Hydrazine-Hydrazone Tautomerism in Phenylhydrazinopyrimidones

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4-Phenylhydrazino-2-pyrimidone and its 1-methyl analog have been shown by ¹H nmr to exist in interconverting hydrazine and hydrazone tautomers in deuteriodimethylsulfoxide solution. Solvent effects indicate that increasing solvent polarity favors the hydrazine forms. In contrast, the 3-methyl analog occurs exclusively as the hydrazone. The hydrazone forms of the parent and 1-methyl derivatives appear to adopt the *syn* rotamers as a result of intramolecular hydrogen bonding. Variable temperature studies showed relatively high free energy barriers to tautomerization in these compounds, resulting both from solvation and intramolecular hydrogen bonding. The ¹H nmr spectra of 1-methyl-4-hydrazino-2-pyrimidone suggest that it exists predominately as the hydrazone in deuteriodimethylsulfoxide:deuteriochloroform solution, although the barrier to tautomerization is similar to those for the phenylhydrazino compounds.

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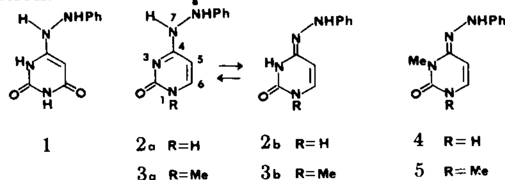
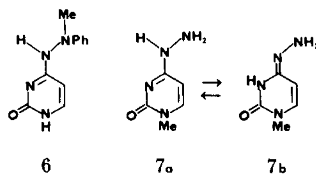
We have been interested in the structures of phenylhydrazinopyrimidines and related compounds that are inhibitors of bacterial DNA polymerase III [1] and animal cell DNA polymerase α [2]. 6-(Phenylhydrazino)uracils, for example **1**, have been proposed [3] to form hydrogen bonds with cytosine residues in the DNA template through the 1-H, 2-oxo and 6-NH groups as an essential part of their mechanism of inhibition. In an attempt to evaluate the influence of structure on inhibitory activity we prepared a 4-deoxy analog of **1**, *viz,* 4-phenylhydrazino-2-pyrimidone (**2**). This compound showed no activity as an inhibitor of bacterial DNA polymerase III, a result in accord with the expectation that it would exist in the 1-H amino-oxo form (**2a**) which would be incapable of hydrogen bonding with cytosine. The 60 and 90 MHz ¹H nmr spectra of **2**, however, showed two sets of resonances in deuteriodimethylsulfoxide solution at ambient temperature suggestive of a slow exchange process between two forms, a process also seen in the spectrum of the 1-methyl analog, **3**. It is proposed that this exchange is a result of hydrazine-hydrazone (amino-imino) tautomerism between forms **2a** and **2b**, and evidence from nmr and ultraviolet spectroscopy is presented to support this proposal. Evidence is also presented that 1-methyl-N^7-aminocytosine (**7**) may exist largely in the imino form in dimethylsulfoxide:chloroform solution.





Results.

The 90 MHz ¹H nmr spectra of **2** and its methylated derivatives at ambient temperature are summarized in Table 1. Two doublets of nearly equal intensity were observed in the spectrum of **2** at δ 5.48 and 5.82, the region expected for 5-H resonances [1,4], and extra peaks were observed in the far downfield, NH region cf the spectrum. The additional resonances were not caused by an impurity because repeated crystallization of **2** produced the same spectrum, and because two substituted analogs, *p*-bromo and *p*-methyl (phenyl ring substituted), also showed analogous extra resonances (results not shown). Elevation of the temperature caused gradual broadening and eventual coalescence of the resonances at δ 5.48 and 5.82 leading to a sharp doublet at δ 5.63 at 368°K. The relative intensities of the doublets δ 5.48 and 5.82 depended upon the solvent: addition of deuterium oxide to a solution of **2** in deuteriodimethylsulfoxide caused an increase in the intensity of the downfield doublet at the expense of that upfield, while addition of deuteriochloroform produced the opposite effect.

Three possibilities were considered to account for this phenomenon. Tautomerism involving 1-H and 3-H forms of **2** was not likely because all evidence for cytosine and other amino-oxo pyrimidines favors the mobile H on N¹ [4,5] and because the ultraviolet spectra of **2** and the fixed 1-H tautomeric analog **3** are nearly identical (Table 2). Furthermore the fixed N¹-methyl analog **3** also showed extra peaks in its nmr spectrum (Table 1), doublets in the 5-H region being observed at δ 5.56 and 5.84, nearly identical to the chemical shifts of **2**, but of clearly unequal intensities.

Hindered rotation about the C⁴—N⁷ bond was a second possibility, leading to different chemical shifts of *syn* and *anti* rotamers of **2**. Hindered rotation has been observed for N^7-methylcytosine and N^7,N^7-dimethylcytosine [6] and the cations of 1-methylcytosine, 1,3-dimethylcytosine and 1,N^7-dimethylcytosine [7,8]. Even in the case of N^7-methylcytosine, however, the *syn* rotamer predominated by 95%

Table 1

NMR Chemical Shifts of 4-Phenylhydrazino-2-pyrimidones at 298°K (Me_2SO-d_6; δ ppm)

Substituents	Compound	1—H	3—H	7—H	8—H	6—H	5—H	Me
	2a	10.24	—	9.36	8.00	7.39	5.82	
	2b	9.64	9.64	—	8.44	n.o. (a)	5.48	
1—Me	**3a**	—	—	9.23	8.00	7.67	5.84	3.24
	3b	—	9.78	—	8.47	n.o.	5.56	3.11
3—Me	**4**	10.08	—	—	8.43	7.00	6.08	3.19
1,3—diMe	**5**	—	—	—	8.42	7.11	6.13	3.18, 3.23
8—Me (b)	**6a**	11.06	—	9.57	—	7.46	5.88	3.12
	6b	9.69	9.69	—	—	n.o.	5.78	3.18

(a) n.o. — not observed. (b) Spectrum run in 1:1 deuteriodimethylsulfoxide:deuteriochloroform at 272°K.

and separate signals for *syn* and *anti* rotamers were only observed at -19° in deuteriodimethylformamide solution [6a]. However, the more electronegative anilino group on N^7 in **2** and **3** might be expected to lower the free energy barrier to rotation in these compounds relative to cytosine or N^7-methylcytosine. Indeed, the nmr spectra of the deuteriochlorides of **2** and **3** in deuteriodimethylsulfoxide at ambient temperature (Table 3) showed only one form of each compound present. Apparently even in the cations, conjugation between N^7 and the pyrimidine ring is insufficient to cause observable hindered rotation under these spectral conditions, conjugation which would be even less in the neutral forms of **2** and **3**.

It is proposed that **2** and **3** undergo slow exchange between hydrazine and hydrazone forms of the molecules in deuteriodimethylsulfoxide. Certain cytosine derivatives substituted on the exocyclic nitrogen (N^7) have been shown to have altered tautomeric structures. N^7-Methyl derivatives possessed the amino-oxo structure [7], but N^7-amino- and N^7-hydroxycytosines were proposed [9] to exist as mixtures of amino and imino forms in water; these authors determined that the amino form predominated for N^7-aminocytosine (K_T *ca.* 30) whereas the imino form was preferred by N^7-hydroxycytosine (K_T *ca.* 10), and concluded that increasing electronegativity of the N^7 substituent led to progressively greater stability of the imino form. Evidence from ultraviolet spectroscopy also led Janion to conclude that N^7-hydroxy- and N^7-methoxycytosines exist largely in the imino forms [10]. Direct proof of these conclusions was obtained from the observation of the imino-oxo form of 1,5-dimethyl-N^7-hydroxycytosine in the solid state [11].

Assignment of NMR Resonances of **2** and **3**.

The greater deshielding of ring protons expected of the fully aromatic forms of **2** and **3** (forms a.) indicated that the downfield 5-H resonances should be assigned to the hydrazine tautomers and those upfield to the hydrazone tuatomers, **2b** and **3b**. For the same resasons the lone doublets at δ 7.39 and 7.67 were assigned to the 6-H of the hydrazines, **2a** and **3a**, respectively. Apparently, the 6-H resonances of the hydrazones are buried under the phenyl multiplets. Indeed, increasing probe temperature led to movement of the observed doublets *upfield* (see, for example, Figure 1 for **3**), where they presumably coalesce with 6-H resonance from the hydrazone tautomers. The downfield shift of the doublet at δ 7.39 of **2** caused by methylation (0.28 ppm) confirms that this resonance arises from the 6-H of the corresponding form.

The downfield, N-H region of the spectrum of **2** is complex. The peak at δ 8.00 in **2** was ascribed to N^8-H of **2a** and that at δ 8.44 to N^8-H of **2b**. These peaks are absent in the spectrum of the N^8-Me analog, **6** (Table 1). The resonance analogous to N^8-H of **2a** has also been observed at δ 7.90 in the nmr spectrum of the corresponding uracil derivative **1** (unpublished results). That the more downfield of these peaks results from the hydrazone **b** form is confirmed by the presence in the spectrum of the fixed hydrazone **5** of a peak at δ 8.42, undoubtedly due to N^8-H. Resonances corresponding to N^8-H of **3a** and **3b** were observed at δ 8.00 and 8.47, respectively, and their relative intensities were identical to those of the corresponding 5-H resonances observed for **3**.

Three broad downfield resonances in the spectrum of **2** were assigned as follows. The peaks at δ 9.36 and 10.24 were ascribed to N^7-H and 1-H of the hydrazine **2a**, and that at δ 9.64 to the overlapped resonances of 1-H and 3-H of the hydrazone **2b**. In 6-(phenylhydrazino)uracil (**1**) the resonance corresponding to N^7-H was observed at δ 8.37 (unpublished results), significantly upfield compared to that assigned here to N^7-H of **2a**. Conjugation involving N^3 of **2a** to produce a dipolar form can readily account for this significant downfield shift. By analogy with the above arguments the resonance at δ 9.23 in the spectrum of **3** is assigned to N^7-H of the hydrazine form **3a**, and that at δ 9.78 to 3-H of the hydrazone, **3b**.

Structures of **2** and its *N*-Methyl Analogs.

The existence of hydrazine and hydrazone forms of **2** in deuteriodimethylulfoxide is a result of roughly equal stabilization of the forms by the solvent. Thus K_T, defined as the ratio **2a/2b** and determined by integration of 5-H resonances, is *ca.* 1.3 and is invariant both with concentration over a tenfold range and with temperature. The effect of solvent is profound, however, K_T decreasing to 0.23 in 1:1 deuteriodimethylsulfoxide:deuteriochloroform. The more polar hydrazine form **2a** would be expected to be less favored in the less polar solvent mixture. The 1-methyl analog, **3**, in contrast, has K_T of *ca.* 3.5 in deuteriodimethylsulfoxide, a value which decreases to 0.9 in 1:1 deuteriodimethylsulfoxide:deuteriochoroform. Presumably, an even greater difference in polarity between **3a** and **3b** contributes to a greater stabilization of the hydrazine in the pure solvent. Unexpectedly, the spectrum of the 3-methyl analog, **4**, showed the presence of only one form which must be the hydrazone, based largely on the chemical shift of the N^8-H resonance at δ 8.43 (Table 1): comparison of the spectrum of **4**, with that of the fixed hydrazone (**5**), where N^8-H appears at δ 8.42, confirms the assignment.

Ultraviolet absorption spectra of **2** and its *N*-methyl analogs in methanol (Table 2) confirm that the 3-methyl compound (**4**) exists only as the hydrazone by comparison of its spectrum with that of **5**. However, **2** and **3** appear to exist primarily as the hydrazines in methanol. Their spectra are similar to those of **7** (Table 2) and 1,7-dimethylcytosine [λ max 283 nm (ϵ = 7470)] in ethanol [9].

Table 2

Ultraviolet Absorption Spectra of Hydrazinopyrimidones in Methanol

Compound	λ max (ϵ)
2	238 (10,950), 277 (7000)
3	238 (17,000), 284 (12,500)
4	239 sh (a) (6400), 260 (9300), 280 sh (8700), 349 (3700)
5	234 sh (11,600), 263 (17,200), 284 sh (14,200), 355 (6300)
7 (b)	278 (8470)

(a) sh, shoulder. (b) From reference 4 (in ethanol).

Syn-anti Isomerism in 2b-5b.

The chemical shifts of the 5-H resonances of the hydrazones of **2-5** appear in two groups. In **2b** and **3b** the 5-H resonances occur at δ 5.48 and 5.56, whereas in **4** and **5** there is a shift to δ 6.08 and 6.13 (Table 1). The downfield appearance of 5-H in **4** and **5** is undoubtedly due to the predominance of the *anti* isomer in these compounds, defined with respect to the proximity between the N^7-N^8 and N^3-C^4 bonds. The presence in each of the latter compounds of the 3-methyl group might be expected to constrain them in the *anti* form, and the anisotropy of the N-N bond would deshield the 5-H resonances. Consequently, **2b** and **3b** exist in the *syn* form (or in rapidly interconverting rotamers), probably stabilized by intramolecular hydrogen bonding between N^3-H and N^8. Indeed, such an intramolecular hydrogen bond was observed in the crystal structure of 1,5-dimethyl-N^7-hydroxycytosine between N^3-H and oxygen [11]. In the latter compound the 5-methyl group may contribute to the *syn* conformation by steric repulsion; similarly, repulsion by the 3-methyl groups of **4** and **5** may contribute to the observed *anti* conformations. No evidence was obtained for *syn-anti* isomerization in the hydrazine forms, **2a** and **3a**.

Spectra of the cations of **2-5** in deuteriodimethylsulfoxide (Table 3) indicate a single, common structure of these compounds. Only in one case, the 8-methyl analog (**6**), were separate resonances observed, suggesting slow interconversion of *syn* and *anti* rotamers by analogy with the corresponding resonances in the 1,7-dimethylcytosine cation (Table 3).

Table 3

NMR Chemical Shifts of Deuteriochlorides at 298°K (Me_2SO-d_6 ; δ ppm)

Compound·DCl	6—H	5—H
2	7.76	6.22
3	8.00	6.25
4	7.85	6.34
5	8.00	6.43
6a	7.82	6.19
6b	7.72	6.05
1,7-diMecytosine (a) **a**	8.32	6.40
b	8.02	6.35

(a) From reference 8.

Variable Temperature Studies.

Free energies of activation for the hydrazine-hydrazone tautomerism of **2** and **3** were estimated from the coalescence temperatures of 5-H resonances, as depicted for **3** in Figure 1. The 1-methyl derivative **3** was studied in 1:1 deuteriodimethylsulfoxide:deuteriochloroform for two reasons. First, this solvent gave a nearly equal proportion of tautomers leading to less uncertainty in the coalescence temperature of 5-H doublets. Second, a lower temperature was achieved than for deuteriodimethylsulfoxide itself, providng a more accurate determination of $\Delta\delta$ in the absence of exchange. Compound **2** was insufficiently soluble in the mixed solvent at low temperatures; however, the nearly equal proportion of tautomers in deuteriodimethylsulfoxide partly compensated for this problem.

Coalescence temperatures for 5-H resonances of **2** and **3** (see Figure 1 for **3**) were 333 and 313°K, respectively. Employing the equations for rate constants for the forward and back reactions at T_c:[$k_1 = (\pi/\sqrt{2})$ and $k_{-1} = (\pi/K\sqrt{2})$] and the Erying formulation [12] gave average $\Delta G^{\ddagger}$ for tautomerization of **2** and **3** of 16.9 and 15.8

kcal/mole, respectively. (K_T values near unity have $k_1 = k_{-1}$). While these equations are approximate, they have been found to give reliable estimates of free energies of activation for both coalescing singlets and doublets compared with complete line shape analysis [13]. These free energy barriers are higher than those expected for a simple, solvent-mediated tautomeric exchange, and probably reflect the need to break intermolecular hydrogen bonds in the hydrazine forms and intramolecular hydrogen bonds in the hydrazone forms in the *syn* conformation. For compound **3** in deuteriodimethylsulfoxide solution, T_c was *ca.* 353°K, giving $\Delta G^{\ddagger}_{avg}$ of 18.4 kcal/mole. This higher value than that in the less polar solvent mixture further supports disruption of solvation as contributing to the barrier to tautomerization.

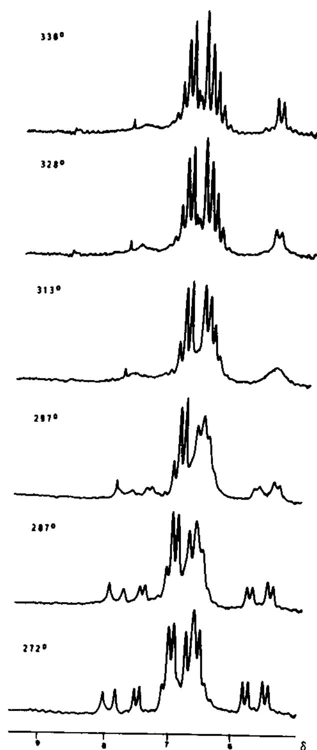


Figure 1. 90 MHz nmr spectra of 1-methyl-4-phenylhydrazino-2-pyrimidone (**3**) in 1:1 deuteriodimethylsulfoxide:-deuteriochloroform.

A lower barrier to tautomerization appears to exist for the N^8-methyl derivative, **6**. At 298°K in deuteriodimethylsulfoxide only one form of this compound was observed, likely the hydrazine by comparison of its chemical shifts with those of **2a** and **2b** (data not shown). At lower temperature (272°K) in 1:1 deuteriodimethylsulfoxide:deuteriochloroform, two forms were observed (Table 1) assigned as the hydrazine **6a** and the hydrazone **6b**, although K_T **(a/b)** is *ca.* 3.5. The influence of the electron releasing methyl group of **6** increases the stability of the hydrazine form in accord with the conclusion of Brown, *et al.* [9].

Solution Structure of N^7-Aminocytosine.

Readily observable interconversion of tautomers of **2** and **3** led us to apply similar experimental approaches to the solution structure of N^7-aminocytosine (4-hydrazino-2-pyrimidone). The 1-methyl derivative (**7**) was sufficiently soluble in deuteriodimethylsulfoxide:deuteriochloroform to achieve low temperatures for ^{1}H nmr work. As shown in Figure 2, at ambient temperature (298°K) peaks in the 90 MHz spectrum of **7** were very broad, with barely discernible doublets at δ 5.65 and 7.32 representing 5-H and 6-H resonances, respectively. The spectrum sharpened appreciably at elevated temperatures (Figure 2). At low temperatures, the 5-H and 6-H resonances separated into

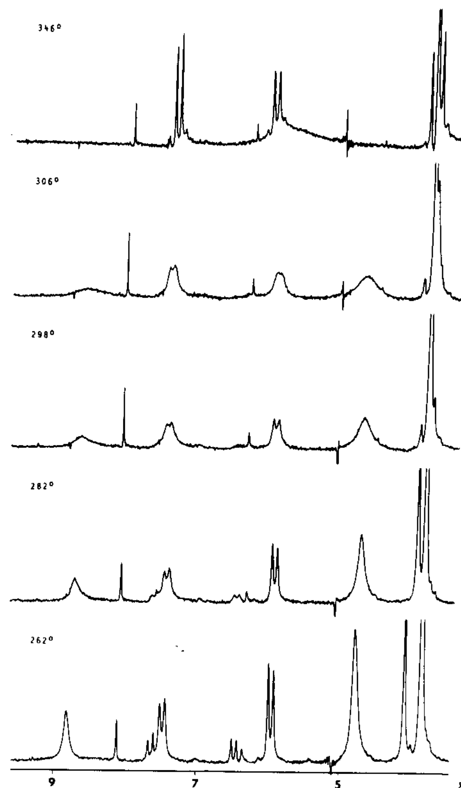


Figure 2. 90 MHz nmr spectra of 1-methyl-4-hydrazino-2-pyrimidone (**7**) in 1:1 deuteriodimethylsulfoxide:deuteriochloroform. The singlet at δ 6.35 is an impurity.

pairs of doublets of unequal intensities. At 272°K the major component showed doublets at δ 7.38 and 5.68 and the minor component (K_T = 0.12) at δ 7.56 and 6.26, respectively. Based on the chemical shift assignments adduced above for the phenylhydrazino compounds (Table 1), the major component of **7** is expected to be the hydrazone **7b**. Approximate free energies of activation for the exchange

of **7** under these conditions, calculated independently from the coalescence temperatures of 5-H (T_c 316°K) and 6-H (T_c 298°K), yielded $\Delta G^{\ddagger}_{1}$ = 15.4 kcal/mole and $\Delta G^{\dagger}_{-1}$ = 14.1 kcal/mole. $\Delta G^{\dagger}$ values for the forward and back reactions are distinctly different because of the large idifference in populations compared with **2** and **3**, but there was excellent agreement in the values calculated from both coalescence temperatures. The barriers, furthermore, are similar to that obtained for **3**, although there is a large difference in K_T between **7** and **3**. The relatively greater stability of the hydrazone form of **7** is reflected in a free energy difference of *ca.* 1.3 kcal/mole between **7a** and **7b**, and may result from more efficient solvation of **7b** compared to **3b** and to a greater contribution by the intramolecularly hydrogen bonded *syn* form of **7b**.

Conclusions.

4-Phenylhydrazino-2-pyrimidones have been shown to exist in slowly interconverting hydrazine and hydrazone forms in deuteriodimethylsulfoxide. Increasing solvent polarity favors the more polar hydrazine forms. The 3-methyl analog appears to exist only in the hydrazone form by analogy with the fixed hydrazone, 1,3-dimethyl-4-phenylhydrazino-2-pyrimidone. The hydrazone forms of the parent (**2b**) and 1-methyl (**3b**) compounds exist as the *syn* rotamers as a result of intramolecular hydrogen bonding involving 3-NH and N^8. Free energy barriers for hydrazine-hydrazone tautomerization in these compounds suggest that extensive solvation, although presumably the vehicle for proton transfer between forms, and intramolecular hydrogen bonding play major roles in slowing the process relative to the nmr time scale.

Reexamination of the nmr spectrum of 1-methyl-4-hydrazino-2-pyrimidone (**7**) has shown that it exists in two interconverting forms in 1:1 deuteriodimethylsulfoxide:deuteriochloroform solution, and these forms are proposed to be the hydrazine (**7a**) and hydrazone (**7b**) structures, the latter being the major form. This result is in accord with expected solvent effects on hydrazine-hydrazone tautomerism observed with the phenylhydrazino compounds (see above), but not with recent evidence that the hydrazine form of N^4-aminocytosine exists in the solid state [14]. However, this result could reflect the predominance of the hydrazine form in aqueous solution as proposed by Brown, *et al.* [9] (the crystals were obtained from aqueous solution) as well as intermolecular forces in the solid state, for example the hydrogen bond observed between N^4-H and O^2 from adjacent molecules [14]. Indeed, evidence from ultraviolet spectroscopy (Table 2 and reference 4) has indicated that **2**, **3** and **7** exist largely as the hydrazines in alcoholic solutions. Further experiments are in progress with model compounds to determine solvent effects on the nmr spectrum of **7** in order to resolve this contradiction.

EXPERIMENTAL

Pyrimidines were purchased from Vega Biochemical Corporation. Elemental analyses were done by Schwarzkopf Microanalytical Laboratories, Woodside, New York. Melting points were determined on a Meltemp apparatus and are uncorrected. 1-Methyl-4-hydrazino-2-pyrimidone (**7**) was prepared by a literature method [9].

Ultraviolet spectra were measured at 25° on a Beckman Model 25 spectrophotometer. The nmr spectra were obtained with a Bruker SPX-90 FT spectrometer and temperatures, measured with a copper-constantan couple, are considered accurate to ±1°. Solutions containing 0.13 *M* of test compounds were prepared in solvents previously stored over molecular sieves. Chemical shifts were measured relative to internal tetramethylsilane. Deuteriochlorides were prepared by dissolving the corresponding pyrimidines in 19% deuterium chloride in deuterium oxide, evaporating the solutions to dryness *in vacuo* and storing the products over phosophorus pentoxide.

4-Phenylhydrazino-2-pyrimidone, **2**.

A mixture of cytosine (0.7 g, 6.3 mmoles) and phenylhydrazine (1.36 g, 12.6 mmoles) was heated at reflux in water (20 ml) containing acetic acid (0.76 g, 12.6 mmoles). After 4 hours the precipitated product was collected by filtration and crystallized from 75% acetic acid to give 0.5 g (40%) of cream-colored solid, mp 246-248°.

Anal. Calcd. for $C_{10}H_{10}N_4O$: C, 59.40; H, 4.98; N, 27.71. Found: C, 59.31; H, 5.13; N, 27.52.

1-Methyl-4-phenylhydrazino-2-pyrimidone, **3**.

1-Methylcytosine and phenylhydazine, under conditions described for the synthesis of **2**, afforded, after crystallization from water, a 45% yield of light purple plates, mp 213-216°.

Anal. Calcd. for $C_{11}H_{12}N_4O$: C, 61.10; H, 5.59; N, 25.91. Found: C, 60.90; H, 5.64; N, 25.79.

3-Methyl-4-phenylhydrazino-2-pyrimidone, **4**.

3-Methylcytosine and phenylhydrazine, under conditions described for the synthesis of **2**, gave, after crystallization from 40% ethanol, a 37% yield of greenish plates, mp 208-211°.

Anal. Calcd. for $C_{11}H_{12}N_4O$: C, 61.10; H, 5.59; N, 25.91. Found: C, 61.07; H, 5.61; N, 25.61.

4-(α-Methylphenylhydrazino)-2-pyrimidone, **6**.

α-Methylphenylhydrazine and cytosine, under conditions described for the synthesis of **2**, yielded, after crystallization from water, 22% of nearly colorless crystals, mp 231-233°.

Anal. Calcd. for $C_{11}H_{12}N_4O$: C, 61.10; H, 5.59; N, 25.91. Found: C, 60.90; H, 5.56; N, 25.95.

1,3-Dimethyl-4-phenylhydrazino-2-pyrimidone, **5**.

A solution of 1,3-dimethyl-4-thiouracil [15] (0.3 g, 1.9 mmoles) and methyl sulfate (0.35 g, 2.8 mmoles) in ethanol (5 ml) was heated at reflux for 30 minutes. Phenylhydrazine (0.35 g, 3.2 mmoles) was added and reflux continued for 3 hours. The solvent was removed *in vacuo* and the semisolid residue was dissolved in water (*ca.* 2 ml). The solution was neutralized with sodium bicarbonate and a yellow oil separated. Chilling of the mixture and trituration produced a yellow solid which was collected by filtration and crystallized from 30% ethanol giving 60 mg (14%) of bright yellow crystals, mp 143-146°.

Anal. Calcd. for $C_{12}H_{14}N_4O$: C, 62.59; H, 6.24; N, 24.18. Found: C, 62.59; H, 6.13; N, 24.33.

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