Torsional Barriers in 6-Amino-5-formamidopyrimidin-4(3*H*)-ones

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The chemical shifts of 6-amino-5-formamidopyrimidin-4(3*H*)-ones show that two rotamers are present in solution, their formyl signals being of unequal intensity. The two forms are stable both as neutral molecules and as cations, in contrast to the behaviour of *N*-alkylformamides. Exchange rates and barriers to rotation have been calculated. The reasons for the unequal distribution of the rotamers are discussed.

6-AMINO-5-FORMAMIDOPYRIMIDIN-4(3*H*)-ONES are useful intermediates in the synthesis of hypoxanthines and related purines. They belong to the class of *N*-substituted formamides which have served as classical models for the study of exchange rates of amide rotamers.[1-3] Here we report the n.m.r. spectra of four derivatives of 6-amino-5-formamidopyrimidin-4(3*H*)-one and of one uracil derivative (5). In these derivatives the formamido-group, owing to a dipolar contribution, exhibits a slow rotation about the C–N bond. This process is temperature-dependent and its rate is within the n.m.r. time-scale.

The chemical shifts of the neutral and cationic forms of compounds (1)—(5) are given in Table 1. Assignments were made as follows. In the spectra of the cations of (1) and (2), the formyl bands can be identified by comparison with the cations of (3) and (4) (see Table 1). In that of the neutral form of (2), the 2-H band was assigned with the aid of the nuclear Overhauser effect. Irradiation at the frequency of the NMe signal (δ 3.47) increased the area of the band at 8.24 by 44%, while the sum of the areas of the bands at δ 8.29 and 7.99 was enlarged by an amount not more than corresponded to the intrinsic error in the measurements (*ca.* 10%). In the spectrum of the neutral form of (1), assignment of the three signals between δ 8.14 and 7.85 was based on integration. The area underneath the band at δ 7.85 was equal to the sum of the areas of the bands at 8.14 and 8.02.

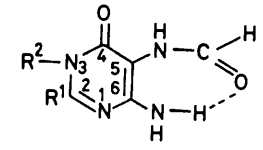
Table 1 shows that in the spectra of all compounds

[1] H. Gutowsky and C. H. Holm, *J. Chem. Phys.*, 1956, **25**, 1228.

[2] A. G. Whittaker and S. Siegal, *J. Chem. Phys.*, 1965, **42**, 3320.

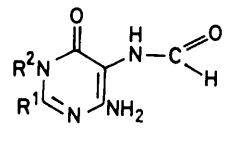
[3] T. Drakenberg and S. Forsén, *J. Phys. Chem.*, 1970, **74**, 1.

studied two formyl proton signals are observed, while the protons in the pyrimidine moiety (2-H, 3-Me, and 2-SMe)

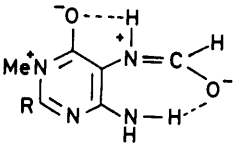


(A)

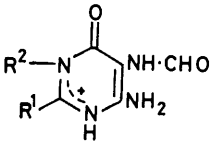
(1) $R^1 = R^2 = H$
(2) $R^1 = H, R^2 = Me$
(3) $R^1 = SMe, R^2 = H$
(4) $R^1 = SMe, R^2 = Me$
(5) $R^1 = SH, R^2 = H$



(B)

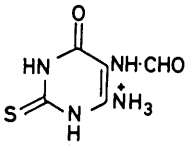


(A')

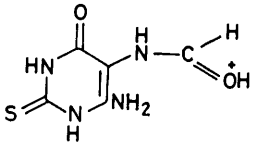


(C)

[cations of (1)–(4)]



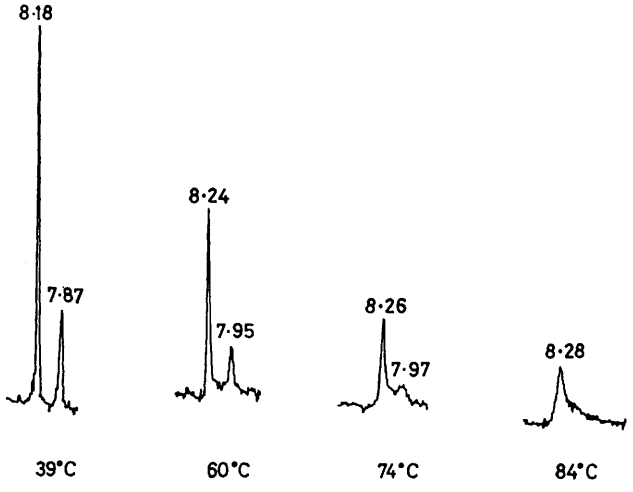
(D)



(E)

give rise to singlets. Separate formyl signals are present in both neutral and cationic forms.

two bands serve as a measure of the population distribution in the equilibrium (A) ⇌ (B). It is assumed that in the ground state, form (A) is stabilised by an intramolecular hydrogen bond and therefore is the predominant conformer, *i.e.* it is represented by the lower field signal (Figure).



Coalescence of the two formyl signals of 6-amino-5-formamido-2-thiouracil (5) with increasing temperature; the rotamer with the more shielded formyl proton disappears around 84 °C

In general, acid lowers the barrier for rotation about the amide bond to such a degree that interconversion of conformers becomes very rapid and the signals coalesce.[4-7] In *N*-methylformamide this applies to both the formyl and the NMe signals.[8] Although in the cations of such simple amides the proton is bound predominantly to the

TABLE 1

U.v. and n.m.r. spectra of 6-amino-5-formamidopyrimidin-4(3*H*)-ones

	$\lambda_{max.}$/nm				δ (neutral form) [b]					δ (cation) [d]				
Compd.	N [a]	A [a]	C [a]	p*K*	2-H	CHO	NMe	SMe	Ratio	2-H	CHO	NMe	SMe	Ratio [c]
(1)	263	266	257	−1.4	7.85	8.14			4 : 3	9.08 [f]	8.52			4 : 8
				>13 [e]		8.02					8.34			
(2)	260	255	256	−1.4	8.24	8.29	3.47		6 : 1	9.10 [g]	8.54	3.84		
				>13 [e]		7.99					8.35			
(3) [h]	231	266	257	−2		8.11		2.61	6 : 5		8.43		2.79	6 : 5
	274		285	8.0		7.93					8.27			
(4)	228	*i*	232	+1.7		8.15	3.42	2.60	4 : 1		8.53	3.75	2.87	4.5 : 1
	275		295			7.93					8.30			
(5)	243/4	>−2				8.18			5 : 1		8.84 [j]			7 : 1
	281/2	6				7.87					8.53			

[a] N, neutral molecule; A, anion; C, cation. [b] Measurements at 30 °C in $(CD_3)_2SO$–D_2O (9 : 1 v/v). [c] Approximate evaluation from the areas underneath the two formyl signals. The first figure always relates to the more deshielded band. [d] In $CF_3{\cdot}CO_2D$. [e] Unstable at pH values above 13. [f] Similar chemical shifts were obtained in 27N-D_2SO_4. [g] These measurements have to be performed rapidly, since in acidic solutions slow cyclisation to 1-methylhypoxanthine takes place. Therefore evaluation of the ratio [(A)] : [(B)] in the cation is not possible. [h] The anion of (3) was studied in buffer of pH 11: δ_{CHO} 8.34 and 7.97; δ_{SMe} 2.50; tautomer ratio 6 : 1. [i] Unstable in alkaline solutions. [j] Since this compound is only very slightly soluble in $CF_3{\cdot}CO_2H$, the chemical shifts of the cation were determined in 27N-D_2SO_4.

The two formyl bands have different shapes and intensities, owing to the presence of unequal populations of the two rotamers (Figure). The areas underneath the carbonyl oxygen, lowering of the rotational barrier is thought to be due to the presence of the minor *N*-protonated form, which is in equilibrium with the major *O*-protonated species. In contrast, in the formamides

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[6] G. Fraenkel and C. Niemann, *Proc. Nat. Acad. Sci. U.S.A.*, 1958, **44**, 688.
[7] A. Berger, A. Loewenstein, and S. Meiboom, *J. Amer. Chem. Soc.*, 1959, **81**, 62.
[8] G. Fraenkel and C. Franconi, *J. Amer. Chem. Soc.*, 1960, **82**, 4478.

studied here, the barrier is maintained in the cations. This is due to the fact that protonation takes place outside the amide group. For compounds (1)—(4), *N*-1 is involved in cation formation, as shown by the following data. In (1) and (2), protonation is characterised by a downfield shift of the 2-H signal of *ca.* 1 p.p.m. (Table 1). This implies formation of an amidinium-like cation (C), in agreement with earlier assignments of amidinium-like structures to the cations of 1- and 3-methylpyrimidin-4(3*H*)-one [9] and of 3-methylhypoxanthine.[10] For compounds (3) and (4), protonation at N-1 is indicated by the relatively large shift of the 2-SMe signal to lower field (0.18 and 0.27 p.p.m., respectively), We have previously shown for 2-methylthiohypoxanthines that protonation in the pyrimidine moiety shifts the SMe band downfield by 0.20—0.30 p.p.m., while cation formation in the imidazole ring deshields the 2-SMe signal by only 0.02—0.1 p.p.m.[11] Thus we conclude that in compounds (1)—(4) protonation takes place outside the formamido-group.

In the cation of compound (5) ($pK > -2$), both formyl resonances are shifted downfield by 0.66 p.p.m., *i.e.* about twice the difference observed for cation formation in compounds (1)—(4). It appears possible that the cation of (5) is a mixture of tautomers (D) and (E), but this cannot be decided at present.

The exchange between rotamers (A) and (B) becomes faster with increasing temperature and finally the formyl proton signals coalesce. The rate constants k_c [for the reaction (A) ⟶ (B)] and k_{-1} [for the conversion of (B) into (A)] at the coalesence point were evaluated with the help of the approximations (i) and (ii) where $\Delta\nu$ represents

$$k_c = \frac{\pi}{\sqrt{2}}\Delta\nu \text{ (i)}; \quad k_{-1} = \frac{\pi}{\sqrt{2K_{eq}}}\Delta\nu \qquad \text{(ii)}$$

the difference between the two formyl signals and K_{eq} is the equilibrium constant = population ratio [(A)]/[(B)]. It has been shown that free energies of activation ($\Delta G^{\ddagger}$) derived from the above approximations are in good agreement with the results of complete lineshape analysis,[12] even for the coalescence of signals with unequal intensities, *i.e.* when the two rotamer populations differ. According to the results of Kost *et al.*[12] an error of 25% in rate constants produces deviations of $\Delta G^{\ddagger}$ of only 0.1 kcal mol^{-1} at 300 K and an experimental error of ± 2 K in the temperature measurements corresponds to a deviation of about 1% in $\Delta G^{\ddagger}$ or *ca.* 0.1—0.2 kcal mol^{-1}. Our experimental data and the calculated values of $\Delta G^{\ddagger}$ and ΔG° are given in Table 2. For compound (2), coalescence of the formyl signals was not reached at 95 °C. which is the upper limit attainable with the solvent used. In this case the coalescence temperature was estimated by extrapolation.

Our data reveal a striking parallel between the difference in rotamer populations (Table 1) and the height of the rotation barriers (Table 2). This may be explained as follows. A strong hydrogen bond between the 6-amino-group and the carbonyl of the formamido-substituent, as shown in structure (A), raises the barrier by stabilising the ground state of one rotamer over that of the other.

TABLE 2

Dynamic n.m.r. parameters of the unchanged forms of some 6-amino-5-formamidopyrimidin-4(3*H*)-ones [a]

Compd.	T_c/K [b]	$\Delta\nu$ of CHO signals [c]	$\Delta G(T_c)^{\ddagger}$/ kcal mol^{-1} [d]	$\Delta G(T_c)^{\ddagger}$/ kcal mol^{-1} [e]	ΔG°/ kcal mol^{-1} [f]
(1)	332	12	17.3 ± 0.2	17.5 ± 0.2	0.2
(2)	> 365 [g]	30	18.4 ± 0.4	19.7 ± 0.4	1.3
(3)	343	18	17.6 ± 0.2	17.8 ± 0.2	0.1
(4)	348	22	17.8 ± 0.2	18.6 ± 0.2	1.0
(5)	360	31	18.2 ± 0.2	19.3 ± 0.2	1.1

[a] In $(CD_3)_2SO$–D_2O (9 : 1 v/v). The coalescence temperatures were determined only for the neutral molecules; the cations in acid solution undergo slow hydrolysis, which is accelerated by raising the temperature. [b] ±3 K. [c] At very low exchange rates. [d] Calculated from k_c. [e] Calculated from k_{-1}. [f] Calculated from $\Delta G^{\circ} = RT \ln K_{eq}$. [g] ±6 K; this value of T_c was obtained by extrapolation and therefore is only approximate.

Furthermore, the dipolar structure (A′) increases the energy of the transition state, in which rotation about the C–N bond has to take place. In addition, in the dipolar form (A′) the hydrogen bond is strengthened still further. A combination of these various factors is operative when the more stable rotamer (A) is to be converted into (B). On the other hand, for the reverse reaction (B) ⟶ (A) only the dipolar form of (B) raises the barrier. Tables 1 and 2 show that compounds (2), (4), and (5) exhibit large differences in rotamer populations as well as higher barriers to rotation in either direction. In the case of (2) and (4) we may assume that 3-methylation, by polarising the grouping CH_3–N(3)–C(4)=O ⟷ CH_3–N^+=C–O^-, stabilises the dipolar forms of both (A) and (B). In this way the energy of the ground states is lowered and that of the transition states is elevated, *i.e.* the barrier for the reaction in either direction is raised. However the increased contribution of the dipolar structure in turn stabilises the hydrogen bond in (A′). The corresponding effect is missing for rotamer (B).

No explanation is put forward at present for the high ratio of (A) to (B) in compound (5), since other pyrimidines with related structures have not been studied.

EXPERIMENTAL

Microanalyses were performed by M. Goldstein, Jerusalem. N.m.r. spectra were measured with a JEOL MH-100 instrument (sodium 3-trimethylsilyl[2,2,3,3-$^2H_4$]propionate as internal standard). Because of limited solubility of the neutral compounds in water, a 9 : 1 mixture of $(CD_3)_2SO$ and D_2O was used as solvent. Cations were studied in trifluoroacetic acid or in D_2SO_4. pK Values were derived from plots of $\lambda_{max.}$ or log $\varepsilon_{max.}$ against pH.

[9] Y. Inoue, N. Furutachi, and K. Nakanishi, *J. Org. Chem.*, 1966, **31**, 175.
[10] D. Lichtenberg, F. Bergmann, and Z. Neiman, *Israel J. Chem.*, 1972, **10**, 805.
[11] U. Reichman, F. Bergmann, and D. Lichtenberg, *J.C.S. Perkin I*, 1973, 2647.
[12] D. Kost, E. H. Carlson, and M. Raban, *Chem. Comm.*, 1971, 656.

Pyrimidines.—Compounds (1),[13] (2),[14] (4),[15] and (5)[15] were synthesised by known methods.

6-*Amino*-5-*formamido*-2-*methylthiopyrimidin*-4(3H)-*one* (3). A solution of 6-amino-5-formamido-2-thiouracil (5) [15] (1 g) in N-sodium hydroxide (10 ml) and dimethyl sulphate (0.5 ml) was stirred at 5—10 °C for 45 min. After neutralisation with hydrochloric acid, the product (3) precipitated, and gave *needles* (98%), m.p. 292—293° (from water); R_F (butan-1-ol–acetic acid–water, 12 : 3 : 5 v/v) 0.34 (Found: C, 34.3; H, 4.45; N, 26.95. Calc. for $C_6H_8N_4O_2S,0.5H_2O$: C, 34.45; H, 4.3; N, 26.8%).

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