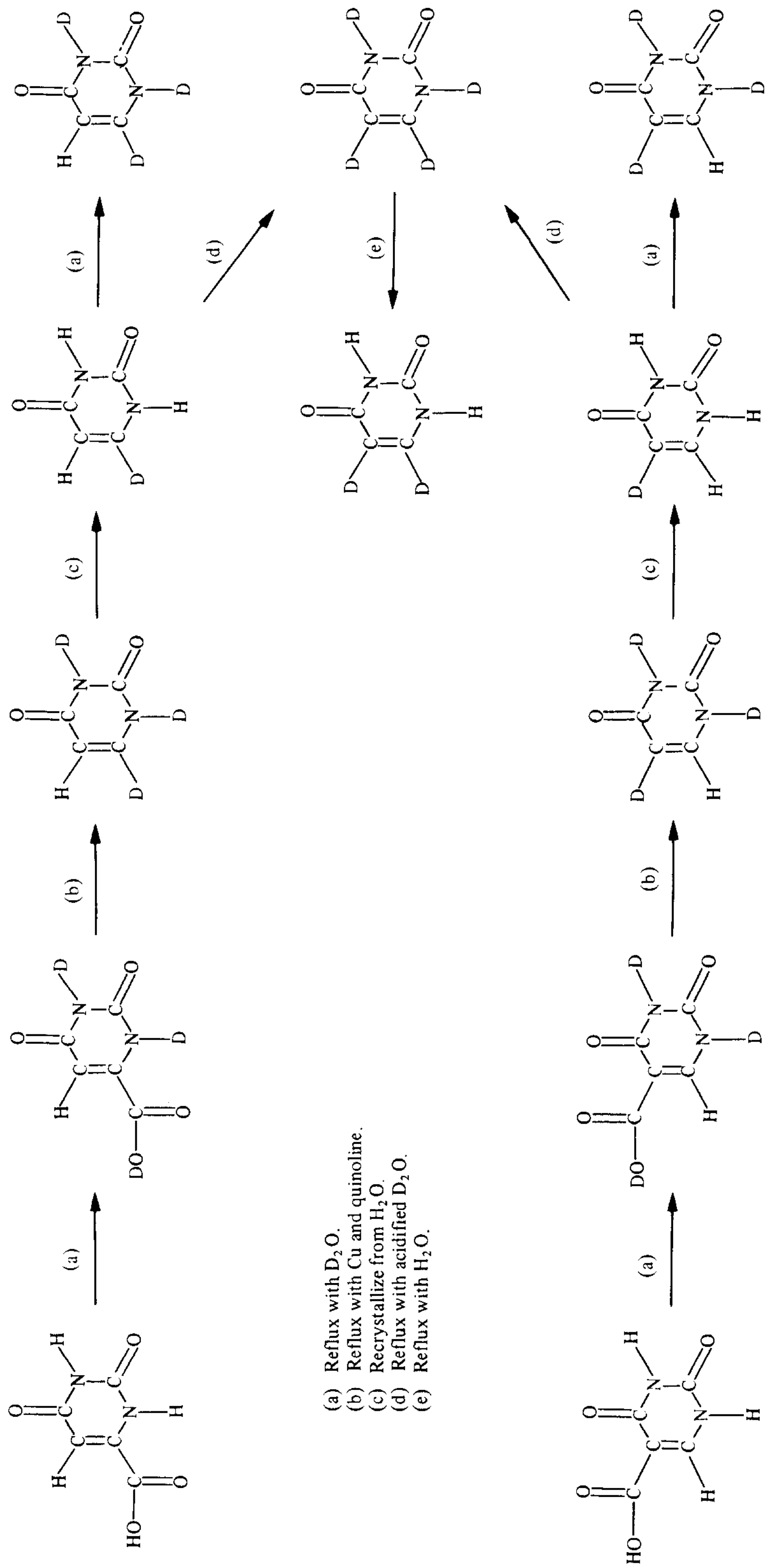
Recently, three molecular orbital calculations of the in-plane vibrational modes of the uracil molecule have been published [17–19]. The MNDO-MOCIC calculation of BOWMAN and SPIRO [17] and the *ab initio* calculation of NISHIMURA *et al.* [19] both reproduced well the observed vibrational frequencies and are in qualitative agreement with each other, although the calculated potential energy distributions differ considerably. However, the quantum mechanical calculations are based on an isolated uracil molecule whereas the observed vibrational frequencies are generally taken from the solid phase, where considerable perturbation from intermolecular hydrogen bonding [23, 24] can be expected (only limited spectral information has been reported for uracil in the vapour phase [12] since the elevated temperature required leads to extensive rotational broadening). BANDEKAR and ZUNDEL [22] have attempted to take account of these intermolecular interactions in the solid phase by carrying out a normal coordinate analysis of the unit cell of uracil, including out-of-plane and lattice modes as well as the in-plane vibrational modes.

Matrix isolation vibrational spectroscopy has proved to be of great value in hydrogen bonding studies [25]. Trapping the solute in an inert solid matrix at low concentration enables the i.r. spectrum of the monomer to be obtained, while raising the concentration allows the changes in the spectrum resulting from intermolecular hydrogen bonding to be observed. Previous studies of maleimide [26] and barbituric acid derivatives [27–29] have demonstrated the value of complementary Raman spectroscopic studies, as well as isotopic substitution, in arriving at a vibrational assignment. We have obtained i.r. and Raman spectra of uracil in both argon and nitrogen matrices and i.r. spectra of seven deuterated derivatives of uracil in argon matrices, over a range of concentrations, in order to assign the vibrational spectrum of the isolated uracil molecule and to observe the changes in the spectrum resulting from intermolecular hydrogen bonding.

After this work had been completed, matrix isolation studies of uracil and 1,3-deuterated uracil were reported by SZCZESNIAK *et al.* [30] and by MALTESE *et al.* [31]. HARSANYI and CSASZAR [32] have calculated both the in-plane and out-of-plane vibrations of uracil by the CNDO/2 force method.

EXPERIMENTAL

Uracil was a commercial product. The 1,3-deuterated compound was prepared by repeated exchange of uracil with D_2O, refluxing for 2–3 h each time. The various C-deuterated uracils were synthesized from orotic acid or iso-orotic acid according to the scheme shown in Fig. 1. Matrix samples were



(a) Reflux with D_2O.
(b) Reflux with Cu and quinoline.
(c) Recrystallize from H_2O.
(d) Reflux with acidified D_2O.
(e) Reflux with H_2O.

Fig. 1. Method of synthesis of C-deuterated uracils.

prepared by heating the solid uracil to a temperature in the range 110–170°C and condensing the resulting vapour simultaneously with a stream of argon or nitrogen matrix gas, using the apparatus described in Ref. [28]. The matrix was deposited on a caesium iodide window *(i.r.)* or a highly polished metal block (Raman) maintained at *ca.* 20 K by a C.T.I. Cryodyne closed-cycle refrigerator. The concentration of the uracil in the matrix was varied by changing the temperature to which the solid uracil was heated, and the time taken to deposit sufficient sample for spectroscopic examination varied from a few min to several h. No evidence of decomposition of the uracil was found in any of the experiments. Doping experiments were carried out by depositing the vapour from the solid uracil simultaneously with a 1 : 1 : 100 mixture of the dopant (nitrogen or water) and argon matrix gas. Infrared spectra were recorded on a Perkin–Elmer 225 spectrophotometer and Raman spectra on a Spex Ramalog 5M spectrometer with a Spectraphysics 171 argon ion laser source, adjusted to give a power of 100–200 mW at the sample.

RESULTS

Infrared and Raman spectra of uracil were obtained in both argon and nitrogen matrices using vaporization temperatures ranging from 110 to 170°C. At the lowest temperature used, primarily monomeric uracil was trapped whereas at higher temperatures a mixture of monomer and associated species was obtained. Infrared spectra of the solid at 20 K, deposited from the vapour phase and annealed at room temperature, and in a KBr pellet at room temperature were also recorded. Comparison of the spectra enabled bands to be assigned to either monomer or associated species and the results obtained are summarized in Table 1 (the assignments will be discussed in detail below).

The effects of possible impurities in the argon matrix spectra were checked by recording spectra of matrices

Table 1. Bands observed (cm^{-1}) in vibrational spectra of uracil in matrices, compared with calculated wavenumbers and the spectra of solid uracil

			Ar (monomer)		N_2 (monomer)		Ar(ass)	solid i.r.	
mode	approx description	calc^a	i.r.	Raman	i.r.	Raman	i.r.	20 K	KBr
ν_1	N_1H stretch	3479	3485 s	3486 m	3468 s		3020 br	3210 sh	3210 br
ν_2	N_3H stretch	3453	3435 s	3436 m	3421 ms			3110 s	3110 s
ν_3	C_5H stretch	3068		3084 m					3080 sh
ν_4	C_6H stretch	3031							
$\nu_5 + \nu_{13}$								2945 w	2935 m
$\nu_8 + \nu_9$								2865 w	2860 w
$2\nu_9$								2830 w	2820 w
$\nu_{10} + \nu_{21}$			1774 s		1778 m				
ν_5	C_2=O stretch	1775	1764 vs 1761 vs	1762 m	1761 vs		1683	1718 vs	1717 vs
$\nu_{15} + \nu_{17}$			1757 vs		1756 ms			1765 w	1768 m
$\nu_{11} + \nu_{21}$			1741 s		1746 ms				
$\nu_{13} + \nu_{18}$			1733 s 1731 s						
$\nu_{12} + \nu_{20}$			1728 vs	1733 m	1735 vs				1740 s
$\nu_{13} + \nu_{19}$			1718 m	1722 m					
ν_6	C_4=O stretch	1748	1706 vs	1707 m	1704 vs	1702 m		1675 s	1673 s
$\nu_{13} + \nu_{20}$			1698 ms		1707 s				
ν_7	C=C str/CH bend	1664	1643 m	1647 s	1644 m	1648 vs		1640 s	1650 sh
				1603 s		1604 s			
$2\nu_{24}$			1515 w		1526 w		1535		1508 mw
ν_8	ring str/NH bend	1483	1472 ms		1477 m			1455 m	1454 m
ν_9	ring str/NH bend	1397	1399 vs		1405 ms	1414 s	1422	1424 s	1418 ms
ν_{10}	CH/NH i.p. bend	1378	1389 m 1386 mw		1388 w	1392 s		1395 mw	1391 m
ν_{11}	ring stretch	1372	1360 vw	1360 m	1368 w	1373 w		1344 vw	1355 vw
$2\nu_{26}$			1313 vw						
			1304 vw						
ν_{12}	CH i.p. bend	1247	1217 w	1220 s		1225 s		1220 sh	1217 w
ν_{13}	ring str/NH bend	1171	1184 vs	1195 s	1190 s	1199 m	1226 1215 1205 1200 1193	1240 vs	1237 s
$2\nu_{18}$			1100 w						
ν_{14}	NH/CH i.p. bend	1069	1073 w 1066 w		1069 w			1095 w	1098 w

Table 1 (*Contd.*)

ν_{15}	ring i.p. def.	978	(982 w)	1001 m	(977 w)	1004 m		1005 w	1006 w
ν_{16}	ring str/NH bend	932	958 w	958 w	962 w		987 966	994 m	993 m
ν_{22}	C_6H o.p. bend	875	842 vw		842 vw		840	825 m	823 m
ν_{23}	C_4=O/CH o.p. bend	832	804 s		811 m 809 m				
ν_{17}	ring breathe	740	759 sh	761 s		766 s		776 w	782 w
ν_{24}	C_2=O o.p. bend	755	757 s		762 s		768	761 m	760 m
ν_{25}	C_5H o.p. bend	684	718 w		724 w				
ν_{26}	N_3H o.p. bend	496	662 s		686 m 682 m		883 br	855 m	850 s
ν_{27}	N_1H o.p. bend	472	551 m		592 mw			805 sh	805 m
ν_{18}	ring i.p. def.	551	559 w 555 w		558 w.		571	538 w	585 w
ν_{19}	C=O i.p. bend	532	536 w		538 w			564 m	566 m
ν_{20}	ring i.p. def.	502	516 m		517 m		529	544 s	549 s
								536 sh	530 sh
ν_{28}	ring o.p. def.	338			400 mw		407	400 sh	428 w
ν_{21}	C=O i.p. bend	374	391 m		393 m		415	435 s	436 m
ν_{29}	ring o.p. def.	119							194 m [b]
ν_{30}	ring o.p. def.	66							

[a] Calculated wavenumbers for the in-plane modes from NISHIMURA *et al.* [19] scaled by 0.90; for the out-of-plane modes from HARSANYI and CSASZAR [32].

[b] Ref. [16].

Abbreviations: vs = very strong; s = strong; ms = medium strong; m = medium; mw = medium weak; w = weak; vw = very weak; br = broad; sh = shoulder; i.p. = in-plane; o.p. = out-of-plane.

doped with nitrogen or water. The main effect of nitrogen impurity was the appearance of additional bands at 3468, 3424, 689 and 582 cm^{-1}, with a corresponding reduction in the intensities of the bands at 3485, 3435, 662 and 551 cm^{-1}. The nitrogen-induced bands are all close to the wavenumbers of the corresponding modes in a pure nitrogen matrix (Table 1). Addition of water led to the appearance of bands at 1698 and 767 cm^{-1}, the band at 757 cm^{-1} decreasing in relative intensity. New absorptions also appeared around 400 cm^{-1}, but it was not clear whether these were due to water itself or perturbed modes of the uracil.

Infrared spectra of 5-, 6-, 5,6-, 1,3-, 1,3,5-, 1,3,6- and *per*-deuterated uracils were obtained in argon matrices at different vaporization temperatures and also in the solid phase. The bands observed for the monomers in argon matrices are summarized in Table 2 and the solid phase spectra of uracil and its 5,6-, 1,3- and *per*-deuterated counterparts are compared in Table 3.

VIBRATIONAL ASSIGNMENT

Uracil has 30 fundamental modes of vibration, which divide into 21 *a′* and nine *a″* modes. The quantum mechanical calculation of the in-plane modes of vibration by NISHIMURA *et al.* [19] proved of great value in assigning the spectra. The calculated wavenumbers scaled by a constant factor of 0.90 (Fig. 2) reproduced closely the observed wavenumbers for uracil and were reasonably close to the observed wavenumbers for most of the modes of the deuterated uracils.

NH *stretching modes*

Infrared spectra of uracil and deuterated derivatives in the NH(ND) stretching region are illustrated in Fig. 3. Monomeric uracil gave a pair of bands at 3485 and 3435 cm^{-1}, close to the wavenumbers reported by NOWAK *et al.* [12] in the vapour phase (3486 and 3445 cm^{-1}). In a nitrogen matrix a similar pair of bands was obtained, shifted to lower wavenumbers (3468 and 3421 cm^{-1}). In both matrices, the higher wavenumber band was more intense, although the difference was smaller in the argon matrix. The quantum mechanical calculations [19] found the higher frequency mode to be the N_1H stretch and the lower frequency mode to be the N_3H stretch, although the calculated splitting (26 cm^{-1}) is rather smaller than that observed either in the gas phase or in matrices (40–50 cm^{-1}). Aggregated uracil in argon or nitrogen matrices exhibited a broad band centred around a prominent negative absorption (Evans hole) at *ca.* 3000 cm^{-1}. The pure solid gave a strong, broad absorption centred around 3110 cm^{-1}. The C-deuterated uracils gave virtually identical spectra in the NH stretching region.

Table 2. Bands observed (cm^{-1}) in the vibrational spectra of monomer uracil and deuterated uracils in argon matrices at 20 K

mode	approx description	uracil	5-d	6-d	5,6-d	1,3-d	1,3,5-d	1,3,6-d	per-d
ν_1	N_1H stretch	3485 s	3485 s	3485 s	3485 s	2593 m	2587 ms	2583 m	2582 m
$\nu_6 + \nu_{16}$							2566 w	2560 w	2567 w
ν_2	N_3H stretch	3435 s	3435 s	3435 s	3435 s	2549 m	2538 w	2542 w	2539 w
ν_3	C_5H stretch	3084[a]							
ν_4	C_6H stretch								
						1768 m			
$\nu_{10} + \nu_{21}$		1774 s		1775 s		1761 s			
						1751 m			
ν_5	C_2=O stretch	1764 vs 1761 vs	1764 vs	1764 vs	1764 vs	1744 vs	1751 vs	1750 vs	1749 vs
$\nu_{12} + \nu_{15}$				1757 vs	1757 vs				
$\nu_{15} + \nu_{17}$		1757 vs	1743 s	1742 s	1742 s	1731 s	1730 s	1730 s	1729 s
$\nu_{11} + \nu_{21}$		1741 s	1724 ms	1724 vs	1724 vs				
$\nu_{13} + \nu_{18}$		1733 s 1731 s	1733 s 1731 s						
$\nu_{12} + \nu_{20}$		1728 vs							
$\nu_{13} + \nu_{19}$		1718 m	1716 s	1714 vs	1713 s				
ν_6	C_4=O stretch	1706 vs	1704 s	1731 vs	1731 vs	1715 vs	1715 vs	1710 vs	1710 vs
						1705 s	1710 vs		
$\nu_{13} + \nu_{20}/\nu_9 + \nu_{21}$		1698 ms	1698 s			1698 m	1701 s		1701 s
			1695 s			1694 m	1692 s		
$\nu_{16} + \nu_{18}/\nu_{12} + \nu_{16}$				1631 m	1631 m			1631 mw	1632 w
ν_7	C=C str/CH bend	1643 m	1631 s	1608 s	1607 s	1631 m	1632 ms	1601 ms	1602 ms
$2\nu_{24}$		1515 w	1512 w	1515 w	1511 vw	1511 mw	1510 w	1510 w	1508 w
ν_8	ring str/NH bend	1472 ms	1461 vs	1462 s	1461 ms	1438 vs	1431 vs	1431 vs	1431 vs
				1457 m				1427 s	1427 s
								1418 m	1418 s
ν_9	ring str/NH bend	1399 vs	1397 vs	1397 ms 1395 s	1397 ms 1395 ms	1326 ms 1316 s	1321 s 1315 ms	1321 m 1315 w	1321 m 1315 mw
				1381 vw	1381 w			1393 vw	1392 w
ν_{10}	CH/NH i.p. bend	1389 m 1386 mw		1318 w		1264 w 1254 m		1240 vw 1234 w	
ν_{11}	ring stretch	1360 vw	1343 w	1343 vw	1343 w	1379 vs	1353 mw	1355 m	1353 w
						1310 w	1347 w		1330 w
$2\nu_{26}$		1313 vw		1312 vw	1312 vw				
		1304 vw	1304 vw	1304 vw	1304 w	1300 w			
				1273 w	1273 w				1240 vw
				1254 w	1254 w				1233 w
ν_{12}	CH i.p. bend	1217 w	1312 w			1210 w 1201 w	1241 m 1232 w		
ν_{13}	ring str/NH bend	1184 vs	1187 vs	1187 s 1184 ms	1187 s	1112 m	1111 m	1111 w	1111 w
$2\nu_{18}$			1169 w	1169 vw	1169 vw				
				1160 vw	1160 vw				
$\nu_{17} + \nu_{21}$			1150 w 1147 w	1150 vw 1147 vw	1150 vw 1147 vw				
			1125 vw	1115 w	1115 w				
$2\nu_{27}$		1100 w	1100 vw	1100 vw	1095 vw				
ν_{14}	NH/CH i.p. bend	1073 w 1066 w	1201 w 1195 w	1078 m	1204 w				
			1082 w						
$2\nu_{20}$				1018 w		1010 vw	1010 vw	1010 vw	1010 vw
ν_{15}	ring i.p. def.	1001[a]	973 w	975 w	968 vw	970 w	959 w	959 vw	959 w
(ν_{14})	ND i.p. bend					918 w	926 w	881 mw	880 mw

Table 2 (*Contd.*)

ν_{16}	ring str/NH bend	958 w	965 w	1047 ms	1047 ms	821 mw	849 m	849 w	849 w
(ν_{10})	C_5D i.p. bend		926 w		926 vw		975 vw		975 vw
$\nu_{19} + \nu_{21}$				926 vw				926 vw	926 vw
				850 w					
				804 mw				804 mw	
ν_{22}	C_6H o.p. bend	842 vw	804 w			838 w	804 w		
(ν_{12})	C_6D i.p. bend			784 w	784 w			784 w	783 w
ν_{23}	C_4=O/CH o.p. bend	804 s	776 ms	777 m	777 m	803 s	776 ms	776 mw	776 m
ν_{17}	ring breathe	761^a			760^a				
				761 ms				758 ms	
ν_{24}	C_2=O o.p. bend	757 s	757 s	758 s	758 s	756 s	755 s	756 s	754 s
ν_{25}	C_5H o.p. bend	718 w		702 w		718 vw			
ν_{26}	N_3H o.p. bend	662 s	662 vs	662 s 658 s	662 ms 658 ms				
(ν_{22})	C_6D o.p. bend								
ν_{18}	ring i.p. def.	559 w 555 w	591 m	591 m	591 m	541 w	575 w	575 w	575 w
ν_{27}	N_1H o.p. bend	551 m	552 w	551 mw	550 w				
ν_{19}	C=O i.p. bend	536 w	539 mw	537 w	537 w	(541 w)	538 vw	539 w	532 w
(ν_{25})	C_5D o.p. bend		520 w		520 w				
ν_{20}	ring i.p. def.	516 m	512 m	515 mw 512 mw	512 m	505 ms	506 ms	507 m	507 mw
(ν_{26})	N_3D o.p. bend					501 m	500 ms	498 m	498 m
								412 w	413 w
(ν_{27})	N_1D o.p. bend					420 w		370 mw	370 mw
ν_{28}	ring o.p. def.		393 w	358 w	358 w	370 s	370 ms	343 w	343 w
ν_{21}	C=O i.p. bend	391 m	390 m	390 m	390 m	388 ms	386 m	386 m	386 ms
ν_{29}	ring o.p. def.								
ν_{30}	ring o.p. def.								

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[a] From Raman spectrum (not observed in i.r.).

Table 3. Bands observed (cm^{-1}) in the vibrational spectra of uracil and deuterated uracils in the solid phase at 20 K

mode	approx description	uracil	5,6-d	1,3-d	per-d
ν_1	N_1H stretch	3210 sh	3210 sh	2330 ms	2302 s
ν_2	N_3H stretch	3110 s	3100 s	2270 s	2265 s
ν_3	C_5H stretch	3080 sh^a	2310 w	3092 m	
ν_4	C_6H stretch		2295 sh	3000 w	
$\nu_6 + \nu_9$				2946 mw	2935 w
$\nu_5 + \nu_{13}$		2945 w	2940 m	2834 m	2830 w
$\nu_8 + \nu_9$		2865 w	2865 w		
$2\nu_9$		2830 w	2830 w		
$\nu_{15} + \nu_{17}$		1765 w			
ν_5	C_2=O stretch	1718 vs	1720 vs	1700 vs	1700 vs
ν_6	C_4=O stretch	1675 s	1675 s	1630 vs	1630 vs
ν_7	C=C str/CH bend	1640 s	1630 s	1600 sh	1600 sh
$2\nu_{24}$		1520 vw	1520 vw	1515 w	1530 sh
ν_8	ring str/NH bend	1455 m	1454 m	1460 m	1455 m
ν_9	ring str/NH bend	1424 s	1420 ms	1335 m	1332 w
ν_{10}	CH/NH i.p. bend	1395 mw		1280 w	

Table 3 (*Contd.*)

ν_{11}	ring stretch	1344 vw	1354 w	1395 w	1352 w
ν_{13}	ring str/NH bend	1240 s	1239 m	1140 m	1140 w
ν_{12}	CH i.p. bend	1220 sh			
ν_{14}	NH/CH i.p. bend	1095 w	1290 w	968 m	955 mw
ν_{15}	ring i.p. def.	1005 w	984 mw	980 sh	975 sh
ν_{16}	ring str/NH bend	994 m	1094 m	878 m	875 w
(ν_{10})	C_5D i.p. bend		895 sh		
ν_{26}	N_3H o.p. bend	855 m	868 m		
(ν_{12})	C_6D i.p. bend		846 w		842 w
ν_{22}	C_6H o.p. bend	825 m		830 m	
ν_{27}	N_1H o.p. bend	805 sh	830 sh		
ν_{23}	C_4=O/CH o.p. bend				
ν_{17}	ring breathe	776 w	777 w	778 w[b]	776 vw
ν_{24}	C_2=O o.p. bend	761 m	761 m	764 ms	763 m
ν_{25}	C_5H o.p. bend			740 w	
(ν_{26})	N_3D o.p. bend			615 w	604 w
(ν_{27})	N_1D o.p. bend			589 w[c]	
(ν_{22})	C_6D o.p. bend		611 vw		
ν_{18}	ring i.p. def.	588 w	580 vw		
ν_{19}	C=O i.p. bend	564 m	562 w	562 m	557 w
(ν_{25})	C_5D o.p. bend		552 sh		
ν_{20}	ring i.p. def.	544 s	538 s	534 s	532 m
ν_{21}	C=O i.p. bend	435 s	434 s	430 s	432 s
ν_{28}	ring o.p. def.	400 sh	400 sh	400 sh	400 sh
ν_{29}	ring o.p. def.	194 m[d]			
ν_{30}	ring o.p. def.				

[a] KBr disc.
[b] Ref. [5].
[c] Ref. [20].
[d] Ref. [16].

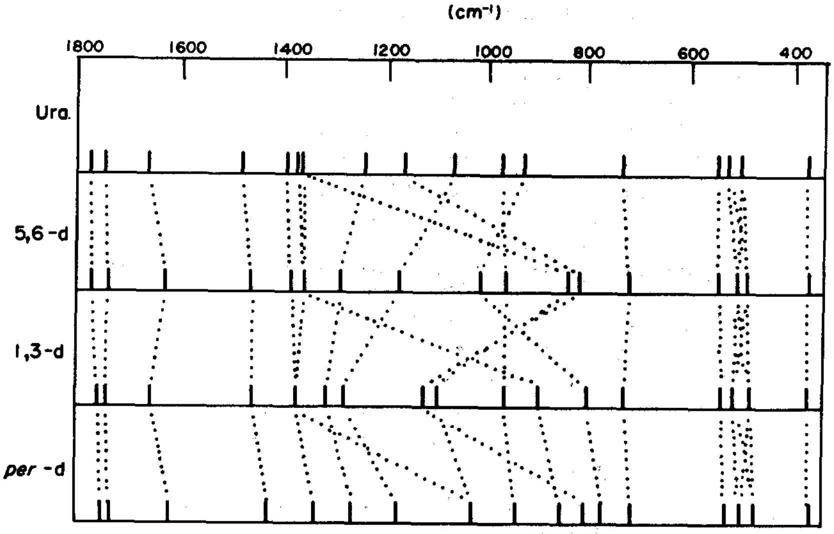
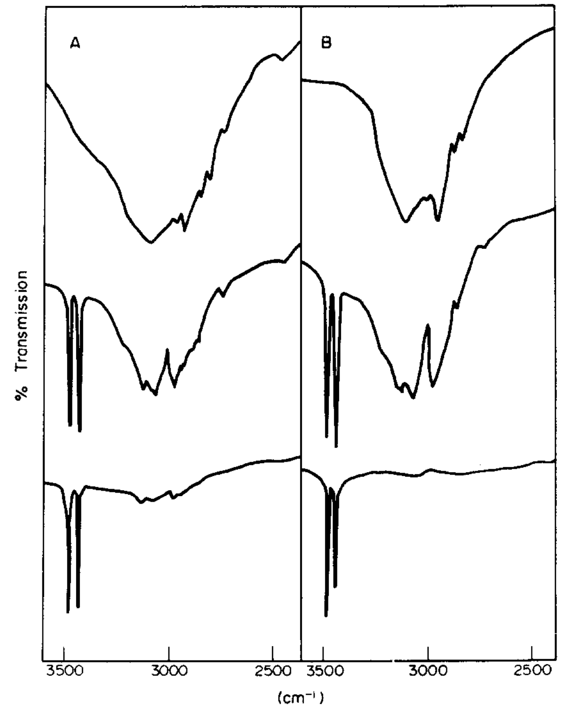


Fig. 2. Calculated vibrational spectra of uracil and deuterated derivatives from NISHIMURA *et al.* [19] (wavenumbers scaled by a constant factor of 0.90).

1,3-Deuterated uracil in an argon matrix exhibited monomer ND stretching bands at 2593 and 2549 cm^{-1}, with relative intensities similar to those of the NH stretching modes of uracil, but in *per*-deuterated uracil the lower frequency component splits to give a pair of weak bands at 2567 and 2539 cm^{-1} (Fig. 3). The 1,3,5- and 1,3,6-deuterated uracils gave a similar pattern, which may be attributed to Fermi resonance between the N_3D stretch and the combination 1710 + 849 cm^{-1}. The aggregated or solid N-deuterated uracils gave a pair of bands around 2300 cm^{-1}.

CH stretching modes

The CH (or CD) stretching modes of the monomeric uracils were too weak to be observed with certainty in the i.r. spectra in argon or nitrogen matrices. Similar behaviour was found in previous studies of maleimide [26] and barbituric acid derivatives [27–29]. In the Raman spectrum of uracil in an argon matrix, a band was observed at 3084 cm^{-1}. NISHIMURA *et al.* [19] calculated 3068 and 3031 cm^{-1} (after scaling) for the C_5H and C_6H stretching modes respectively, thus the single observed band has been assigned tentatively to the C_5H stretch. In the i.r. spectrum of solid uracil, SUSI and ARD [5] assigned the strong band near 3100 cm^{-1} to a CH stretching mode, but comparison with the spectrum of the 5,6-deuterated uracil shows that this should be assigned to NH stretching with the low frequency shoulder (*ca.* 3080 cm^{-1}) as the CH stretching modes. This agrees well with the i.r. spectrum of solid 1,3-deuterated uracil which has a medium intensity band at 3092 cm^{-1}.



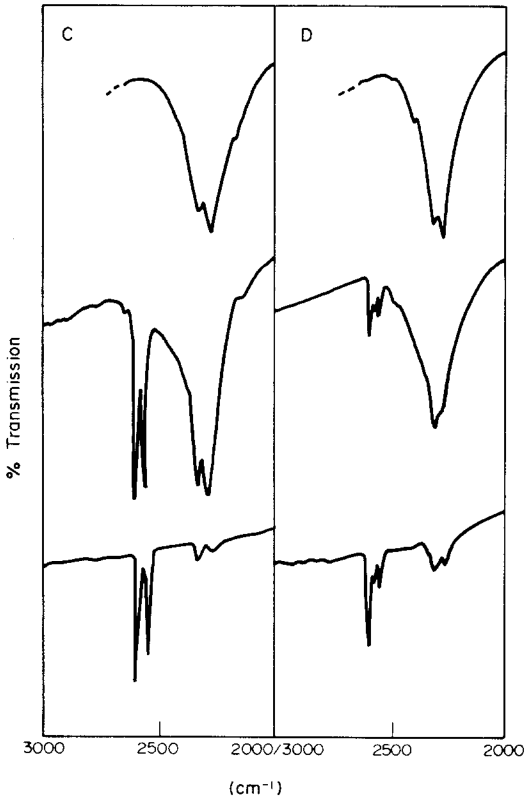


Fig. 3. Infrared spectra in the NH(ND) stretching region of (A) uracil, (B) 5,6-deuterated uracil, (C) 1,3-deuterated uracil, (D) *per*-deuterated uracil. In each case, the top spectrum is the solid at 20 K, the middle spectrum a concentrated argon matrix and the bottom spectrum a dilute argon matrix.

C=O and C=C stretching modes

The 1800–1600 cm^{-1} region of uracil and its deuterated derivatives was found to contain a considerable number of medium to strong bands. Only three fundamentals, which may be approximately described as the $C_2{=}O$, $C_4{=}O$ and C=C stretching modes, are expected in this region. The (scaled) calculated wavenumbers are 1775, 1748 and 1664 cm^{-1} respectively. The C=C stretching mode was readily identified at 1643 cm^{-1} in the i.r. spectrum of uracil in an argon matrix, with a corresponding intense band in the Raman spectrum. This mode was found to shift to 1631–1632 cm^{-1} in the 5-, 1,3- and 1,3,5-deuterated derivatives, but to 1601–1608 cm^{-1} in the uracils deuterated in the 6 position. This agrees well with the potential energy distribution of NISHIMURA *et al.* [19], which shows an appreciable contribution from C_6–H bending. Bands at 1631–1632 cm^{-1} in the uracils deuterated in the 6 position may be assigned to combinations 1047 + 591 cm^{-1} (6- and 5,6-deuterated uracil) or 849 + 783 cm^{-1} (1,3,6- and *per*-deuterated uracil), intensified by Fermi resonance with the C=C stretching mode. The C=O stretching region is very complex in uracil, becoming simpler as the degree of deuteration increases (Fig. 4). There is a general

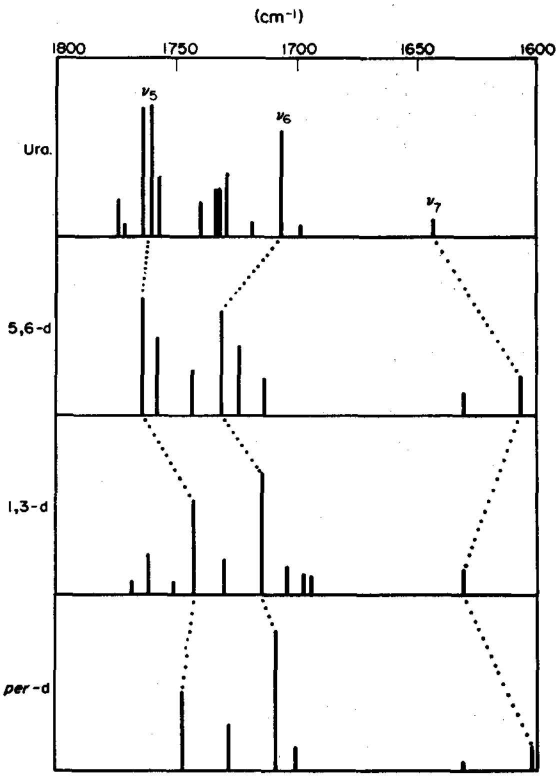


Fig. 4. Schematic representation of the C=O and C=C stretching region of the i.r. spectrum of monomeric uracil and deuterated derivatives in argon matrices. The absorbances have been scaled relative to the band at 757 cm^{-1} in each spectrum.

pattern of two very strong bands (assigned to ν_5 and ν_6) with an intermediate strong band, which has been assigned as $\nu_{15} + \nu_{17}$ (these modes showing only small shifts on deuteration). The deuteration behaviour of the remaining bands allows tentative assignments to be made (Tables 1 and 2), but with a large number of possible combinations and the perturbations resulting from multiple Fermi resonance interactions little weight should be attached to these assignments. In the associated or solid uracils the C=O stretching modes shift to lower frequencies (Table 3) and little evidence remains of the various combination bands observed in the monomer matrix spectra. BANDEKAR and ZUNDEL [21, 22] ascribe the C=O band splittings in solid uracil to C=O transition dipole–dipole coupling. Since the splittings observed in the monomer spectra can only be due to Fermi resonance interactions, it would seem more plausible that any splittings present in the solid phase spectrum have a similar origin.

Other in-plane modes

The calculations of NISHIMURA *et al.* [19] predict strong mixing of the NH and CH in-plane bending modes with the ring stretching vibrations. The observed matrix spectra confirm this general picture. Figure 5 compares the spectra of argon matrices containing a mixture of monomer and associated uracil and containing predominantly monomer uracil. It can be seen that a number of absorptions shift to significantly higher wavenumbers in the associated species, particularly the modes absorbing at 1399, 1184, 958 and 391 cm^{-1} in the monomer which all

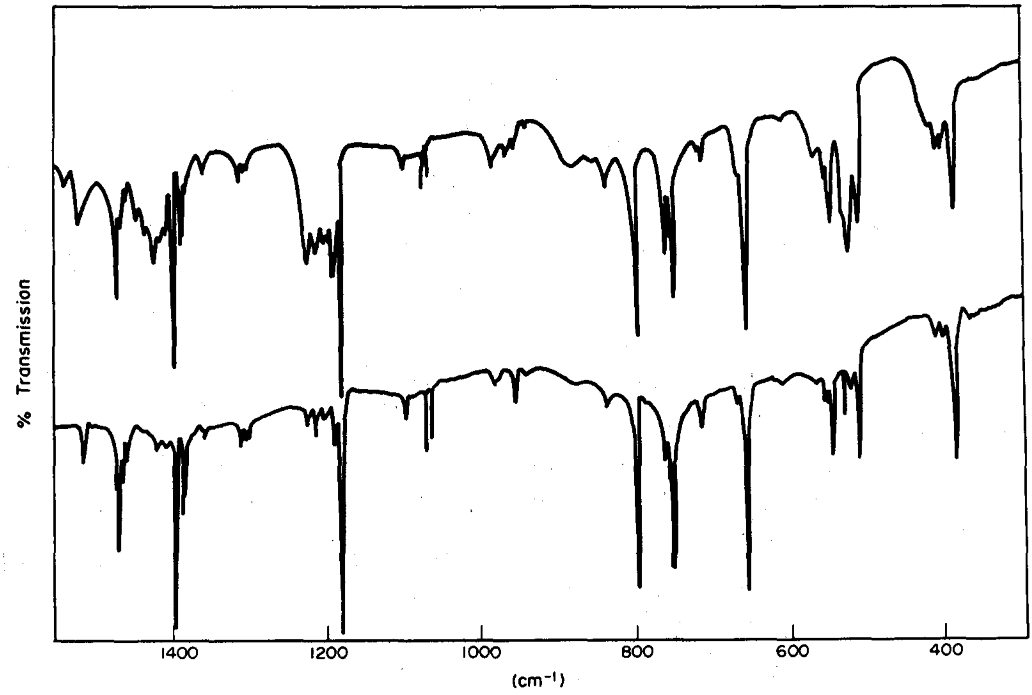


Fig. 5. Infrared spectra of a concentrated matrix (upper) and a dilute matrix (lower) of uracil in argon.

exhibit prominent absorptions due to the associated species *ca.* 25 cm^{-1} above the monomer wavenumber. The NH out-of-plane bending modes also shift to much higher wavenumbers, appearing at around 850 cm^{-1} in the associated species. The monomer spectra of the various deuterated species are compared in Fig. 6.

The highest frequency mode (ν_8) was found at 1472 cm^{-1} for uracil in an argon matrix, shifting to 1461 cm^{-1} in 5,6-deuterated uracil, 1438 cm^{-1} in 1,3-deuterated uracil and 1431 cm^{-1} in the *per*-deuterated derivative. This pattern agrees well with the quantum mechanical calculations [19], which give a PED of ring stretching (50) and N_1H bending (26) with a calculated wavenumber (scaled) of 1483 cm^{-1} for uracil. Also in excellent agreement with these calculations, three fundamentals were found between 1400 and 1350 cm^{-1}: a strong band at 1399 cm^{-1}, a doublet (presumably due to matrix splitting, the corresponding absorption in a nitrogen matrix being a singlet at 1388 cm^{-1}) at 1389 and 1386 cm^{-1}, and a very weak band in the i.r. spectrum, with an intense counterpart in the Raman, at 1360 cm^{-1}. The deuteration behaviour of these bands was entirely consistent with the assignment of the highest and lowest wavenumber bands as ring stretching modes (ν_9, ν_{11}) and the 1389/1386 cm^{-1} doublet as a CH bending/NH bending mode (ν_{10}). The 1399 cm^{-1} mode must have an

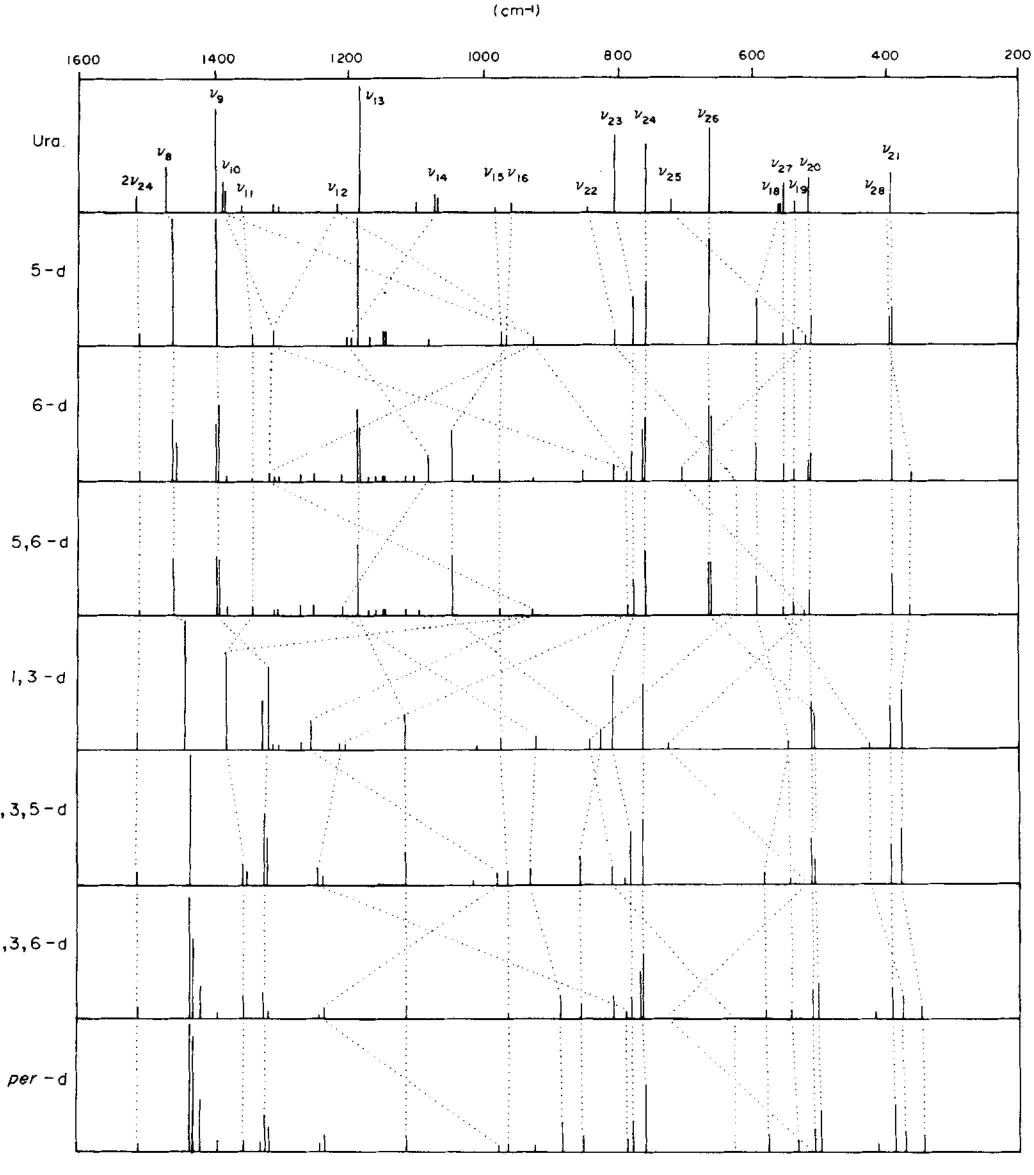


Fig. 6. Schematic representation of the 1600–200 cm^{-1} region of the i.r. spectrum of monomeric uracil and deuterated derivatives in argon matrices. The absorbances have been scaled relative to the band at 757 cm^{-1} in each spectrum.

appreciable contribution from NH bending, shown by its association behaviour and the shift to lower wavenumbers in the 1,3-deuterated derivatives. In 1,3-deuterated uracil, ν_{10} appeared as a doublet at 1264/1254 cm^{-1}.

The next three modes show the poorest agreement with the calculations of NISHIMURA *et al.* [19], which predicted the Kekule ring stretch at 1247 cm^{-1}, the CH out-of-phase bending mode at 1171 cm^{-1} and a ring stretching mode at 1069 cm^{-1}. For uracil, the observed wavenumbers (1217, 1184 and 1073/1066 cm^{-1}) agree reasonably well with the predicted values, but the deuteration behaviour was not as predicted. The very strong band at 1184 cm^{-1} in the i.r. spectrum of uracil (which shifts to higher wavenumbers in the associated species) has obvious counterparts at 1187 cm^{-1} in 5,6-deuterated uracil and at 1111–1112 cm^{-1} in 1,3- and *per*-deuterated uracil. It should thus be described as ring stretching/NH bending (ν_{13}). The 1217 cm^{-1} band (ν_{12}) seems to be mainly CH bending in character, while the 1073/1066 cm^{-1} doublet (the splitting again being a matrix effect, with a single band in a nitrogen matrix at 1069 cm^{-1}) appears to be NH bending mixed with CH bending. In the deuterated uracils, the ND bending and CD bending modes are relatively weak and thus difficult to identify with certainty. Following the behaviour predicted by NISHIMURA *et al.* [19], we assign the bands around 900 cm^{-1} in the 1,3-deuterated uracils to ND bending and bands at 926 cm^{-1} (5,6-deuterated), 975 cm^{-1} (*per*-deuterated) and 783–784 cm^{-1} as the CD bending modes.

The remaining modes are all ring stretching and deformation vibrations. The ring deformation mode ν_{15}, predicted at 978 cm^{-1}, was identified from the Raman spectrum as a band at 1001 cm^{-1} in an argon matrix; only weak bands were found in this region of the i.r. spectrum. The ring stretching mode ν_{16} was found in both i.r. and Raman spectra at 958 cm^{-1} and the ring breathing mode ν_{17} could be readily identified as the intense Raman band at 761 cm^{-1}. The deformation modes ν_{15} and ν_{17} were little affected by deuteration, but the ring stretching mode ν_{16} appeared to mix strongly with the CD and ND bending modes, shifting to 1047 cm^{-1} in 5,6-deuterated uracil, 821 cm^{-1} in 1,3-deuterated uracil and 849 cm^{-1} in the *per*-deuterated derivative. This pattern of shifts is quite similar to that predicted by NISHIMURA *et al.* [19]. Since the combination $\nu_6 + \nu_{16}$ exhibits a Fermi resonance interaction with the N_3D stretching mode (and not the N_1D stretching mode) in the 1,3,5-, 1,3,6- and *per*-deuterated uracils, ν_{16} must contain a large contribution from N_3D bending in these compounds. The ring deformation mode ν_{18} appeared as a doublet at 559/555 cm^{-1} for uracil in an argon matrix (the 551 cm^{-1} band was identified as the NH out-of-plane bending mode from its shift on doping with nitrogen), shifting to 591 cm^{-1} in the C-deuterated derivatives. In 1,3-deuterated uracil ν_{18} appeared to be accidentally degenerate with ν_{19} at 541 cm^{-1}, shifting to 575 cm^{-1} in *per*-deuterated uracil. The remaining three modes were readily identified for uracil in an argon matrix as 536 cm^{-1} (ν_{19}), 516 cm^{-1} (ν_{20}) and 391 cm^{-1} (ν_{21}), all exhibiting only small shifts on deuteration.

Carrying these assignments for monomeric uracil through to the solid phase spectra yielded an assignment (Table 3) which differs in several respects from that given by SUSI and ARD [5]. It is clear from the matrix spectra of uracil and its deuterated counterparts that the band at 1520 cm^{-1} in solid uracil, assigned by SUSI and ARD [5] as ν_8, should be assigned to the overtone $2\nu_{24}$. A very weak band at 1344 cm^{-1} in the i.r. spectrum of solid uracil may be assigned as the counterpart of the 1360 cm^{-1} mode of monomeric uracil. The remaining assignments for solid uracil are substantially the same (although the descriptions of the modes differ) as those of SUSI and ARD [5] apart from ν_{21}. The strong band at 435 cm^{-1} in the solid spectrum clearly corresponds to the monomer band at 391 cm^{-1}, which we have assigned to the in-plane mode since it is unaffected by deuteration whereas the nearby out-of-plane bending mode shifts to lower frequency in the deuterated derivatives (presumably due to mixing with the ND and CD out-of-plane bending modes). Similar differences occur in the assignments for the deuterated uracils.

Out-of-plane bending modes

Although the calculations of NISHIMURA *et al.* [19] were limited to the in-plane modes, the matrix spectra enabled the out-of-plane vibrations to be assigned with reasonable confidence. SUSI and ARD [5] assigned the CH out-of-plane bending modes of solid uracil to strong bands at *ca.* 820 and 760 cm^{-1}. The argon matrix spectrum of monomer uracil has intense bands at 804 and 757 cm^{-1}, but there are corresponding bands in the spectra of all the deuterated uracils (the 803 cm^{-1} absorption shifting to 776 cm^{-1} in the C-deuterated derivatives). These bands must thus be assigned to C=O out-of-plane bending vibrations. Since the higher frequency mode is clearly mixed with the CH out-of-plane bending modes, it is likely to be the C_4=O bending mode rather than the C_2=O bending mode. The CH out-of-plane bending modes seem to be rather weak and have been tentatively assigned to 842 and 718 cm^{-1} for uracil in an argon matrix, the corresponding CD bending modes also being difficult to identify with certainty.

The NH out-of-plane bending modes were easily identified as 662 cm^{-1} and 551 cm^{-1} for uracil in an argon matrix, shifting to 686/682 cm^{-1} and 592 cm^{-1} in a nitrogen matrix; similar shifts occurred in the nitrogen-doped argon matrix spectra. Large shifts of the NH out-of-plane bending modes between argon and nitrogen matrices were found in previous studies of maleimide [26] and barbituric acid derivatives [27–29]. In solid uracil or associated uracil in matrices the NH out-of-plane bending modes shift up to *ca.* 850 cm^{-1}. Comparison with 1-methyl uracil and 3-methyl uracil has shown [20] that the higher wavenumber band in the solid spectrum is the N_3H mode and the lower wavenumber band the N_1H mode; it has been assumed that the same order applies to monomeric uracil. In matrices, there seems to be little mixing of the NH and CH out-of-plane vibrations but in the solid phase the modes lie in the same frequency region and thus there may be appreciable mixing. The normal coordinate calculations of BANDEKAR and ZUNDEL [22] support this view. Comparison of the monomer and solid spectra shows that the band assigned to the $C_2{=}O$ bending mode in the monomer spectrum remains a strong band in the i.r. spectrum of the solid, whereas the band assigned to $C_4{=}O$ bending (mixed with CH bending) in the monomer spectrum is not readily identified in the solid spectrum. Possibly the weak bands in the i.r. spectra at *ca.* 776 cm^{-1} should be assigned to this mode rather than to the ring breathing mode, which was observable only in the Raman spectra for monomeric uracil.

In 1,3-deuterated uracil, the ND out-of-plane bending modes were identified as 501 and 420 cm^{-1} (a shift of *ca.* 1.31) in an argon matrix. In *per*-deuterated uracil there appears to be considerable mixing of the ND, CD and ring bending modes (the ring deformation mode ν_{28} also shifts from *ca.* 400 cm^{-1} in uracil to 358 cm^{-1} in 5,6-deuterated uracil, suggesting mixing with the CD bending modes). In the spectra of the solid 1,3-deuterated uracils, the ND out-of-plane bending modes appear at *ca.* 620 cm^{-1}. The two lowest frequency ring deformation modes, ν_{29} and ν_{30}, were below the range covered by the matrix experiments.

DISCUSSION

The generally excellent agreement between the matrix spectra of monomeric uracil and the calculated spectrum of NISHIMURA *et al.* [19] shows that their force field is a good approximation for the in-plane modes. Although hydrogen bonding shifts many of the modes appreciably, the solid spectra may be assigned analogously to the monomer. The out-of-plane modes could not be assigned with complete certainty, the change in potential energy distribution between the monomer and solid spectra (as a result of the large shift in the NH bending modes) making comparison difficult. The assignment for monomer uracil shows good agreement with the CNDO calculation of HARSANYI and CSASZAR [32].

The spectrum of the associated species formed by uracil in matrices differs significantly from the spectrum of the solid phase. The NH stretching absorption is shifted to lower frequencies than in the solid, and the NH out-of-plane bending to higher frequencies, implying that the strength of interaction is greater in the associated species in matrices than in the solid. There is some evidence for selective participation in the hydrogen bonding in the associated species by particular groups: in dilute matrices the N_1H monomer stretching band is more intense than the N_3H band, whereas in concentrated matrices the intensities reverse, suggesting participation of the N_1H group in hydrogen bonding. Similarly, in the C=O out-of-plane bending region, a prominent aggregate band appeared on the high frequency side of the $C_2{=}O$ mode, whereas the $C_4{=}O$ mode showed no such change. Thus the associated species in matrices is most probably a cyclic dimer, linked by $C_2{=}O \ldots H{-}N_1$ hydrogen bonds, whereas in the solid phase there is a network of hydrogen bonds involving the $C_4{=}O$ and both N–H groups [23, 24].

The spectrum of the water-doped uracil in argon matrix also provided some structural information. As in the associated uracil species, the $C_2{=}O$ out-of-plane bending mode shifted to higher frequency while the $C_4{=}O$ mode was unaffected. Thus the uracil–water complex appears to be formed by the water molecule hydrogen bonding to the $C_2{=}O$ group.

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