Ultraviolet Spectra of N-Heterocyclic Systems. I. The Anions of Uracils[*]

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The keto-enol equilibria of uracil and its anions have been studied in order to clarify the nature of the tautomerism encountered in the complicated derivatives of a natural product[1,2]. Moreover, besides being of interest because of its relation to nucleic acids, uracil contains two potentially dissociable protons, and accordingly it was felt that this simple molecule might provide a convenient means for more complex systems. The keto-enol equilibria of various monohydroxy *N*-heterocyclic bases have already been studied extensively by means of infrared, ultraviolet, and Raman spectroscopy, and other methods[3]. The studies with dihydroxy compounds are much more limited, and although the phenomena have indeed been investigated with uracil, especially by Shugar and Fox[4], the tautomeric equilibrium constants K_T were not taken into consideration.

Six tautomeric forms are conceivable for uracil. However, the following evidence shows that it exists predominantly in the diketo form I (Plate 1) in solution as well as in the solid state. The solid infrared spectrum of uracil (Table I) in the double bond stretching region is very similar to 1, 3-dimethyluracil (VIII) which has a fixed structure; 1-methyluracil (IV) and 3-methyluracil (VI), for which three tautomeric forms are possible, respectively, also show very similar absorptions. The dioxane solution spectra of uracil, 1-methyluracil, and 3-methyluracil are also quite similar to 1, 3-dimethyluracil. Hence, it is easily seen that all four compounds can be represented by the diketo forms both in dioxane and in the solid state. It is well-known that among the possible tautomers, the more polar forms are favored in the solid state and in solutions of high dielectric constants. The fact that the tautomers present in dioxane, a solvent of low dielectric constant (2.101 at 25°C)[5], and in the solid state, are the diketo forms (I, IV, VI) suggests that the same diketo forms are also predominant

TABLE I. INFRARED ABSORPTIONS
(in cm^{-1})

	KBr	Dioxane
Uracil (I)	1715, 1670	1720 sh, 1698
1-Methyluracil (IV)	1695, 1675	1712 sh, 1694
3-Methyluracil (VI)	1705, 1630	1726, 1670
1, 3-Dimethyluracil (VIII)	1712, 1655	1713, 1673

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[*] Paper read in part at the 13th Annual Meeting of the Chemical Society of Japan, Tokyo, April, 1960.

[1] K. Nakanishi, M. Ohashi, S. Kumasaki and S. Yamamura, *J. Am. Chem. Soc.*, **81**, 6339 (1959).

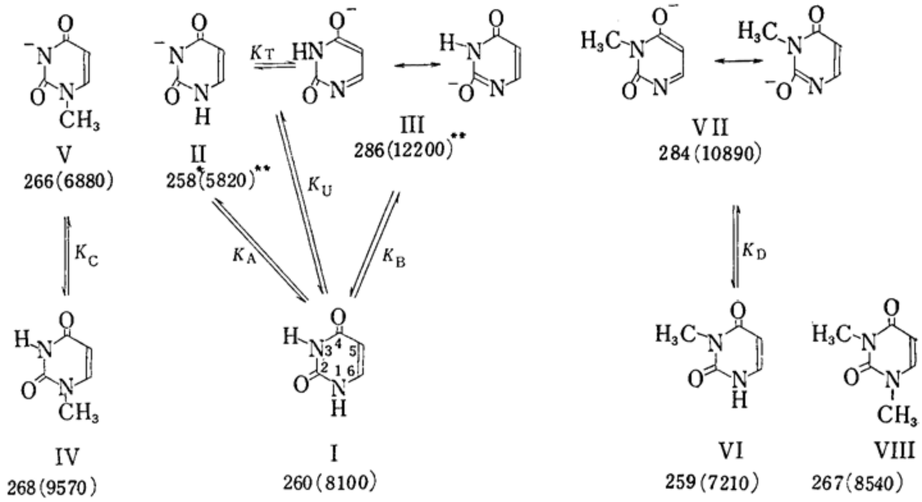
[2] Results pertaining to the natural derivatives will be reported shortly.

[3] Some recent papers on this subject: (a) H. Shindo, *Chem. and Pharm. Bull.*, **7**, 407 (1959); (b) K. Nakamoto and A. E. Martell, *J. Am. Chem. Soc.*, **81**, 5857 (1959); (c) A. Albert and E. Spinner, *J. Chem. Soc.*, **1960**, 1221.

[4] D. Shugar and J. J. Fox, *Biochem. et Biophys. Acta*, **9**, 199 (1952); see also J. J. Fox and I. Wempen in "Advances in Carbohydrate Chemistry", Vol. 14, Academic Press, New York (1959), p. 283.

[5] H. S. Harned and B. B. Owen, "Physical Chemistry of Electrolytic Solutions", Reinhold Publishing Co., New York (1958), p. 161.

Plate 1. Proton equilibria of uracils in water



Data for neutral molecules and anions are for pH 6.86 and 12.0, respectively.
Peak positions (mμ) and intensities (ε) are given.
* Obtained after wing correction (Fig. 4).
** Calculated (see text).

TABLE II. ULTRAVIOLET SPECTRA OF URACILS (25°C)
Wavelengths given in mμ; numbers in parentheses are $\varepsilon \times 10^{-3}$
$\Delta\lambda$ (in mμ) = λ (pH 12.0) − λ (pH 6.86)

	Dioxane	pH 6.86	pH 9.71	pH 12.0	Δλ	pK
1. Uracil	257(8.35)	260 (8.10)	265(5.47)	286 (6.01)	+26	9.51 (at 25°C)
2. Thymine[a)]		265 (7.89)		291 (5.44)	+26	9.9
3. 1-Methyluracil	266(9.70)	268 (9.57)	267(7.64)	266 (6.88)	− 2	9.77 (at 25°C)
4. Uridine[a)]		262(10.1)		262 (8.5)	0	9.25
5. Uracil deoxyriboside[a)]		262(10.2)		262 (7.63)	0	9.3
6. Thymidine[a)]		267 (9.65)		267 (7.38)	0	9.8
7. 3-Methyluracil	257(7.31)	259 (7.21)	255(5.74)	284(10.89)	+25	10.00 (at 25°C)
8. 1,3-Dimethyl uracil	265(8.71)	267 (8.54)				

a) Data from D. Shugar and J. J. Fox, *Biochem. et Biophys. Acta*, 9, 199 (1952).

in water, a solvent of higher dielectric constant[6). This was supported by measuring the ultraviolet spectra in dioxane-water mixtures. Isosbestic points, suggestive of an equilibrium mixture, were absent, and in all cases the only trend observed was a slight red shift in going from 100 percent dioxane to water (pH 6.86, Table II, intermediate dioxane concentrations are omitted); it is well-known that in neutral uncharged molecules the $\pi-\pi^*$ bands are shifted towards the red as the dielectric constant of the solvent is increased.

The ultraviolet spectra are recorded in Figs. 1—3. In going from the neutral to the anionoid

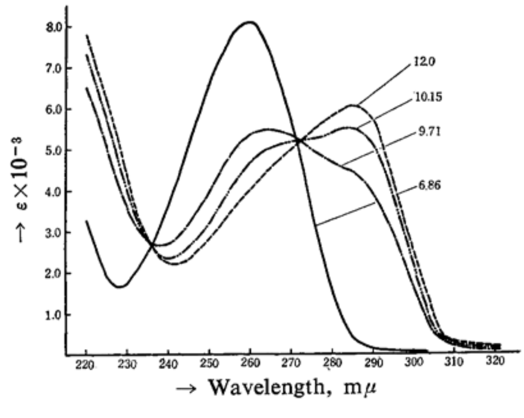


Fig. 1. Ultraviolet spectra of uracil at various pH (numbers beside curves).

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6) Infrared measurements have shown that uridine and uridylic acid exist in the diketo form in D_2O: H. T. Miles, *Biochim. et Biophys. Acta*, **22**, 247 (1956).

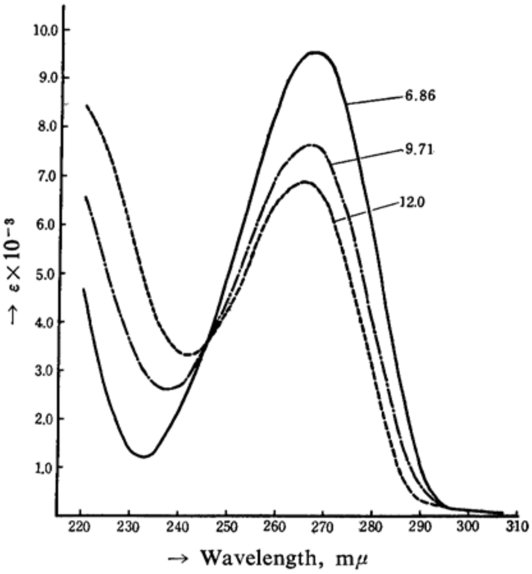


Fig. 2. Ultraviolet spectra of 1-methyluracil at various pH (numbers beside curves).

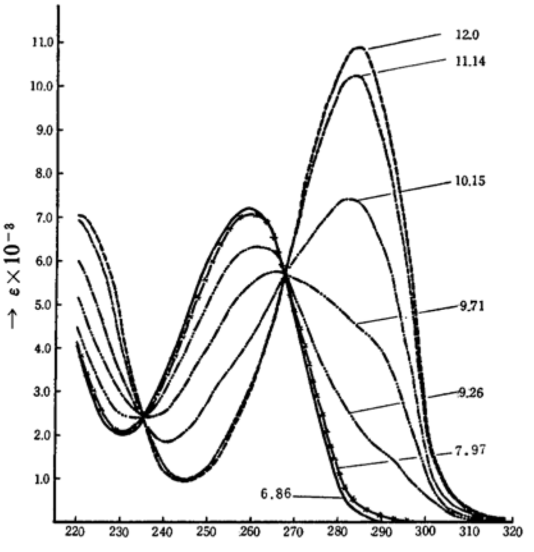
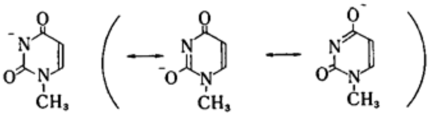
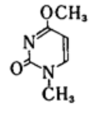


Fig. 3. Ultraviolet spectra of 3-methyluracil at various pH (numbers beside curves).

species in the case of 1-methyluracil, the peak intensity is lowered, but the wavelength is almost unaffected (Fig. 2 and Table II). This rules out the contribution of canonical structures such as Va or Vb; because of a more extended conjugated system, Va and Vb should show absorption at much longer wavelengths than the original species. For example, 1-methyl-4-methoxy-2-pyrimidone (IX) (cf., Experimental), which contains the O=C–N=C–OCH$_3$ system, already absorbs at 275 mμ, and accordingly Va and Vb containing the O=C–N=



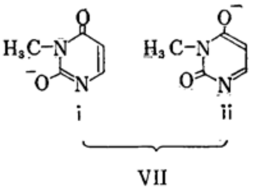
V Va Vb



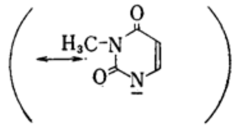
IX

275 mμ
(ε 5,890)
at pH 6.86

C–O$^-$ system would be expected to show absorption at a still longer wavelength. Localization of the negative charge on the nitrogen atom has recently been suggested for the anion of 2-hydroxypyridine[8]. The cause for this localization is probably due to the fact that the intrinsic stability of the two carbonyl bonds outweigh the stabilization gained by an extended conjugation. Similar trends, i. e., lowering of peak intensity and constancy or slight blue shift of band position, are encountered upon anion formation in other 1-substituted uracils (Table II, nos. 4—6) and various uridine phosphates[9] such as uridine-5′-diphosphate (262 mμ, ε 10,000 vs. 261 mμ, 7,900), and uridine-5′-triphosphate (262 mμ, ε 10,000 vs. 261 mμ, 8,100). The anion of 3-methyluracil, on the other hand, shows a considerable red shift and increases in peak intensity. This behavior can be accounted for by canonical structures VII-i or VII-ii[10] but not by VIIa; in VII a conjugated system more extended than Va or Vb is present, and this probably compensates for the carbonyl bond energy.



VII



VIIa

The anion of uracil (Fig. 1) has its peak at 286 mμ (ε 6,010) with a broad hump around 260 mμ (ε ca. 4,000). The peak undoubtedly corresponds to the 284 mμ (ε 10,890) peak of VII; if wing correction (Fig. 4) is carried out by subtracting the curve of 3-methyluracil anion multiplied by 6,010/10,890, a peak is revealed at 258 mμ (ε 2,490). This peak is due to the anion II, the equivalent of V (266 mμ). Thus, the following empirical rule is apparent

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[7] H. McConnell, *J. Chem. Phys.*, **20**, 700 (1952).

[8] E. Spinner, *J. Chem. Soc.*, **1960**, 1232.

[9] R. M. C. Dawson, D. C. Elliott, W. H. Elliott and K. M. Jones, ed., "Data for Biochemical Research," Oxford Univ. Press, London (1959), p. 78.

[10] At present, no conclusion can be drawn as regards the relative weights of contributions of structures VII-i and VII-ii.

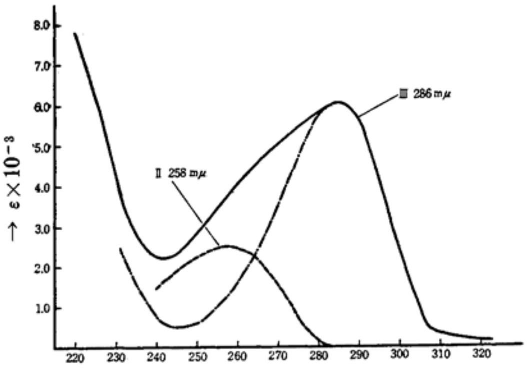

Fig. 4. Ultraviolet absorptions of uracil anions at pH 12.0.

in the ultraviolet spectra of uracils (Plate 1, see also Table II): (1) Alkylation at N-1 results in a red shift of ca. 8 mμ (I vs. IV, II vs. V). (2) Deprotonation at N-1 similarly results in a red shift of ca. 26 mμ (I vs. III, VI vs. VII). (3) Alkylation at N-3 results in a slight blue shift of 1∼2 mμ (I vs. VI, III vs. VII). (4) Deprotonation at N-3 similarly results in a slight blue shift of ca. 2 mμ (IV vs. V, I vs. II). It is interesting to note that this simple calculation is also applicable to the disubstituted 1,3-dimethyluracil (VIII): calculated $\lambda_{\max}$ 266∼267 mμ; observed 267 mμ. The ultraviolet absorption curves of thymine (5-methyluracil) and thymidine[4] show that this general trend is apparent in this series also.

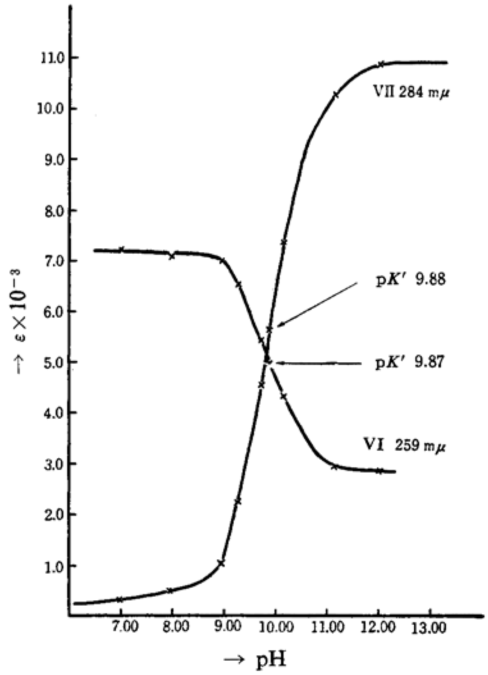

Fig. 5. 3-Methyluracil; variation of peak intensities with pH.

The pK of uracil (pK_U), 1-methyluracil (pK_C), and 3-methyluracil (pK_D) were obtained by spectroscopic measurement of the pK' from the curves at pH 6.86, 9.71 and 12.0 (Figs. 1—3), and correction to thermodynamic pK values (Table II). The pK' values were also obtained by graphical plotting of extinction coefficients versus pH as shown in Fig. 5 for 3-methyluracil; the results are identical with the calculated values and, therefore, are omitted for other samples. The extinction coefficients for the anions II and III, which cannot be obtained experimentally, were calculated as follows:

$$\varepsilon_{\mathrm{III}}/\varepsilon_{\mathrm{I}}=\varepsilon_{\mathrm{VII}}/\varepsilon_{\mathrm{VI}}$$

hence, $\varepsilon_{\mathrm{III}}=8100\times10890/7210=12200$

and the percentage of III present in the uracil anion is

$$\mathrm{III\,(percentage)}=6010/12200\times100=49$$

Accordingly, the ratio of II to III, K_T, is $51/49=1.0$.

Similarly,

$$\varepsilon_{\mathrm{II}}/\varepsilon_{\mathrm{I}}=\varepsilon_{\mathrm{V}}/\varepsilon_{\mathrm{IV}}$$

hence, $\varepsilon_{\mathrm{II}}=8100\times6880/9570=5820$

The percentage of II may be estimated from the intensity of the 258 mμ peak (Fig. 4):

$$\mathrm{II\,(percentage)}=(2490/5820)\times100=43$$

hence, $K_T=\mathrm{II}/\mathrm{III}=43/57=0.8$

The agreement with the ratio 1.0 calculated from the 286 mμ peak is not quite satisfactory, but the 1.0 value is probably more correct because no wing correction is necessary.

If K_A and K_B are the individual dissociation constants leading from I to II and III, respectively (Plate 1), it can be readily shown[11] that

$$K_u=K_A+K_B \quad \text{and} \quad K_T=\mathrm{II}/\mathrm{III}=K_A/K_B$$

Substitution of $K_T=1.0$ gives pK_A=pK_B=9.81.

Occasionally[11,12], K_T is calculated by assuming that K_C or K_D is approximately equal to K_A or K_B, respectively, since substitution of a methyl group for a hydrogen atom would have a minor effect. However, the above results show that the approximation is not so satisfactory. Since the ultraviolet spectra of the other compounds listed in Table II and of the uridine phosphates[9] closely resemble those of the uracils treated in the present paper, the tautomeric behavior can be handled in an identical manner.

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[11] G. F. Tucker and J. L. Irvin, *J. Am. Chem. Soc.*, **73**, 1923 (1951).

[12] E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York (1943), p. 96.

Experimental

Materials.—1-Methyluracil[13] was prepared from uracil through 2,4-dichloropyrimidine, 2,4-dimethoxypyrimidine and 1-methyl-4-methoxy-2-pyrimidone (hydrolysis). The structure of the product as 1-methyluracil has been confirmed by Brown et al.[14].

3-Methyluracil[15] was prepared from ethyl ethoxy methylenemalonate[16] through 3-methyl-5-carbethoxyuracil and 3-methyl-5-carboxyuracil(decarboxylation). Product confirmed by Brown et al.[14]

1,3-Dimethyluracil[17] was prepared by methylation of uracil with dimethyl sulfate.

Spectral Measurements. — The infrared spectra were recorded with a Koken model 301 spectrophotometer. The ultraviolet spectra were recorded with a Hitachi model EP-1 spectrophotometer; a pair of 1 cm. quartz cells with stoppers was used and the cell temperature was maintained at 25±1°C. The error in absorbancy is ±0.002.

Measurement of p*K*.—All pH measurements were carried out at 25±1C° with a Horiba model P pH meter equipped with extension glass and calomel electrodes; the meter was calibrated with a standard Horiba buffer solution of pH 6.86 at 25°C($KH_2PO_4+Na_2HPO_4$). The following buffers were used for the pH indicated: pH 7.97, $KH_2PO_4+Na_2HPO_4$; pH 8.95, 9.26, 9.71 and 10.15, $Na_2CO_3+NaHCO_3$; pH 11.14 and 12.0 (uncorrected), Na_2HPO_4+NaOH. The ionic strength of the solutions was 0.1. The apparent p*K*′ was calculated from:

$$pK' = pH - \log(\varepsilon_{HX} - \varepsilon_{obs})/(\varepsilon_{obs} - \varepsilon_X)$$

where ε_{HX} and ε_X are the molecular extinction coefficients (ε) measured in pH 6.86 and pH 12.0 buffer solutions, respectively, and ε_{obs} is the ε measured in an intermediate pH solution where the pH was chosen so that the value of $(\varepsilon_{HX}-\varepsilon_{obs})/(\varepsilon_{obs}-\varepsilon_X)$ was near unity, i. e., pH 9.71. The wavelength chosen was 260 mμ and 285 mμ for uracil, 265 mμ for 1-methyluracil, and 259 mμ and 284 mμ for 3-methyluracil.

The p*K*′ was also obtained graphically as shown in Fig. 5. These apparent constants were corrected to the thermodynamic scale with the aid of the Debye-Hückel relationship,

$$pK = pK' + \log f_{HX} - \log f_X \tag{1}$$

$$\log f_X = -0.509(\sqrt{I}/1+\sqrt{I}) \quad \text{(at 25°C)}$$

where *f* and *I* represent the activity coefficient and the ionic strength. Since for neutral molecules f_{HX} is approximately unity, the correction becomes

$$pK = pK' - \log f_X = pK' + 0.12$$

Summary

In aqueous solutions uracil exists predominantly in the diketo form, whereas in alkaline solutions it exists as an approximately 1 : 1 mixture of two deprotonated forms. The two deprotonated anions show ultraviolet absorptions similar to the anions of 1-methyluracil and 3-methyluracil, and the main contributing canonical structures which give rise to the general absorption feature of the anions are discussed. The peak shifts accompanying N-alkylation or deprotonation may be summarized in a simple empirical rule. Individual ionization constants have also been estimated.

The results of uracil can be extended to thymine, uridine, thymidine, and their phosphates. The pesent method can probably be extended to other systems containing two dissociable protons.

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[13] G. E. Hilbert and T. B. Johnson, *J. Am. Chem. Soc.*, 52, 2001 (1930).

[14] D. J. Brown, E. Hoerger, and S. F. Mason, *J. Chem. Soc.*, 1955, 211.

[15] C. E. Whitehead, *J. Am. Chem. Soc.*, 74, 4267 (1952).

[16] "Organic Syntheses", Coll. Vol. 3, John Wiley and Sons, Inc., New York (1955), p. 395.

[17] D. Davidson and O. Baudisch, *J. Am. Chem. Soc.*, 48, 2382 (1926).