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URACIL AND ITS 4-HYDROXY-1(*H*) and 2-HYDROXY-3(*H*) PROTOMERS. pK$_a$'s AND EQUILIBRIUM CONSTANTS[1]

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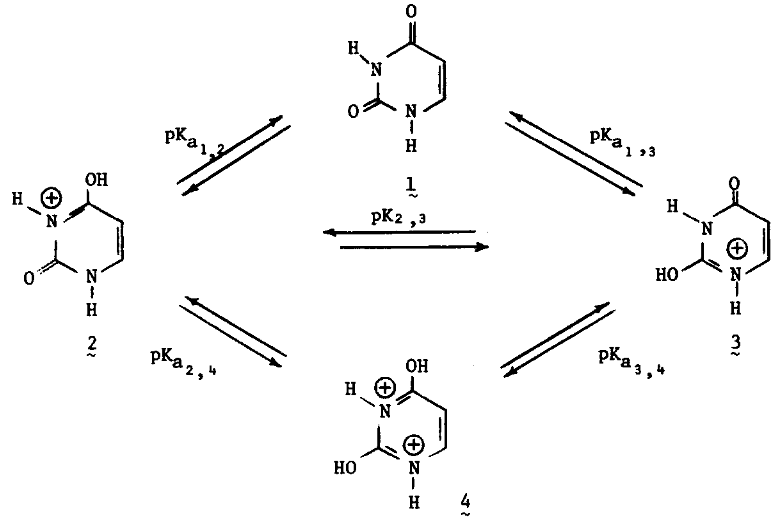
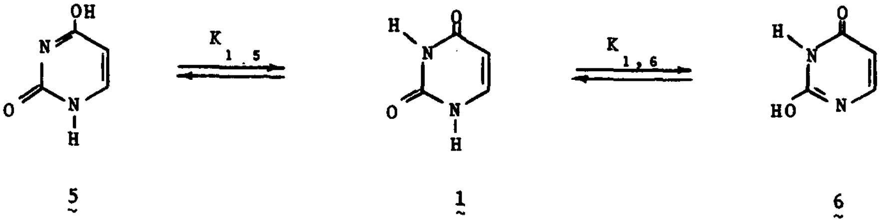
The most stable 2,4-dioxo structure of uracil can be interconverted with five other protomers by hydrogen shifts between oxygen and nitrogen. However, in solution the equilibrium concentrations of the less stable protomers are very small, and the only practical approach for measuring equilibrium constants is the pK method.[3] Protonation of 1 at O_4 to give 2 is favored[4,5] over protonation at O_2. As a result only the equilibrium constant, $K_{1,5}$ between 1 and its 4-hydroxy-1(*H*) protomer (5) has been reported.[3,6]





If $pK_{a_{1,3}}$ were known, a value for the equilibrium constant between 1 and its 2-hydroxy-3(*H*) protomer, $K_{1,6}$, could be obtained. Unfortunately, the equilibrium concentration of 3 is too small to measure directly in the presence of 4. In principle $pK_{a_{1,3}}$ can also be calculated from the pK_a's for deprotonation of dication 4 to monocations 2 and 3 as shown in equations 1 and 2. We now report a procedure for estimating $pK_{a_{2,4}}$ and $pK_{a_{3,4}}$, and consequently

$$pK_{2,3} = pK_{a_{2,4}} - pK_{a_{3,4}} \qquad (1)$$

$$pK_{a_{1,3}} = pK_{a_{1,2}} + pK_{2,4} \qquad (2)$$

pK's for all of the equilibria involving 1 - 4. The pK method is then used to provide equilibrium constants for $1 \rightleftharpoons 5$ and $1 \rightleftharpoons 6$.

pK_a's for the second protonation of uracil, 2-methoxy-4-oxo-3(*H*)-pyrimidine, and 4-methoxy-2-oxo-1(*H*)-pyrimidine are available from a previous study. pK_a's for monoprotonation of the three bases were measured using the same technique and are listed in Table I. Good H_A behavior was found for both protonations of all of the bases. Several values have been reported for

Table I. pK_a's[a]

Compound	pK_a^1	n^1[b]	pK_a^2	n^2[b]
Uracil	-2.9_8	0.88	-7.3_2	c
2-Methoxy-4-oxo-3(*H*)-pyrimidine	$+0.6_0$	1.22	-5.8_4	c
4-Methoxy-2-oxo-1(*H*)-pyrimidine	$+0.6_0$	0.95	-7.3_2	c

[a] Calculated using H_A in H_2O-H_2SO_4-SO_3 solvents. [b] Slope of the log of ionization ratios versus H_A. [c] H_A indicators, n = 1.

deprotonation of 2 to 1. The pK_a reported by us (-2.98) is similar to that obtained by converting Katritzky and Waring's data to H_A (-2.84)[3d] but differs from those published by Antowvskii and coworkers (-2.20)[3a] or Parry and coworkers (~ +0.6).[3c]

As mentioned previously, 2 is the most stable monocation of uracil. 4-Methoxy-2-oxo-1(*H*)-pyrimidine and 2-methoxy-4-oxo-3(*H*)-pyrimidine both add a proton to the basic imidate nitrogens giving O-methyl analogs of 2 and 3, respectively. The nmr spectra of all three monocations are similar. However, the uv spectrum of monoprotonated 2-methoxy-4-oxopyrimidine, λ_{max} 248 nm (ε 5.9 x 10^3, 74.4% H_2SO_4-H_2O is significantly different from those of 2, λ_{max} 274 nm (ε 4.0 x 10^3, 88.2% H_2SO_4-H_2O) and monoprotonated 4-ethoxy-2-oxopyrimidine, λ_{max} 284 (ε 8.9 x 10^3, 10% H_2SO_4-H_2O).[4d] The nmr spectra of 4 and both O-methyl derivatives in H_2SO_4-SO_3 are similar to those reported for the same cationic species in HSO_3F-SbF_5-SO_2.[5a]

pK_a's for the O-methyl analogs of 2 and 3 were substituted for $pK_{a_{2,4}}$ and $pK_{a_{3,4}}$ in equation 1[8] giving $pK_{2,3}$= -1.48 ($K_{2,3}$ = 30). The value for $pK_{a_{1,3}}$ = -4.46 was determined according to equation 2.[9]

pK_a's for dissociation of 2 and 3 to 1 were used to calculate the solution phase protomeric equilibrium constants; $K^{298}_{1,5}$ = 3.8 x 10^3 ($\Delta F^{298^0}_{1,5}$ = -4.9 kcal/mol) and $K^{298^0}_{1,6}$ = 1.1 x 10^5 ($\Delta F^{298^0}_{1,6}$ = -6.9 kcal/mol). Enthalpy differences between 1 and 5 (29.8 kcal/mol) and 1 and 6 (17.9 kcal/mol) have been estimated by semiempirical SCF-MO techniques.[10] Although the free energy differences which were found in solution cannot be directly compared with "gas phase" enthalpies,[11] the discrepancy between our work and the theoretical results seems to be too large to attribute to differences in solvation or in entropy in view of the structural similarities of the monohydroxy protomers.

In conclusion, the pK_a's for monoprotonation of uracil at O_2 and O_4 have been determined and used to calculate the equilibrium constants between uracil and 4-hydroxy-2-oxo-1(*H*)- and 2-hydroxy-4-oxo-3(*H*)-pyrimidine. The technique for estimating the pK_a for protonation of uracil at O_2 be applicable to other heterocyclic systems where the concentration of a minor cationic species cannot be measured directly.

REFERENCES

1. We wish to thank the American Cancer Society and the Public Health Service (RR07092) for financial support.
2. a. Alfred P. Sloan Fellow, 1975-77. b. University of Utah Research Fellow.

3. A. R. Katritzky and J. M. Lagowski, *Advan. Heterocyclic Chem.*, **1**, 311 (1963).

4. (a) V. L. Antonovskii, A. S. Gokovskaya, T. M. Prokopeva, and B. I. Sukhorukov, *Dokl. Akad. Nauk, S.S.S.R.*, **205**, 461 (1972); (b) R. Shapiro and M. Dansig, *Biochem.* **11**, 23 (1972); (c) E. P. Parry, D. H. Herm, and J. G. Burr, *Biochim. Biophys. Acta.*, **182**, 572 (1969); (d) A. R. Katritzky and A. J. Waring, *J. Chem. Soc.*, 1540 (1962).

5. (a) C. D. Poulter and R. B. Anderson, Tetrahedron Letters, 3823 (1972); (b) R. van Wagner and W. von Philipsborn, *Helv. Chim. Acta,* **53**, 299 (1970).

6. Since pyrimidine bases follow the H_A acidity function,[7] estimates for $pK_{a_{1,2}}$ based on H_o are high by approximately 0.5 pK units.

7. C. D. Poulter and G. D. Frederick, *J. Amer. Chem. Soc.*, **97**, 1797 (1975).

8. Apparently replacing an O-hydrogen by methyl does not alter the pK_a appreciably as evidenced by identical pK_a's for the second protonation of **1** and 4-methoxy-2-oxo-pyrimidine.[7]

9. Although the absolute errors in $pK_{a_{2,3}}$ and $pK_{a_{3,4}}$ may be ± 0.2 pK units, the relative errors are much less. The error in $pK_{2,3}$ should be $< \pm$ 0.05 pK units. The error in $pK_{a_{1,3}}$, is $\leq$ 0.1 pK units. Since $pK_{a_{1,3}}$ is the difference between $pK_{a_{1,2}}$ and $pK_{2,3}$, comparisons involving $pK_{a_{1,2}}$ and $pK_{a_{1,3}}$ should be good to within 0.2 pK units. The maximum error in $K_{2,3}$, $K_{1,5}$, and $K_{1,6}$ should be no more than a factor of 1.7.

10. N. Bodor, M. J. S. Dewar, and A. J. Harget, *J. Amer. Chem. Soc.*, **92**, 2929 (1970).

11. (a) P. Beak, D. S. Mueller and J. Lee, *J. Amer. Chem. Soc.*, **94**, 3867 (1974); (b) P. Beak and F. S. Fry, Jr., ibid., 93, 1700 (1973); (c) P. Beak, J. Bonham, and J. T. Lee, Jr., *ibid.*, **90**, 1569 (1968).