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TAUTOMERIC EQUILIBRIA OF 2(4)-MONOOXOPYRIMIDINES IN THE GAS PHASE, IN LOW-TEMPERATURE MATRICES AND IN SOLUTION

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ABSTRACT

IR absorption spectra, including the NH, OH and C=O stretching regions, have been recorded for 4-oxo-6-methyl- and 2-oxo-4,6-dimethyl pyrimidines and several related derivatives, in the gas phase, in low-temperature inert matrices, and in several liquid solvents.

All the 4-oxopyrimidines in the gas phase, and 4-oxo-6-methylpyrimidine in low-temperature matrices, exhibit comparable populations of the keto and enol forms. By contrast the 2-oxopyrimidines are predominantly in the enol forms. Both classes of compounds are predominantly in the keto form in liquid solvent systems. The tautomeric equilibrium constant (K_T) in the vapour phase for 4-oxo-2,6-dimethylpyrimidine is about 2, and for the other 4-oxopyrimidines is about 1. For 4-oxo-6-methylpyrimidine, the equilibrium constant in inert matrices varies slightly with the activity of the matrix gas, with the keto tautomer favoured in the more active matrix. From the temperature-dependence of K_T, the free energy difference between the two tautomeric forms of 4-oxo-6-methylpyrimidine in the vapour phase has been calculated. Heats of vaporization have also been calculated for the various compounds and related to their abilities to associate by hydrogen bonding in the condensed phase.

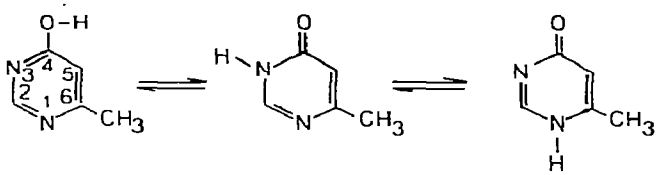
The UV absorption spectra of some of the foregoing have also been recorded in the gas phase, but these were of only limited value in studies of tautomeric equilibria, as compared to the IR spectra.

INTRODUCTION

The influence of the molecular environment on tautomerism in purines and pyrimidines is directly relevant to the role of these heterocyclic compounds in nucleic acid structure and function. These exist, under physiological conditions, in aqueous media whereas, following incorporation into nucleic acids, they are frequently in the aprotic environment prevailing in the interior of a helical structure. The striking role of the molecular environ-

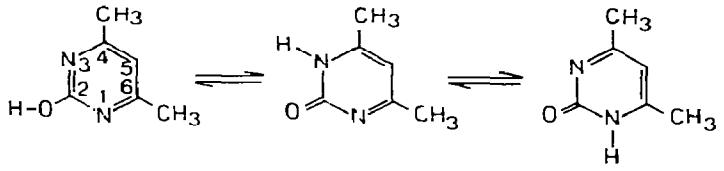
ment on tautomeric equilibria in nitrogen heterocycles has been well documented [1—15]. In particular it has been shown that the vapour phase protomeric equilibria for oxo- and mercapto-pyridines [10, 12, 14, 16—18] and pyrimidines [11, 12, 16] may differ from the corresponding equilibria for such systems in solution by factors of the order of 10^3—10^5.

We present here the results of a study, with the aid of IR and UV spectroscopy, of the keto—enol equilibria of 4-oxo-6-methylpyrimidine



1a 1b 1c

and 2-oxo-4,6-dimethylpyrimidine



2a 2b 2c

and related compounds in the gas phase, in several fluid solvents and in a variety of low-temperature matrices. The matrix studies are of relevance to the effects of weakly-interacting aprotic environments on the tautomeric equilibria, thus bridging the gap between data for the gas phase and for polar solvents. It should be noted that information about such equilibria in polar solvents is somewhat limited, in part because of solubility considerations. The emphasis in this study has been on IR spectroscopy, which permits direct observation of C=O, NH and OH frequencies involved in keto—enol tautomerism, with results more reliable than those obtained by UV spectroscopy [10, 11]. Apart from their intrinsic physico-chemical and biological interest, the results should prove useful in attempts to improve quantum chemical calculations.

EXPERIMENTAL

Compounds

4-oxo-6-methylpyrimidine and 4-oxo-pyrimidine were prepared from 4-thio-6-methyluracil and 4-thiouracil, respectively, by reduction with Raney nickel in methanol, followed by crystallization from ethanol—acetone [19]. Diazomethane treatment of 4-oxo-6-methylpyrimidine in methanol yielded 4-oxo-3,6-dimethylpyrimidine as the major product; following its isolation,

the mother liquors were subjected to preparative thin-layer chromatography to give 4-oxo-1,6-dimethylpyrimidine (5% yield) and 4-methoxy-6-methylpyrimidine (10—15% yield).

Published procedures were used for the synthesis of 2-oxopyrimidine [20], 2-oxo-4,6-dimethylpyrimidine [21] and 2-oxo-1,4,6-trimethylpyrimidine [21].

All compounds, obtained in crystalline form, were compared with published data. They were chromatographically homogeneous in several solvent systems, and exhibited the expected UV absorption spectra at different pH values in aqueous medium.

Solvents

Spectral grade CCl_4 (for IR spectroscopy, from P.O.Ch., Gliwice, Poland) was dried by distillation over P_2O_5, and then over metallic sodium. $CDCl_3$ (from the Institute for Nuclear Studies, Swierk, Poland) was dried over molecular sieves. Toluene and hexane, both analytical grades (P.O.Ch., Gliwice) were redistilled over molecular sieves. All of these were degassed by several cycles of freezing and thawing under vacuum.

Matrix gases

Spectral grade argon and krypton were obtained from Air Products Ltd. (Cambridge, England). CO_2 was prepared by thermal decomposition of magnesium carbonate; the resulting water was collected in a condenser at —50°C, and the CO_2 further dried by passage over P_2O_5 and then with anhydrous $CaCl_2$.

IR spectra of vapours

The IR spectra in the region of —OH and —NH stretching frequencies were obtained with the aid of cylindrical quartz cells 3 cm in diameter and with 7 cm pathlength. The material to be examined was introduced through a side tube which was sealed off after the cell had been evacuated to a pressure of about 10^{-5} mm Hg. The cell, together with an identical control, was mounted in a chamber heated through a thermoregulator. A copper—constantan thermocouple attached to the cell wall was employed for measurements of temperature, which was monitored continuously during an experiment and did not vary by more than 5°C. The quartz windows (Infrasil) of these cells were sufficiently transparent down to 2500 cm^{-1} to permit measurements of —OD and —ND stretches following deuteration of the compounds.

Measurements in the region below 2000 cm^{-1} were made with demountable cells consisting of a stainless steel cylinder 2 cm in diameter and with 9 cm pathlength, to the ends of which were fitted NaCl or CaF_2 windows. A copper—constantan thermocouple introduced through the cell wall was employed to control and record the temperature, which had an observed variation of about 10—15°C.

UV absorption spectra in the gas phase

These made use of similar demountable cells of 7 cm pathlength fitted with quartz windows. Subsequently these measurements were checked in a sealed quartz cell.

In all instances reported below, recording of the vapour phase spectrum was followed by cooling, and testing for evidence of thermal decomposition by UV spectroscopy in solution and, occasionally, by chromatography.

Matrices of samples

Matrices were made with various gases by controlled condensation of a mixture of matrix gas and the vapour of the sample, on a cooled CsI or CaF_2 window [22]. The sample was placed in a small, electrically-heated glass tube placed in the vacuum chamber slightly below the cold window. The rate of vaporization of the sample was regulated by the rate of heating. For argon and CO_2 matrices the window was cooled to about 10 K with a liquid helium cryostat or to about 20 K with a Spectrim-Cryodyne CTI-21 system. For matrices formed from other gases, the window was cooled with a liquid nitrogen cryostat to about 70 K. The concentration of the sample in the matrix was controlled by the rate of heating of the furnace. The temperature of the window was measured with an Allan—Bradley resistor (with the liquid helium cryostat) or a copper—constantan thermocouple (with the liquid nitrogen cryostat). The time for deposition of a suitable film of sample and matrix gas was usually about 2—4 h. The deposited layer was measured against a reference film consisting of the matrix gas alone.

IR spectra were recorded on a Zeiss (Jena, GDR) UR-20 spectrophotometer, and the UV spectra on a Zeiss Specord UV-vis instrument.

RESULTS AND DISCUSSION

4-oxo-6-methylpyrimidine

IR vapour spectra

Direct information regarding the presence of tautomers such as **1a**, **1b** and **1c** may be obtained from an examination of the 3400—3600 cm^{-1} region embracing NH and OH stretching frequencies. The vapour phase spectrum of **1** exhibits two such bands at 3578 cm^{-1} and 3432 cm^{-1}, which are not detectable in the spectra of the fixed tautomeric forms, i.e. the N_1- and N_3-methyl derivatives and, thus, can be assigned to OH and NH stretching vibrations, respectively. Additional evidence for the origin of these two bands was provided by partial deuteration of 4-oxo-6-methylpyrimidine, leading to the appearance of two new bands, with comparable intensities, at 2640 cm^{-1}

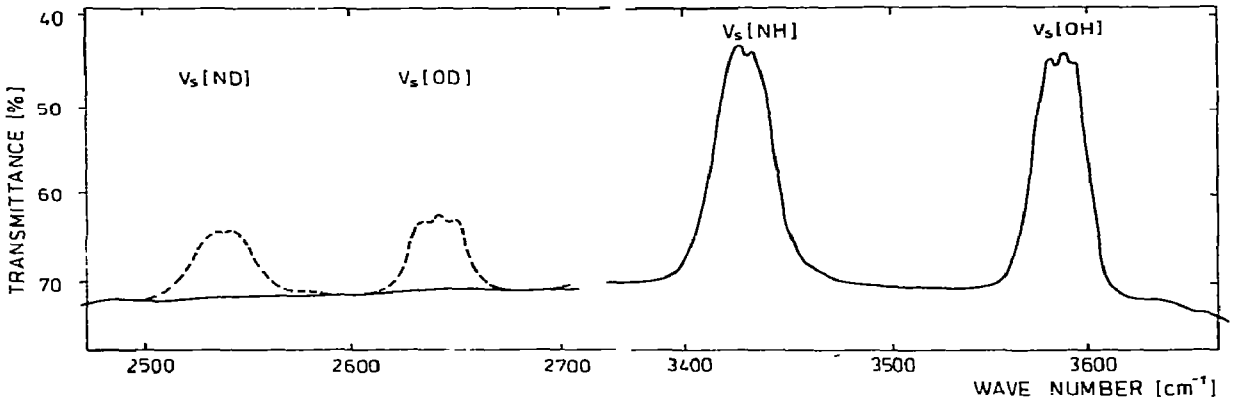


Fig. 1. IR absorption spectra, in the OH and NH (and OD and ND) stretching regions, of 4-oxo-6-methylpyrimidine in the vapour phase (230°C): —, undeuterated compound; ---, partially deuterated compound.

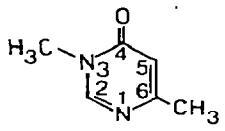
and 2539 cm^{-1} (Fig. 1)*. When the deuterated sample was cooled, back-exchanged with H_2O, and again brought to the vapour phase, the bands at 2640 and 2539 cm^{-1} decreased in intensity, with a concomitant increase in the intensity of the 3578 and 3432 cm^{-1} bands. Both the NH and OH (and ND and OD) bands in Fig. 1 exhibit characteristic structure and band half-widths similar to those observed in the gas phase with phenol, pyrazole and imidazole [24], indicating that the observed structure is due, not to different vibrational bands, but rather to the presence of *P*, *Q* and *R* rotational branches.

The high frequencies of the bands, their small half-widths, and their rotational structure, together suggest that they might originate from monomer species. No significant absorption which might be assigned to hydrogen-bonded species was noted in the lower frequency region 2600—3300 cm^{-1}.

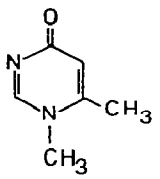
The keto tautomer may exist in one, or a mixture of two, forms with the proton located on N_1 and/or N_3 (**1b** or **1c**, Scheme 1). Each of these would be expected to exhibit slightly different NH stretching frequencies, as observed in the case of uracil, viz. 3486 cm^{-1} for N_1H and 3445 cm^{-1} for N_3H [25]. The presence in the spectrum of 4-oxo-6-methylpyrimidine of only one ν_s(NH) band clearly points to the existence of only one tautomeric form in the gas phase. The closeness of the observed ν_s(NH) frequency, 3432 cm^{-1}, to that of $\nu_s(N_3H)$ of uracil suggests, but does not necessarily prove, that the N_3H tautomer (**1b**) is that existing in the vapour phase.

An additional effort to resolve this problem, by comparing the C=O stretches of the fixed tautomeric forms 4-oxo-3,6-dimethylpyrimidine and 4-oxo-1,6-dimethylpyrimidine (Scheme 3), was unsuccessful. The two carbonyl

*The extent of deuteration is probably higher than appears from a visual inspection of the integral absorbances of the OH and OD, and NH and ND, bands. This follows from the fact that the absorption coefficient of an XD band is usually lower than for the corresponding XH band [23].



3a



3b

frequencies were virtually indistinguishable, 1715 cm^{-1} and 1716 cm^{-1}, respectively (see below, Fig. 2b, c). Further attempts to resolve this problem with the aid of UV spectroscopy gave, at best, uncertain results (see below, Fig. 6).

In the spectral region 1600—1800 cm^{-1} (Fig. 2a), 4-oxo-6-methylpyrimidine exhibits an intense band at 1734 cm^{-1} which is clearly due to the carbonyl stretching frequency. The relatively high frequency of this band, by comparison with that for the same compound in the solid state (1670 cm^{-1}), is consistent with the conclusion reached above that it is largely the monomeric species that is present in the gas phase.

For the purposes of comparison, Figs. 2(b) and 2(c) show the spectra of the fixed tautomeric forms of 4-oxo-1,6-dimethylpyrimidine and 4-oxo-3,6-dimethylpyrimidine in this region (Scheme 3). It will be noted that, while the frequencies of the C=O bands for these derivatives are only slightly lower (1716 and 1715 cm^{-1}) than for the parent 4-oxo-6-methylpyrimidine (1734 cm^{-1}), as might have been anticipated, there are marked differences in the ratios of the integral absorbances of these bands to those at about 1615 cm^{-1}, as compared with this ratio for 4-oxo-6-methylpyrimidine.

In the case of 4-oxo-6-methylpyrimidine (Fig. 2a) the absorption at 1734 cm^{-1} originates uniquely from the keto tautomer **1b** whereas the absorption at about 1615 cm^{-1} is a composite band derived from a mixture of tautomers 1a and 1b. The ratio of the integral intensities of these bands is about 3. For the fixed keto forms 4-oxo-1,6-dimethylpyrimidine and 4-oxo-3,6-dimethylpyrimidine these ratios are about 5 and 6, respectively. The lower ratio for 4-oxo-6-methylpyrimidine is consistent with its existence in the gas phase as a mixture of two tautomers, only one of which exhibits the carbonyl band.

Equilibrium constant in the vapour phase

The keto—enol equilibrium constant, K_T, may be estimated from the relation

$$K_T \left[\frac{NH}{OH}\right] = \frac{A(OH)}{A(NH)} \frac{I(NH)}{I(OH)}$$

where the A's are integral molar absorption coefficients, and the I's measured integral absorbance values, for the NH and OH bands. It has usually been assumed that the value of A is identical for NH and OH stretching vibrations [11, 16, 17].

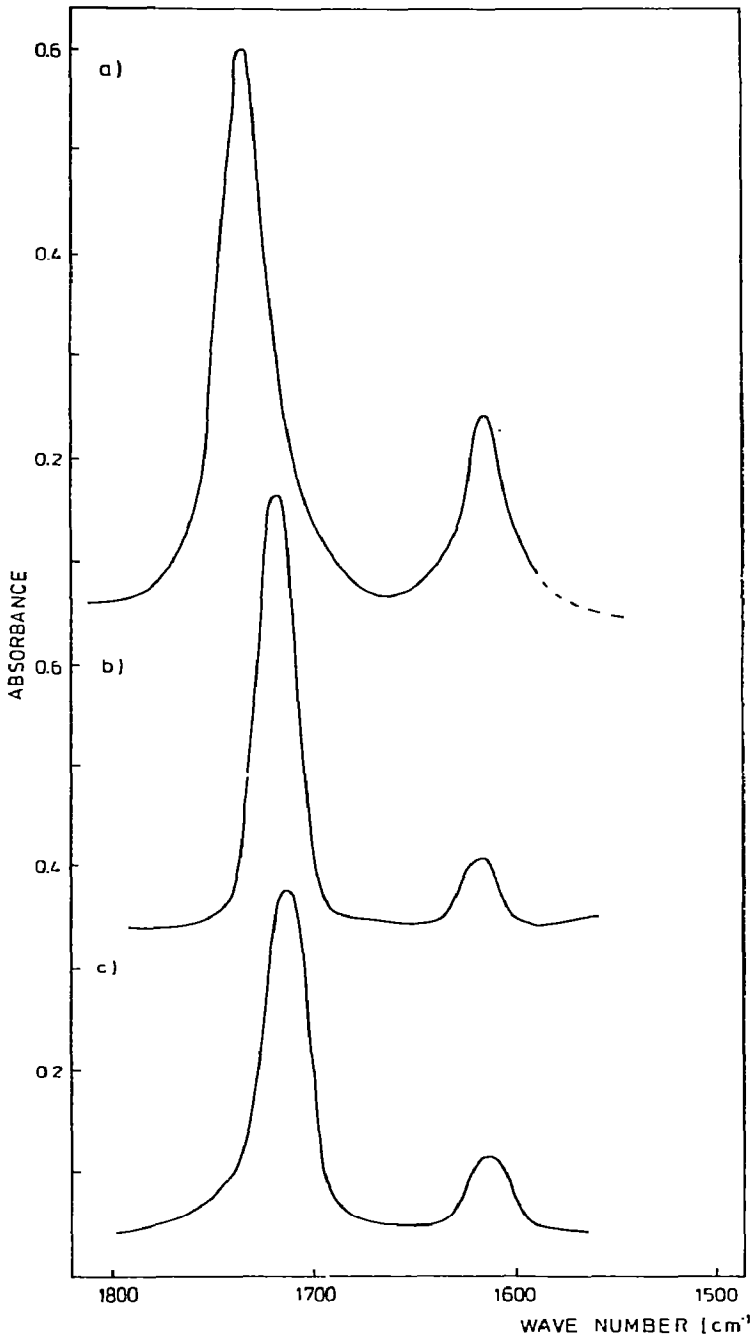


Fig. 2. IR absorption spectra, in the C=O stretching region (1600—1800 cm^{-1}), in the vapour phase of: (a) 4-oxo-6-methylpyrimidine (170°C); (b) 4-oxo-3,6-dimethylpyrimidine (130°C); (c) 4-oxo-1,6-dimethylpyrimidine (130°C).

We have measured the A values using two reference compounds: for 1-methyluracil, which exists in the vapour phase uniquely in the keto form [25], $A(N_3H) = 5.3 \times 10^6$ cm mol^{-1}, while for 2-oxo-4,6-dimethylpyrimidine,

which exists in the vapour mainly as the enol form (see below), A(OH) = 3.5 × 10^6 cm mol^{-1}. The latter value is reasonably close to that reported for the A(OH) of phenol [26] and 4-hydroxohydrazine [18].

For 4-oxo-6-methylpyrimidine the measured value of I(NH)/I(OH) is about 1.1. The resulting value of K_T[NH/OH] = 0.7 ± 0.2 at 245°C.

An independent estimate of K_T, albeit less accurate (due to overlapping of bands), may be derived from the spectra in the 1600—1800 cm^{-1} region (Figs. 2a and 2b), based on the ratios I(C=O)/I(1615) for 4-oxo-6-methylpyrimidine and the fixed keto form 4-oxo-3,6-dimethylpyrimidine. The value obtained in this way is $K_T \cong 1$.

Thermodynamic parameters

The spectrum of 1a—1b in the OH and NH regions was examined over the temperature range 140—260°C. From Fig. 3 it will be noted that, whereas the absorbances of both bands increase by a factor of more than 20 in this temperature range, their ratio is only slightly modified. The initial pronounced increase in the absorbance of the two bands with temperature is clearly related to the increased vaporization of the sample with temperature. Following total vaporization of the compound, the absorbances of the two bands are almost insensitive to increase in temperature.

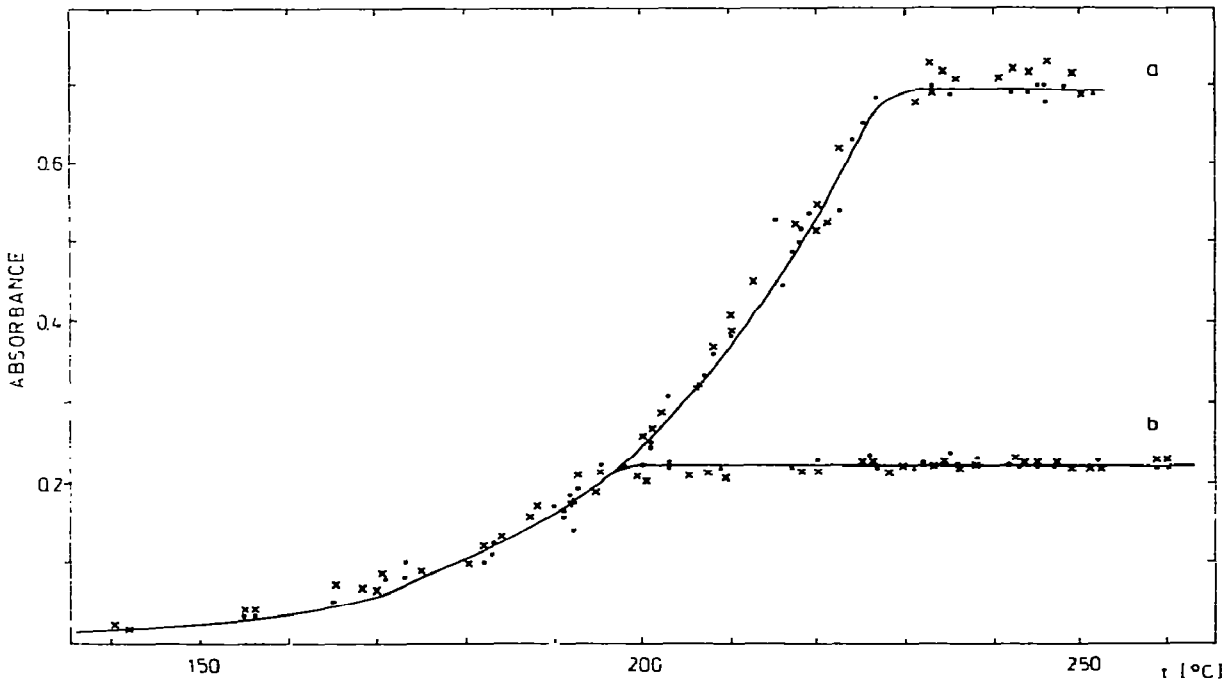


Fig. 3. Temperature-induced increase of the absorbance of stretching bands I(OH) (×) and I(NH) (•) of 4-oxo-6-methylpyrimidine vapour: (a) 7.5 mg and (b) 3 mg of the compound in the cell.

The differences in enthalpy (ΔH), entropy (ΔS) and free energy (ΔG) between the tautomers **1a** and **1b** were determined at elevated temperatures, under conditions where all the material in the cell was in the vapour phase, from the temperature-dependence of ln K_T, using the standard relations [27]

$$\ln K_T = -\Delta H/RT + \Delta S/R$$

$$-\Delta G = -\Delta H + T\Delta S$$

The temperature-dependence of ln I(NH)/I(OH) may be followed experimentally under these conditions. The dependence of ln K_T on $1/T$ is presented in Fig. 4, the resultant straight line being derived by the least-squares method. The resulting thermodynamic parameters (at 230°C) are: $\Delta H = -0.7 \pm 0.2$ kcal mol^{-1}; $\Delta S = -0.7 \pm 0.5$ e.u.; and $\Delta G = -0.3 \pm 0.3$ kcal mol^{-1}.

While the calculated value of ΔH is not dependent on the ratio of the molar absorption coefficients, A(OH)/A(NH), it was obtained on the basis of the usual assumption that the ratio of the absorption coefficients is independent of temperature. The value of ΔH reflects the difference in chemical binding energies of **1a** and **1b** (cf. refs. 11 and 12) and is rather low, the keto tautomer **1b** being slightly more stable.

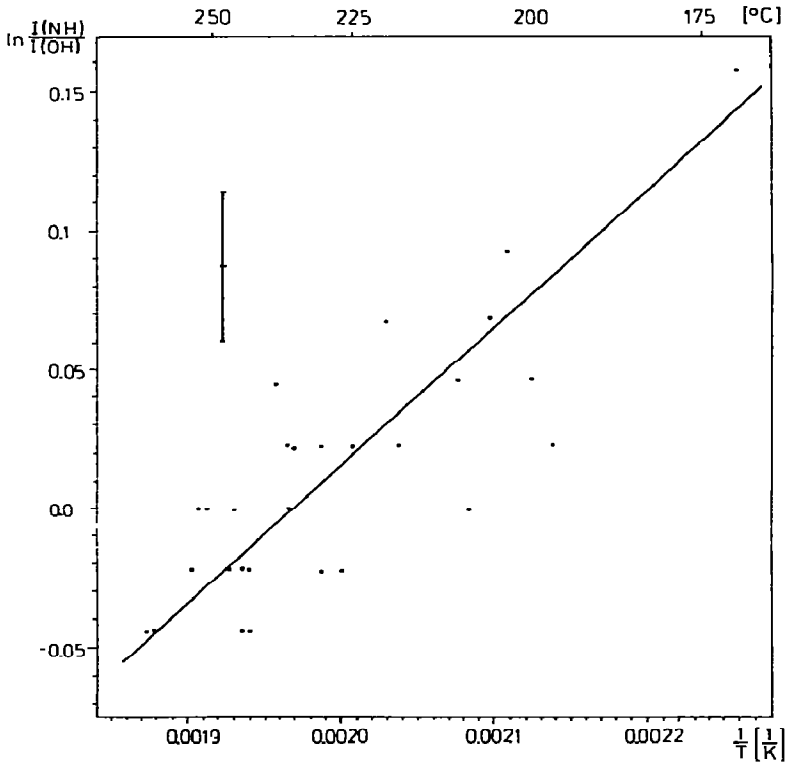


Fig. 4. Dependence of ln I(NH)/I(OH) on the reciprocal of the absolute temperature for 4-oxo-6-methylpyrimidine, under conditions where all the material is in the gas phase.

Heat of vaporization

The initial, very marked, temperature-induced increase in absorbance of the NH and/or OH bands (Fig. 3) may be used to evaluate the heat of vaporization of the compound from the slope of a plot of ln p vs. $1/T$, where p is proportional to the vapour pressure. The product of the band area, I (or the absorbance at the maximum when the band width is not significantly altered with temperature) and temperature, $I \cdot T$, is proportional to the vapour pressure. Figure 5 exhibits a plot of ln $(I \cdot T)$ vs. T^{-1} for the NH band of 4-oxo-6-methylpyrimidine. The slope of the plot gives a value for the heat of vaporization for the compound of about 21 ± 2 kcal mol^{-1}, a value similar to that reported for uracil and substituted uracils [25, 28].

For purposes of comparison, the heat of vaporization of the fixed tautomeric form of 4-oxo-3,6-dimethylpyrimidine was measured under identical conditions. This compound cannot associate in the melt via hydrogen bonding, and would consequently be expected to exhibit a lower heat of vaporization. The measured value was, in fact, 12 ± 1 kcal mol^{-1}, i.e. about one-half that for the parent 4-oxo-6-methylpyrimidine, and close to that obtained for 1,3-dimethyluracil [25].

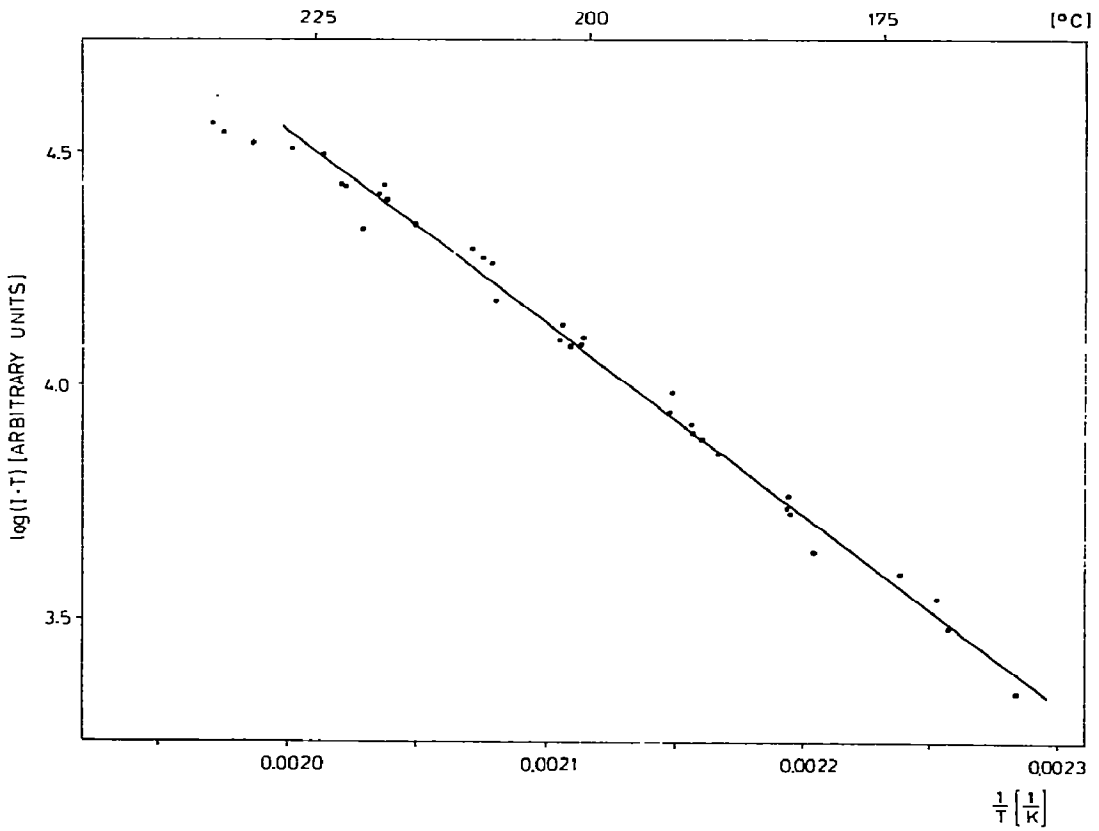


Fig. 5. Logarithmic dependence of the product of the ν_s(OH) band absorbance I and temperature T on the reciprocal of the temperature for 4-oxo-6-methylpyrimidine.

UV spectra in the gas phase

The gas phase UV spectra of 4-oxo-6-methylpyrimidine 1a—1b and the model compounds [29, 30] 4-oxo-1,6-dimethylpyrimidine (3b), 4-oxo-3,6-dimethylpyrimidine (3a) and 4-methoxypyrimidine [11] are presented in Fig. 6. Comparison of the vapour spectrum of 1a—1b with those of model compounds 3b and 4-methoxypyrimidine shows that absorptions attributable to both tautomers are observed in the vapour.

Interpretation of the UV spectra of 1a and 1b is, however, not straightforward due to the fact that the spectrum of the model compound 4-oxo-1,6-dimethylpyrimidine shows increased absorption in the spectral range where absorption of 1a is expected (ca. 250 nm). However, taking into account the results of the IR studies (see discussion below), the absorption near 250 nm of the parent compound is probably due to the 1a, not the 1c, tautomer. Because of overlap of the absorptions attributable to 1a and 1b, no attempt was made to estimate the equilibrium constant from the UV vapour spectrum.

Effect of substituents

The effect of substitution on the tautomeric equilibrium of 4-oxopyrimidine was examined with the aid of the IR and UV spectra of 4-oxo-2,6-dimethyl-

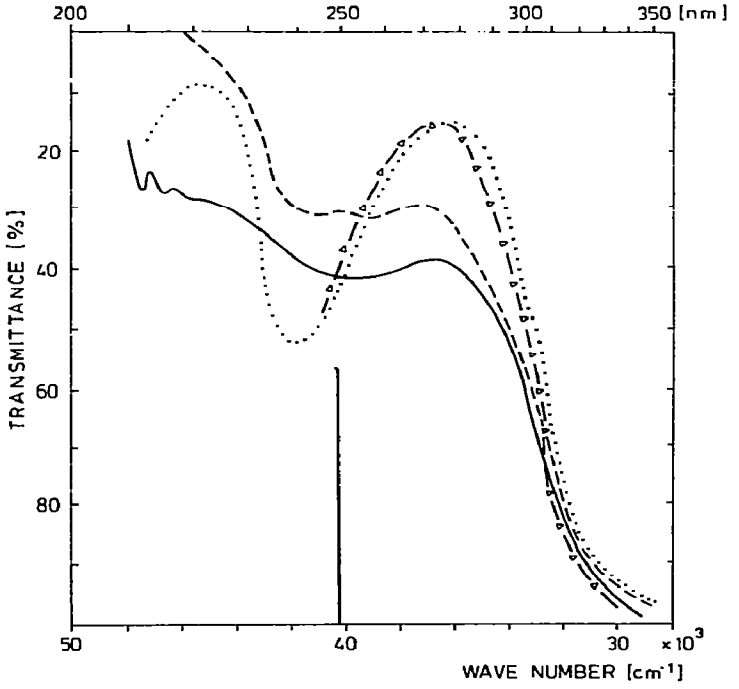
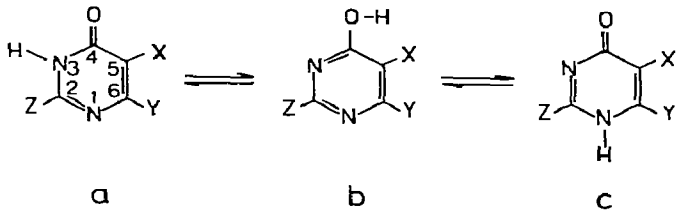


Fig. 6. UV absorption spectra of: ----, 4-oxo-6-methylpyrimidine in the gas phase; —, 4-oxo-1,6-dimethylpyrimidine in the gas phase; ...., 4-oxo-3,6-dimethylpyrimidine in the gas phase; —△—, 4-oxo-6-methylpyrimidine solution in $CDCl_3$. The vertical line shows the position of the absorption of 4-methoxypyrimidine vapour [11].

pyrimidine, 4-oxo-6-methylpyrimidine and 4-oxo-5-bromo-6-methylpyrimidine (Scheme 4: (4), (5), (6), (7)).



a b c

(4) X, Y, Z=H
(5) X=H, Y, Z=CH_3
(6) X=H, Y=CH_3, Z=H
(7) X=Br, Y=CH_3, Z=H

The corresponding NH and OH band frequencies, the ratios of I(NH)/I(OH), and the UV spectral parameters for the compounds 4—7 are listed in Table 1. It will be seen that the ratio I(NH)/I(OH) is about 2 for 4-oxo-2,6-dimethylpyrimidine, and approximately 1 for the remaining three compounds. The corresponding differences in enthalpy between the keto and enol forms of the 4-oxopyrimidine derivatives are given in Table 1.

In the electronic absorption spectra of 4—7, the long wavelength absorption comprises two strong, partially overlapping, bands. One (at about 270 nm) is due to the keto form, the other (at about 250 nm) to the enol tautomer (see Table 1). These bands are of approximately equal intensity for three of these compounds; only in the case of the 2,6-dimethyl derivative is the band intensity at 255 nm lower than that at 270 nm, in agreement with the lower intensity of the OH, relative to the NH, band in the IR spectrum. It is of interest that introduction of a methyl substituent at the 6-position, and or even a strongly electronegative bromine atom at the 5-position, does not detectably affect the tautomeric equilibrium of 4-oxopyrimidine in the gas phase, whereas an additional methyl group at the 2-position does markedly shift this equilibrium. The absence of any effect due to a bromine substituent

TABLE 1

Effect of substituents on the keto—enol equilibrium of 4-oxopyrimidines

Compound	M.p. (°C)[b]	ν_s(OH) (cm^{-1})	ν_s(NH) (cm^{-1})	$\frac{I(NH)}{I(OH)}$	λ max (nm)	ΔH_v[c] (kcal mol^{-1})	ΔH[d] (keto enol) (kcal mol^{-1})
4-oxopyrimidine	163—165	3580	3430	1	200, 255, 275	—	—
4-oxo-6-methylpyrimidine	148—150	3577	3429	1.1	220, 250, 270	21	—0.7
4-oxo-2,6-dimethyl-pyrimidine	194—201	3579	3423	2	215, 255, 270	26	—2
4-oxo-5-bromo-6-methyl-pyrimidine	[a]	3580	3430	—	227, 260, 283	—	—

[a]At about 200°C thermal decomposition occurs. [b]Ref. 19. [c]ΔH_v, heat of vaporization. [d]ΔH (keto/enol), difference of the enthalpies of the keto and enol tautomers.

at the 5-position is consistent with the previously noted lack of any effect due to a 5-fluoro- [25] or a 5-bromo- [31] substituent on the tautomeric form of uracil in the gas phase.

IR spectra in low-temperature matrices

Apart from gas phase studies, solvent and association effects may also be minimized in low-temperature matrices consisting of argon or other relatively inert gases [32]. An additional inherent advantage of this procedure is that the absorption bands of matrix-isolated species are fairly sharp, thus permitting resolution of bands with small frequency differences which usually overlap in solution, and in the vapour phase.

The IR spectrum of 4-oxo-6-methylpyrimidine in an argon matrix is exhibited in Fig. 7, along with the corresponding spectrum in the vapour phase. The characteristic ν_s(OH), ν_s(NH) and ν_s(C=O) bands exhibit frequencies slightly lower in the matrix than in the vapour. Their frequencies are collected in Table 2, together with the frequencies of other vibrations of **1a** ⇌ **1b** in an argon matrix and in vapour and solid phases for comparison. The ν_s(OH) and ν_s(NH) bands in the matrix are also split into several components, as commonly observed in argon matrix spectra [19], and this may be attributed to interactions between neighbours and/or different trapping sites in the matrix, or to rotational isomers. The band splitting observed here cannot be due to formation of dimers or higher aggregates, since this would lead to much lower ν_s(OH) and ν_s(NH) frequencies. The presence of hydrogen-bonded species probably accounts for the weak, diffuse absorption in the region 2600—3200 cm^{-1}, which is similar to that observed for the pure solid compound (see Table 2). In the C=O stretching region, absorption corresponding to monomeric species was found at 1720 cm^{-1} and that due to the hydrogen-bonded species at about 1679 cm^{-1} (Table 2).

The presence of distinct ν_s(OH) and ν_s(NH) bands in the argon matrix spectrum of 4-oxo-6-methylpyrimidine testifies to the existence under these conditions of the keto—enol equilibrium observed in the gas phase. From Fig. 7 it can be seen that the absorbances of the two bands are similar in magnitude and, in fact, the ratio I(NH)/I(OH) = 1.3 is close to that observed in the vapour. Accurate estimation of K_T in this instance is difficult because we do not know the molar extinction coefficients for the two bands under these conditions. If we assume that their ratio is similar to that in the vapour (see above), K_T [NH/OH] = 0.9.

The OH and NH regions of the spectrum of 4-oxo-6-methylpyrimidine in several other low-temperature matrices are shown in Fig. 8. The ν_s(OH) and ν_s(NH) bands are observed in all matrices, their frequencies and absorbance ratios I(OH)/I(NH) varying with the nature of the matrix gas (Table 3). With increasing polarity of the medium (see below for discussion of the effect of the medium) the frequency of the ν_s(NH) and ν_s(OH) absorptions decreases, and the frequency shifts $\Delta\nu_s$(OH) and $\Delta\nu_s$(NH) are related to the energy of

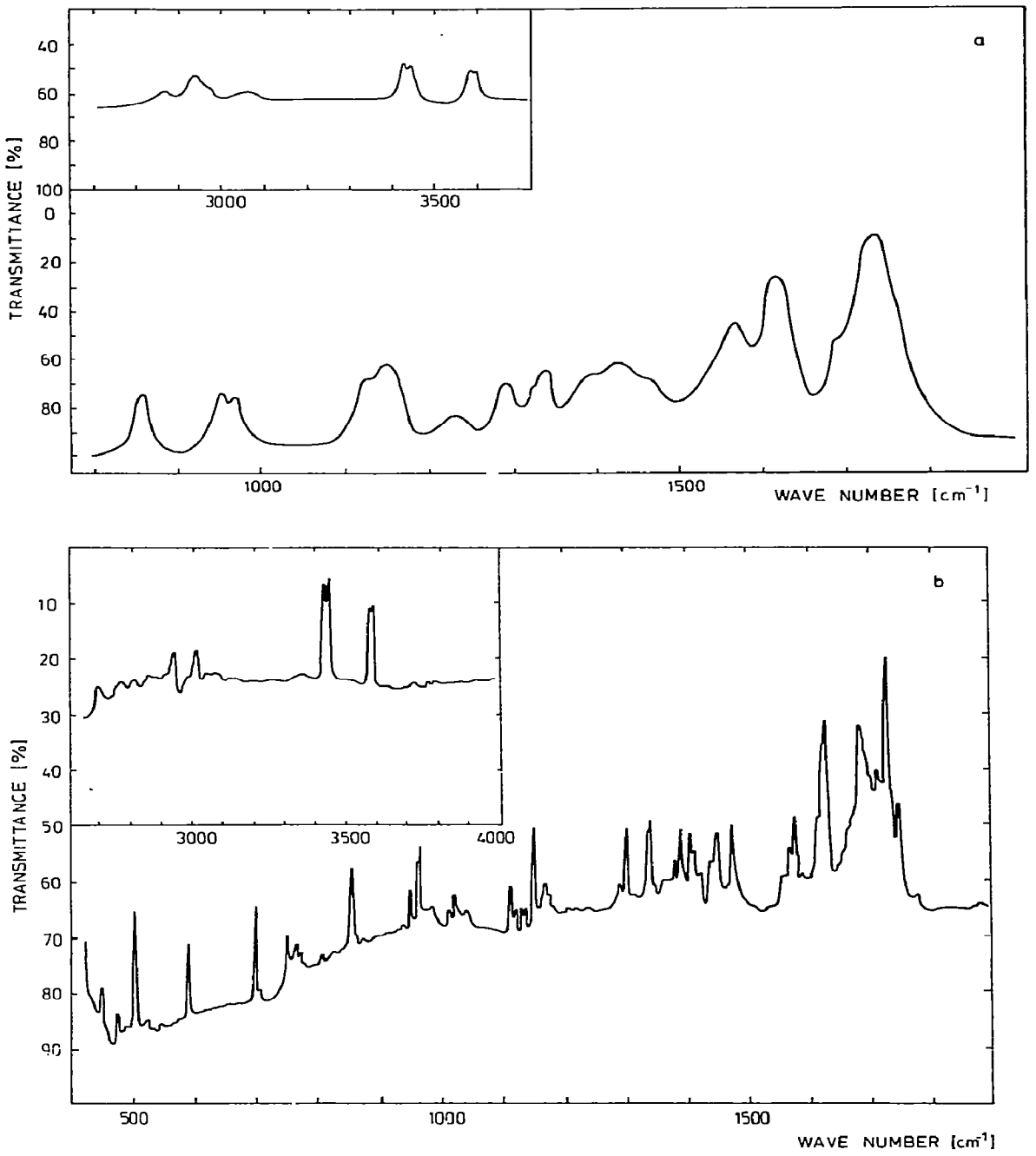


Fig. 7. IR absorption spectra of 4-oxo-6-methylpyrimidine (a) in the vapour phase, (b) in an argon low-temperature matrix.

interaction of **1a** and **1b** with the environment [33]. The spectra presented in Fig. 8 are consistent with previous studies of the effects of the medium on protomeric equilibria in solution [14]. As the polarity of the medium increases, the more polar isomer is stabilized relative to the less polar isomer.

TABLE 2

Frequencies[a] and assignments of IR absorption bands of 4-oxo-6-methylpyrimidine in different phases

Vapour	Ar matrix	Solid	Assignment
3585 m	3580 m		
3578 m	3573 m		Free OH stretch
3570 m	3569 m		
3437 m	3434 m		
3432 m	3430 m		Free NH stretch
3423 m	3422 m		
	3110 w		
3045 w	3050 w	3045 w	
2994 w	2982 w	2995 w	CH stretch
2930 w			
		2810 m	
	2798 w		
	2750 w	2750 m	As. NH stretch
	2676 w	2680 m	
	1740 w		
1734 s	1720 s		Free C=O stretch
	1707 w	1706 sh	
	1679 m	1670 s	As. stretch C=O
	1657 sh		
1617 m	1618 m	1618 m	
	1571 sh		Ring stretch
1565 m	1554 m	1556 m	
		1547 m	Ring stretch or NH bend
1470 m	1471 m	1473 w	Ring stretch
	1443 m		
1430 m	1434 m	1430 w	
	1420 w		NH bend
	1412 m	1411 m	
	1402 m		
1390 m	1386 w		
	1372 w	1370 w	
	1358 w		CH bend
1340 m	1331 m	1331 m	
1296 m	1296 m	1308 w	
1225 w			?
	1167 m	1165 m	As. CH bend
1156 s	1150 s		
	1135 w		
1128 sh	1132 w		OH bend or C—O stretch
	1118 w		
	1113 m		

TABLE 2 (continued)

Vapour	Ar matrix	Solid	Assignment
	1040 w		
	1018 m	1025 m	
	1012 w		
	985 w	980 w	
965 m	965 m	968 m	Ring bend
950 m	950 m		C—O bend (?)
856 m	856 m	850 m	

[a]s, strong; m, medium; w, weak; sh, shoulder.

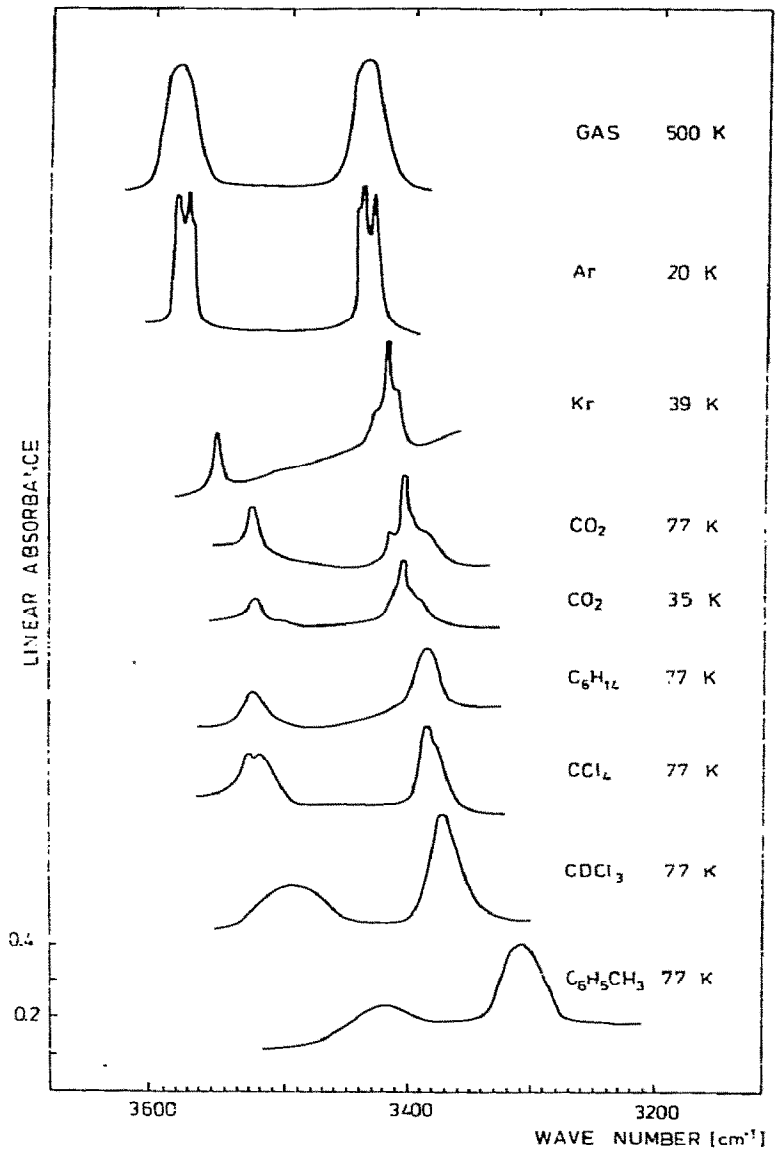


Fig. 8. Comparison of IR absorption spectra of 4-oxo-6-methylpyrimidine in the ν_s(OH) and ν_s(NH) region for various low-temperature matrices.

TABLE 3

NH, OH and C=O frequencies, and ratios of absorbances of NH to OH bands, of 4-oxo-6-methylpyrimidine, in the vapour phase, in low-temperature matrices and in fluid solvents

Environment	Proton affinity (eV)	ν_s(NH) (cm^{-1})	ν_s(OH) (cm^{-1})	$\frac{I(NH)}{I(OH)}$	ν_s(C=O) (cm^{-1})
Vapour phase					
		3437	3585	1.1	
		3432	3578		1734
		3423	3570		
Matrices					
Argon	3.8	3434	3580		1720
		3430	3573	1.3	
		3422	3569		
Krypton	4.5	3426	3557		1705
		3418		1.7	
		3412			
Carbon		3411		5.5	
dioxide	4.7	3390	3535		1709
Hexane	6.4[a]	3395	3530	3.1	1703
Carbon	7.1[b]	3397	3522	1.3	1702
tetrachloride			3534		
D-chloroform	7.1[b]	3367	3488	1.4	1700
Toluene	7.3 + 8	3318	3434	1.5	1700
Solutions					
D-chloroform	7.1[b]	3385 unassoc.	—	—	
		2950 assoc.	—	—	1664
Benzene	7.1 + 7.5[c]	3345 unassoc.	—	—	1700
		2950 assoc.	—	—	1668
D-acetone	8.25[c]	3250	—	—	
D-pyridine	10. [c]	2800	—	—	

[a]Estimated from the data for C_nH_{2n+2} series from ref. 40. [b]Estimated from the data for halogenomethanes from ref. 40. [c]Proton affinity of hydrogenated analogue from ref. 40.

IR solution spectra of 4-oxo-6-methylpyrimidine

An examination was also made of the IR spectra of 4-oxo-6-methylpyrimidine in several solvents. The spectra are displayed in Fig. 9 and the appropriate data given in Table 3. The spectrum in dilute $CDCl_3$ solution (Fig. 9a) exhibits the ν_s(NH) of non-associated molecules, and broad absorption comprised of several sub-bands, probably due to Fermi resonance [35], corresponding to self-associated species. The breadth and structure of this

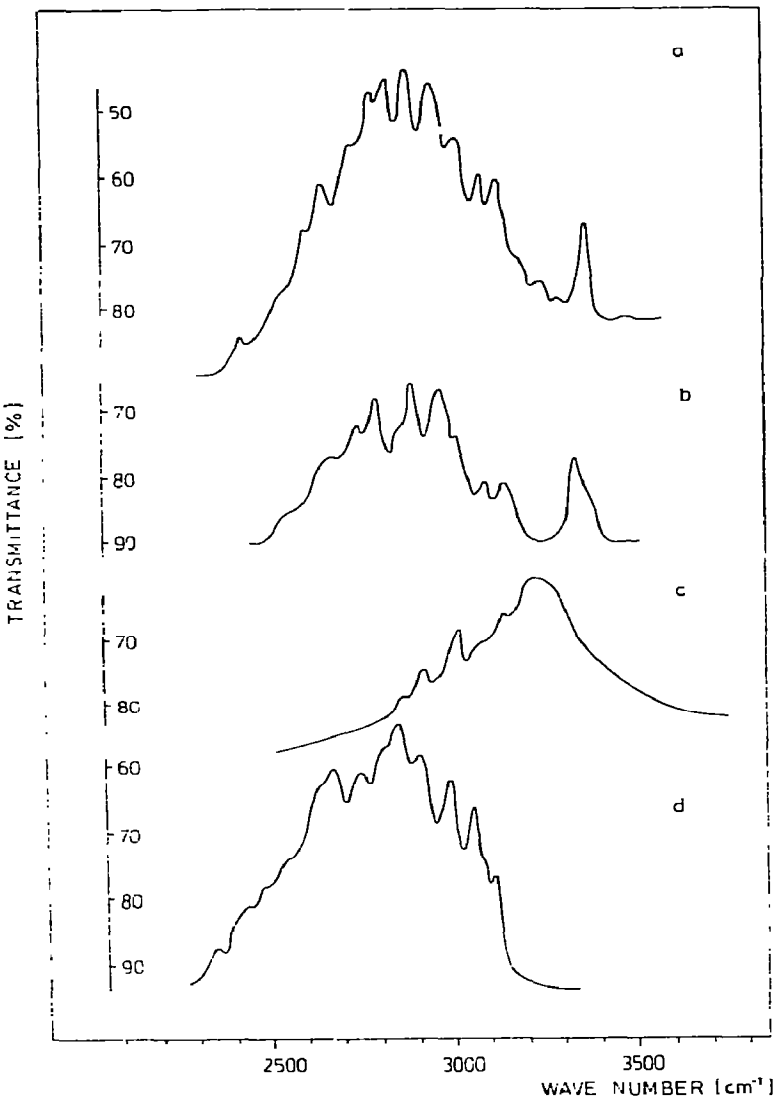


Fig. 9. IR absorption spectra of 4-oxo-6-methylpyrimidine, in the 3000 cm^{-1} region, diluted in (a) $CDCl_3$; (b) C_6D_6; (c) $(CD_3)_2CO$; (d) C_5D_5N; l = 1 mm, concentration 10^{-2} M.

absorption make it difficult to establish whether it originates from only one hydrogen-bonded tautomer (1a or 1b), or both. While the intense absorption in the C=O stretching region points to the presence of the keto tautomer, it is not feasible to exclude the presence of the enol form. However, the UV spectrum suggests that it is mainly the keto form which is present under these conditions.

An examination of the influence of temperature on the redistribution of the intensity of the ν_s(NH) band of non-associated molecules, and that of the band due to associated molecules in $CDCl_3$ solution, led to a value for the enthalpy of self-association of $\Delta H = -5.1$ kcal mol^{-1}, in reasonable agreement with values reported by others for similar compounds [34].

A rather striking illustration of the effect of molecular environment is furnished by a comparison of the tautomeric equilibria in $CDCl_3$ solution and in a $CDCl_3$ matrix. Only the keto tautomer is observed in solution, whereas in the matrix both tautomers are present in approximately equal proportions (Fig. 8). The predominance of the keto tautomer in solution is probably due to self-association [15].

The spectrum in dilute d_6-benzene solution was similar to that in chloroform, but the values of ν_s(NH) for the self-associated and non-associated species were shifted to lower frequencies (Fig. 9b and Table 3). The presence of ν_s(C=O) in benzene solution and its high integral intensity point to **1b** as the predominant form under these conditions, but does not exclude a small proportion of the enol form.

In dilute d_6-acetone solution the spectrum was markedly different (Fig. 1c), and consisted of one relatively broad band centred at about 3250 cm^{-1}, a frequency significantly higher than that corresponding to self-associated molecules. Solvent absorption in this case did not permit observation of the region embracing carbonyl stretching frequencies. It is consequently not clear which tautomeric form(s) account for the broad absorption band at about 3250 cm^{-1}.

In dilute d_5-pyridine solution, the compound exhibited broad absorption (Fig. 9d) with characteristic sub-bands [35]. The origin of this absorption is not clear, as in the previous case. The presence of a strong band in the carbonyl stretching region, however, points to the keto tautomer as the predominant species.

Effects of the medium

It is clear that, as in the case of other compounds [1—3, 11, 12, 14], the tautomeric equilibria of the 4- and 2-oxopyrimidines are sensitive to the molecular environment. With a view to characterization of such effects, gas-phase proton affinities have been proposed as a fundamental operational measure of the activity of the medium [36]. We have examined the relationship between the proton affinity of the medium and the frequency shifts of ν_s(OH) and ν_s(NH), which are related to the energy of interaction with the environment [29]. The results, based on data from Table 3, are shown in Fig. 10. It will be seen that the shifts of both $\Delta\nu_s$(OH) and $\Delta\nu_s$(NH) increase with increasing proton affinity (PA) of the medium. The correlation between $\Delta\nu_s$(OH) and PA covers only media with lower PA values where the enol tautomer is present; that between $\Delta\nu_s$(NH) and PA embraces a broader range of PA values. Unfortunately, with the present state of development of the theory of molecular interactions, it is not feasible to analyze the interaction energies of the species involved on the basis of proton affinities.

However, the correlation shown in Fig. 10 allows an estimate of the proton affinity of the keto tautomer **1b**, from the frequency shift $\Delta\nu_s$(NH) for self-associating species. The PA of **1b** obtained in this way is about 9.5 eV

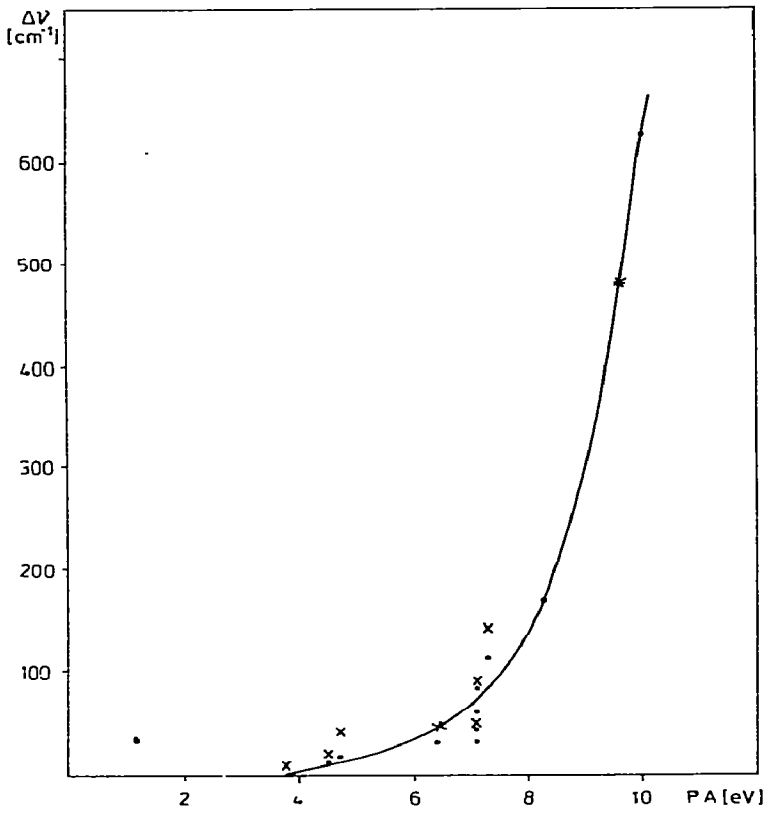


Fig. 10. Correlation between the frequency shift $\Delta\nu_s$ of the NH (x) and OH (•) stretching vibrations of 4-oxo-6-methylpyrimidine and the proton affinity (PA) of the matrix and/or solution.

(221 kcal mol^{-1}), a value higher than those for *N*-methyl- and *O*-methyl-2-oxopyrimidines; 216 and 218 kcal mol^{-1}, obtained by pulsed ICR [37].

No absorption due to the self-associated species of the enol form 1a could be detected in the spectrum of 4-oxo-6-methylpyrimidine, either in matrices or in solution, so that an evaluation of the PA value was not feasible.

2-oxo-4,6-dimethylpyrimidine

In sharp contrast to 4-oxo-6-methylpyrimidine, the gas-phase spectrum of this compound exhibited an intense band at 3605 cm^{-1}, corresponding to an OH stretch, with a molar absorption coefficient of 3.4×10^6 cm mol^{-1}, and only an extremely weak band at about 3430 cm^{-1}, probably due to the ν_s(NH) of a very low proportion of the keto tautomer 2b (Fig. 11). The low intensity of this latter band made it difficult to estimate K_T from the ratio I_s(NH)/I_s(OH). Recourse was therefore made to the spectra in the region of carbonyl and valence bond frequencies for 2-oxo-4,6-dimethylpyrimidine and its fixed keto form 2-oxo-1,4,6-trimethylpyrimidine. The equilibrium constant was then obtained from the formula:

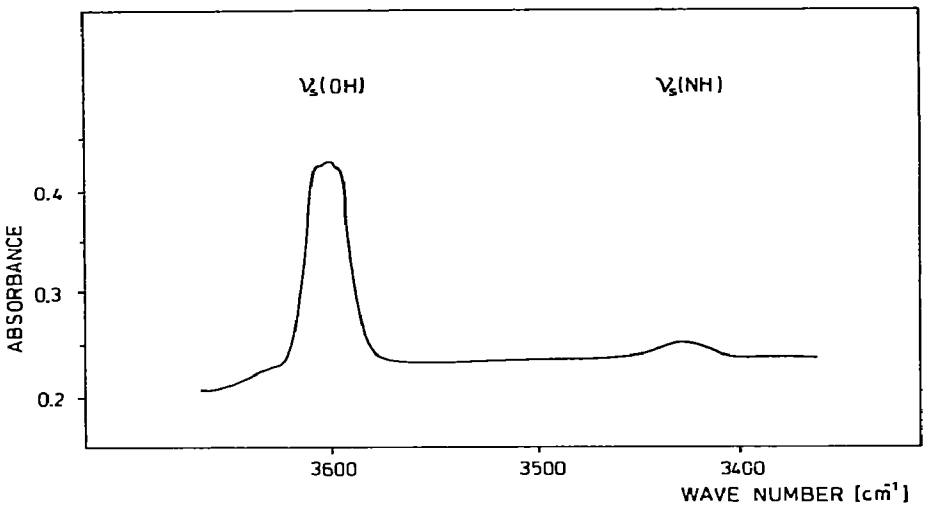


Fig. 11. IR absorption spectrum in the OH and NH stretching regions, of 2-oxo-4,6-dimethylpyrimidine in the vapour phase (215°C).

$$K_T(\text{keto/enol}) = \frac{I'(C{=}O)/I'(C{-}C)}{I''(C{=}O)/I''(C{-}C) - I'(C{=}O)/I'(C{-}C)}$$

where the I's are intensities and the primed values refer to 2-oxo-4,6-dimethylpyrimidine and the double-primed values to the trimethyl fixed keto form. This gave a value for K_T of $(6 \pm 1) \times 10^{-2}$, i.e. two orders of magnitude lower than that for 4-oxo-6-methylpyrimidine.

From the temperature-dependent increase in absorbance of ν_s(OH), due to increased transfer of substance to the gas phase, the heat of vaporization of 2-oxo-4,6-dimethylpyrimidine was calculated as 19 kcal mol^{-1}, i.e. 2 kcal mol^{-1} lower than that for 4-oxo-6-methylpyrimidine, and close to that for 3-methyluracil, 18 kcal mol^{-1} [25].

In matrices of CCl_4 and toluene at 77 K, the compound exhibited sharp bands at 3556 cm^{-1} and 3473 cm^{-1}, respectively, corresponding in each case to an OH stretch. There was no detectable absorption in the 3300—3400 cm^{-1} region (NH stretch), nor in the carbonyl stretching region which was partly obscured by the matrix absorption. This therefore points to an absence of significant amounts of the keto form.

In dilute $CDCl_3$ solution at room temperature, a broad band centred at about 2800 cm^{-1} was assigned to self-associated species, and a clearly defined band at 3380 cm^{-1} to the NH stretch of the monomeric species. Consistent with this was the presence of an intense band at 1645 cm^{-1} assigned to ν_s(C=O), so that the keto tautomer 2b predominates under these conditions.

The vapour phase UV spectrum of 2-oxo-4,6-dimethylpyrimidine and the parent 2-oxopyrimidine both exhibited maxima at 260 nm and to the violet of 220 nm. The first of these corresponds to a similar band reported by Beak et al. [11, 12] for 2-methoxypyrimidine in the gas phase. In methanol,

aqueous and $CDCl_3$ solutions, the 260 nm band was replaced by another at 290 nm, close to that recorded by Beak et al. [11] for the fixed keto form 2-oxo-1-methylpyrimidine in the gas phase. The UV results are consistent with the IR data in showing the presence of only the enol form in the gas phase, and the keto form in solution.

CONCLUSIONS

One of the more interesting findings of the present investigation is the marked difference in tautomeric forms between 2-oxo- and 4-oxo-pyrimidines, both in the gas phase and in low-temperature matrices, as compared to the similar predominant keto tautomeric forms in solutions of various polarities. This is in sharp contrast to the behaviour of some natural nucleic acid bases, which exhibit the same tautomeric forms under all the conditions considered above [25], and further emphasizes the differing effects of molecular environment even between members of a similar class of compounds (cf. ref. 12). Additional significance attaches to the observation that the effect of molecular environment, both polar and non-polar, correlates with the gas phase proton affinity values.

It is also clear that the matrix-isolation method is undoubtedly of use in examining tautomeric equilibria in non-polar and slightly polar media, since the results in an argon matrix are similar to those prevailing in the vapour phase. This should prove of practical utility, inasmuch as the matrix method may be applied to studies on tautomeric equilibria of compounds insufficiently thermostable to permit transfer of an adequate quantity to the gas phase.

Finally, the results for the 5-bromo-4-oxo derivative are of some biological significance in the light of the demonstration that 5-fluoro- and 5-chloro-2-oxopyrimidines are effective mitotic inhibitors [38, 39]. It would, indeed, be of interest to examine whether the *N*-methylated fixed keto forms of these compounds exhibit similar biological activity. If so, this would probably make it possible to establish whether the active keto form is the N(1)—H or N(3)—H tautomer.

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REFERENCES

1 A. R. Katritzky and J. M. Lagowski, Adv. Heterocycl. Chem., 1 (1963) 2.
2 A. Psoda and D. Shugar, Biochim. Biophys. Acta, 247 (1971) 507.
3 J. Elguero, C. Marzin, A. R. Katritzky and P. Linda, Adv. Heterocycl. Chem. Suppl., 1 (1975).
4 L. P. Yahontov, D. M. Krasnokutskaya, E. M. Peresleni, Y. N. Sheinker and M. V. Rubcov, Dokl. Acad. Nauk SSSR, 172 (1967) 118.
5 L. P. Yahontov, D. M. Krasnokutskaya, E. M. Peresleni, Y. N. Sheinker and M. V. Burcov, Dokl. Acad. Nauk SSSR, 176 (1967) 613.
6 Y. N. Sheinker, E. Peresleni, I. S. Rezchinkova and N. P. Zosimova, Dokl. Acad. Nauk SSSR, 192 (1970) 1295.
7 A. Gordon and A. R. Katritzky, Tetrahedron Lett., (1968) 2767.
8 J. Sepiol, Z. Kazimierczuk and D. Shugar, Z. Naturforsch., 310 (1970) 361.
9 M. J. Cook, A. R. Katritzky, P. Linda and R. D. Tack, J. Chem. Soc., Perkin Trans. 2, (1972) 1295.
10 P. Beak and F. S. Fry, Jr., J. Am. Chem. Soc., 95 (1973) 1700.
11 P. Beak, F. S. Fry, Jr., J. L. Lee and F. Steele, J. Am. Chem. Soc., 98 (1976) 171.
12 P. Beak, Acc. Chem. Res., 10 (1977) 186.
13 P. Beak, J. C. Covington and S. G. Smith, J. Am. Chem. Soc., 98 (1976) 3284.
14 P. Beak and J. B. Covington, J. Am. Chem. Soc., 100 (1978) 3969.
15 P. Beak, J. B. Covington and J. M. Zeigler, J. Org. Chem., 43 (1978) 177.
16 E. S. Levin and G. N. Radionova, Dokl. Acad. Nauk SSSR, 164 (1965) 584.
17 E. S. Levin and G. N. Radionova, Dokl. Acad. Nauk SSSR, 172 (1967) 607.
18 E. S. Levin and G. N. Radionova, Dokl. Acad. Nauk SSSR, 174 (1967) 1132.
19 D. J. Brown, Pyrimidines, Wiley, New York and London, 1962.
20 R. R. Hunt, J. F. W. McOmie and E. R. Sayer, J. Chem. Soc., (1959) 525.
21 W. J. Hall and A. G. Williams, J. Am. Chem. Soc., 37 (1915) 594.
22 S. Cradock and A. J. Hinchcliffe, Matrix Isolation, Cambridge University Press, Cambridge, 1975.
23 W. B. Person, Molecular Spectroscopy, Vol. 2, Chemical Soc., London, 1974, p. 428.
24 Unpublished results from our laboratory.
25 M. J. Nowak, K. Szczepaniak, A. Barski and D. Shugar, Z. Naturforsch., Teil C, 33 (1978) 876.
26 K. Szczepaniak and W. J. Orville-Thomas, J. Chem. Soc. Faraday Trans. 2, 70 (1974) 1175.
27 G. M. Barrow, Physical Chemistry, McGraw Hill, New York, 1961.
28 L. B. Clark, G. G. Peschel and I. Tinoco, J. Phys. Chem., 69 (1965) 3615.
29 E. Spinner and G. B. Yeoh, J. Chem. Soc. B, (1971) 279.
30 H. Specker and H. Gawrosch, Ber., 75 (1942) 1338.
31 M. J. Nowak, Ph.D. Thesis, University of Warsaw, 1979.
32 S. T. King, W. L. Dilling and N. B. Tefertiller, Tetrahedron, 28 (1972) 5859.
33 M. S. Mazarri and R. S. Drago, J. Am. Chem. Soc., 92 (1970) 7086 and refs. therein.
34 J. Dachesne (Ed.), Physico-chemical Properties of Nucleic Acids, Vol. 2, Academic Press, London and New York, 1973, p. 42.
35 J. L. Wood, in J. Yarwood (Ed.), Spectroscopy and Structure of Molecular Complexes, Plenum Press, London, 1973, p. 303.
36 E. M. Arnet, E. J. Mitchel and T. S. S. R. Murty, J. Am. Chem. Soc., 96 (1974) 3875.
37 M. J. Cook, A. R. Katritzky, M. A. Taagepera, T. G. Singh and R. W. Taft, J. Am. Chem. Soc., 98 (1976) 6048.
38 E. Wibe, R. Oftebro, T. Christensen, S. G. Laland, E. O. Petersen and T. Lindmo, Cancer Res., 38 (1978) 560.
39 R. Oftebro, P. Grimmer, T. B. Pyen and S. G. Laland, Biochem. Pharmacol., 21 (1972) 2451.
40 L. V. Gurvich, G. V. Karachavcev, V. N. Kondratiev, I. A. Lebiediev, V. A. Medviediev and I. S. Hodiejev, Dissociation Energies of Chemical Bonds. Ionization Potentials and Electron Affinities, Nayka, Moskow, 1974, (in Russ.)