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ESR Studies of Electron and Hydrogen Adducts of Thymine and Uracil and Their Derivatives and of 4,6-Dihydroxypyrimidines in Aqueous Solution. Comparison with Data from Solid State. The Protonation at Carbon of the Electron Adducts

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Abstract: With the use of the in situ radiolysis ESR method the coupling constants were determined of the radicals produced in aqueous solution by reaction of the hydrated electron with uracil and thymine and with their nucleosides and nucleotides. From the coupling constants of these electron adducts it is evident that the unpaired spin density at C(6) of the pyrimidine ring is much larger than that at C(5). Substitution with methyl, carboxyl, or ribosyl groups at C(5), C(6), and N(1), respectively, has little effect on the distribution of the unpaired spin. The splittings of the radicals measured in aqueous solution are very similar to those previously reported for the same radicals in the solid state, which shows that the latter data are of predictive value also for the aqueous phase. At pH 7 in the presence of phosphate the electron adducts are converted into 6-dihydropyrimidin-5-yl radicals by protonation on C(6). The protonation reaction has previously been observed to occur in the solid state. In comparison, the hydrogen atom reacts with uracil to give the 5-dihydro-6-yl radical. The uracil isomer, 4,6-dihydroxypyrimidine, and its 2- and 5-methyl derivative react with the hydrated electron to give delocalized radical anions, which on protonation by H^+ are converted into the 5-dihydro-2-yl radicals. This conversion, which can also be catalyzed by phosphate, is more efficient than in the case of uracil. The hydrogen atom reacts with the 4,6-dihydroxypyrimidine system by addition at C(5) to give the same radical as that from the reaction with the hydrated electron followed by protonation. With the electron adduct of 4,6-dihydroxypyrimidine, an OH^- catalyzed protonation by water on C(5) is observed.

During the last three decades the radiation chemistry of the nucleic acid bases has been the focus of a large amount of interest and attention.[3] This, obviously, reflects the relevance of the radical chemistry of the nucleic acid constituents to the radiation-induced in vivo inactivation of DNA.

Over this period, much of the progress has developed in parallel in two distinct areas that have remained essentially independent of each other: one is the realm of (aqueous) solution chemistry where product analysis and pulse radiolysis[3a-c] have predominantly been applied, and the other field is that of solid-state (including frozen solutions) electron spin resonance (ESR).[3d,e] Liquid-solution ESR has so far not been able to contribute very much to the understanding of the free radical chemistry of the nucleic acid bases, essentially because of insufficient sensitivity. As a consequence, in most cases the relevance for the in vivo situation of the radical species identified in the solid state by highly sophisticated ESR techniques could not be evaluated in a satisfactory way and is, therefore, still under debate.[3e] In a way, liquid-phase ESR of the free bases, nucleosides, and nucleotides constitutes the most simple of several "missing links" between the solid-state radical chemistry and radiation-induced cell death. This refers not only to problems of the structure of the radicals but also to their reactivity, which has, in the solid state, been characterized in detail in many instances.

A further reason for the need for liquid-phase ESR data is to provide coupling constants more precise than those obtainable from solid-state studies. This more detailed information is necessary in order to assess the quality of theoretical (MO) calculations on spin and charge distribution in nucleic acid radicals and on their geometries.

Of the numerous nucleobase radicals detected in the solid state, those formed by e^- addition (the "π anions") have been most thoroughly studied and most unambiguously identified. It is also very well-established that the electron adducts of the pyrimidines react further, typically by addition of a proton at C-6 to give pyrimidin-5-yl radicals. Very recently, evidence from pulse radiolysis experiments has been presented[4] that such a reaction

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(3) For reviews see: (a) Scholes, G. In "Photochemistry and Photobiology of Nucleic Acids"; Wang, S. Y., Ed.; Academic Press: New York, 1976; p 521. (b) Hüttermann, J.; Köhnlein, W.; Téoule, R.; Bertinchamps, A. J., Eds. "Effects of Ionizing Radiation on DNA"; Springer-Verlag: Berlin, 1978. (c) von Sonntag, C.; Schuchmann, H.-P. *Int. J. Radiat. Biol.*, in press. (d) Box, H. C. "Radiation Effects. ESR and ENDOR Analysis"; Academic Press: New York, 1977. (e) Bernhard, W. A. *Adv. Radiat. Biol.* **1981**, *9*, 199.

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Scheme I

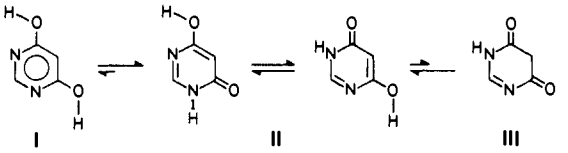


Table I. Acid–Base Properties of 4,6-Dihydroxypyrimidines[a]

compound	pK_a(1)	pK_a(2)	pK_a(3)
4,6-DHP	−0.26[b]	5.4[b,c]	12.0[d]
		5.3[d]	
		5.35[e]	
2-Me-4,6-DHP	0.21[b,e,f]	6.35[b,e,f]	12.9[d]
	0.1[d]	6.4[d]	
5-Me-4,6-DHP	−0.51[e,f]	6.01[e,f]	

[a] pK(1) relates to the cation (4,6-DHPH$^+$); pK(2) and pH(3) are for production of mono- and dianion, respectively. [b] Reference 29. [c] Reference 8. [d] This work, determined by spectrophotometric titration monitoring the OD change at 250–270 nm. [e] Reference 30. [f] Reference 31.

occurs also in aqueous solution. This shows that solid-state data may be of predictive value for the solution chemistry of pyrimidine radicals not only with respect to structure but also with respect to reactivity. Further experiments in this direction, therefore, appeared to be appropriate.

Experimental Section

The compounds were all commercially available from Aldrich, Fluka, Merck, or Sigma, except 5-methyl-4,6-dihydroxypyrimidine, which was synthesized as described.[5] The deoxygenated aqueous solutions typically contained 10–100 mM formate (to scavenge OH and H) or 0.1–0.5 M *tert*-butyl alcohol (to scavenge the OH radicals) and 0.5–2 mM of the pyrimidine to react with e^-_{aq} or H atoms. The solutions were irradiated with a beam (diameter 1 mm) of 3 MeV electrons with the method described by Eiben and Fessenden.[6]

Pulse radiolysis experiments were performed as previously described.[7]

Results and Discussion

1. The 4,6-Dihydroxypyrimidine System. 4,6-Dihydroxypyrmidine (4,6-DHP) exists in aqueous solution predominantly in the monoketo/monoenol form.[8] It is a stronger acid than its isomer, uracil. The pK_a values of 4,6-DHP are 5.3 and 12.0. Substitution of H atoms by methyl groups decreases the acidity of the DHP system (Scheme I). The relevant data are collected in Table I.

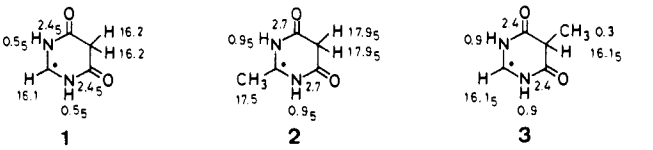
1. a. Reactions with the Hydrogen Atom. 4,6-DHP was reacted with H atoms by irradiating deaerated aqueous solutions containing 1 mM 4,6-DHP and 50 mM *tert*-butyl alcohol at pH 1–3. Under these conditions the OH radicals produced by the radiation are scavenged by *tert*-butyl alcohol according to eq a, and the hydrated electrons are converted to hydrogen atoms by reaction with protons (eq b).

$$(CH_3)_3COH + OH \rightarrow (CH_3)_2C(OH)\dot{C}H_2 + H_2O \quad (a)$$

$$e^-_{aq} + H^+ \rightarrow H\cdot \quad (b)$$

The experimentally observed ESR spectrum consisted of a doublet (a(H)$_{C(2)}$ = 16.1 G) of 1:2:1 triplets (a(H)$_{C(5)}$ = 16.2 G) of 1:2:1 triplets (a(H)$_{N(1),N(3)}$ = 0.55 G) of 1:1:1 triplets (a(N)$_{N(1),N(3)}$ = 2.45 G) with g = 2.0032. It is assigned to radical **1**, formed by addition of H to C(5) of 4,6-DHP (Table II). The assignment of the large triplet with the splitting of 16.2 G to the methylene protons at C(5) rather than at C(2) is based on the effect on the splitting pattern exerted by substitution of H by methyl at C(2) and C(5), i.e., on a comparison with the coupling constants observed for the corresponding radicals **2** and **3** (see Figure 1) as obtained from the reaction of H with 2-methyl- and 5-methyl-4,6-dihydroxypyrimidine (Table II). This assignment is supported by a comparison with the similar coupling constants[9] or OH adducts to 4,6-DHP, 2-Me-4,6-DHP, and 5-Me-4,6-DHP. These radicals differ from **1**, **2**, and **3** by replacement at C(5) of one H by OH.

Table II. Structures and Coupling Constants (in Gauss) of H Adduct Radicals Derived from 4,6-Dihydroxypyrimidines



1 g = 2.0032 **2** a **3** g = 2.0039

[a] Not accurately determinable due to extreme polarization of the spectrum.

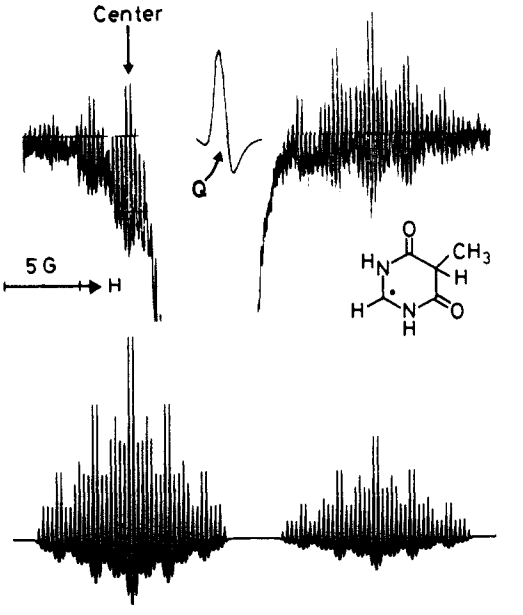


Figure 1. ESR spectrum obtained on reaction of H with 2 mM 5-Me-4,6-DHP in the presence of 0.1 M *tert*-butyl alcohol at pH 2 and ≃5 °C. Q denotes the signal from the quartz cell. The computer-simulated spectrum is shown for comparison.

The coupling constants for H–C$_\alpha$ and H–C$_\beta$ and those for N(1) and N(3) as well as for H–N(1) and H–N(3) (Table II) are similar to those[10] for aliphatic peptide radicals. On this basis, the unpaired spin in the radicals **1**, **2**, and **3** can be considered to be localized to C(2). However, if a purely aliphatic structure is assumed, the splittings of the protons at C(5) appear to be too large. This discrepancy may be resolved on the basis of a contribution via a pseudo π orbital of the methylene hydrogen orbitals to the system C(2)(NHCO)$_2$ (we thank referee I for making this suggestion).

The symmetric and "aliphatic" type structure of the H adducts **1**, **2**, and **3** of the 4,6-DHP's may be compared with the unsymmetric and conjugated structure II of the parent compounds. With II, the conjugated system extending from C(2) to C(5) is destroyed by addition of a radical at C(5); it is interesting that under these conditions the diamide type structure **1–3** with the unpaired spin being localized is thermodynamically more stable than the delocalized monoketo/monoenol type radical that is initially produced

(4) (a) Das, S.; Deeble, D. J.; Schuchmann, M. N.; von Sonntag, C. *Int. J. Radiat. Biol.* **1984**, *46*, 7. (b) Deeble, D. J.; Das, S.; von Sonntag, C. *J. Phys. Chem.*, in press.

(5) Hull, R.; Lovell, B. J.; Openshaw, H. T.; Todd, A. R. *J. Chem. Soc.* **1947**, 41.

(6) Eiben, K.; Fessenden, R. W. *J. Phys. Chem.* **1971**, *75*, 1186.

(7) Jagannadham, V.; Steenken, S. *J. Am. Chem. Soc.* **1984**, *106*, 6542.

(8) Hurst, D. T. "Chemistry and Biochemistry of Pyrimidines, Purines and Pteridines"; J. Wiley and Sons: Chichester, 1980; p 18.

(9) Novais, H. M.; Steenken, S., manuscript in preparation.

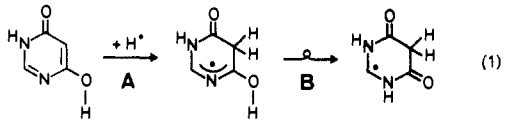
(10) Livingston, R.; Doherty, D. G.; Zeldes, H. *J. Am. Chem. Soc.* **1975**, *97*, 3198. Doherty, D. G.; Livingston, R.; Zeldes, H. *J. Am. Chem. Soc.* **1976**, *98*, 7717.

Table III. Coupling Constants (Gauss[a]) of Electron Adducts in Aqueous Solution at ≈5 °C and pH 7–11

compound	H(2)	H(5)	H(6)	N(1)[b]	N(3)	N–H
uracil[c]		0.90	12.65	1.50	0.90	
2-thiouracil		1.65	12.85	2.60	1.30	0.35
1-methyluracil		1.50	12.30	2.25	0.75	
uridine		1.98	12.30	1.00	0.23	0.23
uridine-5′-phosphate		1.90	12.40	1.05	0.25	0.25
2′-deoxyuridine-5′-phosphate		2.00	12.40	1.15	0.24	0.24
3-methyluracil		1.80	14.95	2.50	0.60	0.30 (CH_3)
thymine		0.90 (CH_3)	11.80	1.35	0.90	0.20 (2)
isoorotic acid[d]						
monoanion			13.98	1.72	0.63	0.44; 0.79
dianion			12.79	1.35	0.51	0.51; 1.02
6-methyluracil		1.25	15.60 (CH_3)	1.88	0.63	
orotic acid[d]		1.97		1.41	0.39	1.43; 1.26
4,6-DHP	3.80	2.88		1.90	1.90	0.70
2-Me-4,6-DHP	14.40 (CH_3)	3.00		2.50	2.00	0.60
5-Me-4,6-DHP	6.50	2.40 (CH_3)		2.00	1.20	0.37

[a] The accuracy of the measured coupling constants is estimated to be ± 40 mG. [b] The assignment of the coupling constants to the nitrogen atoms based on INDO calculations (ref 16, 20, 21). [c] The *g*-factor of the radical is 2.0030. [d] Values of coupling constants for orotic and isoorotic acids were taken from ref 11 and are presented for comparison.

on radical addition at C(5). On the basis of a typical lifetime of ≈1 ms for radicals under steady-state conditions, the rate constants for the keto–enol rearrangement eq 1B must be $\geq 10^3$ s^{-1}.



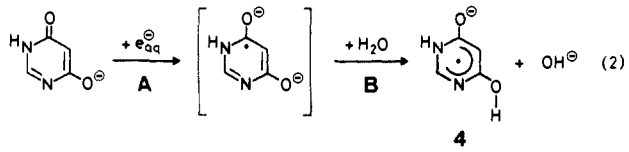
(1)

As mentioned above, the diketo or diamide type structure is also observed with the OH adducts of the 4,6-DHP's. In this case it was possible to measure the pK_a value of the OH adduct of 4,6-DHP itself. The pK_a value of the radical is almost 3 units higher than that of the parent compound.[9] This situation is in agreement with a keto but not with an enol structure of the radical.

1.b. Reactions with the Hydrated Electron. These were carried out by irradiating deaerated aqueous solutions containing 10–100 mM formate and, e.g., 1 mM 4,6-DHP. Under these conditions the OH and H radicals are quantitatively scavenged by the formate to yield the reducing radical $CO_2^{-}\cdot$ whereas e^-_{aq} reacts with 4,6-DHP. (At pH 8.6 the rate constant for this process was determined to be 10^{10} M^{-1} s^{-1} by monitoring the decay kinetics of e^-_{aq} at 650 nm.)

Between pH 6 and 11 an ESR spectrum was observed which consisted of a doublet ($a(H)_{C(2)}$ = 3.80 G) of doublets ($a(H)_{C(5)}$ = 2.88 G) of doublets ($a(H)_{N-H}$ = 0.70 G) of 1:2:3:2:1 quintets $a(N)_{N(1),N(3)}$ = 1.90 G). The spectrum is assigned to the ketyl-type radical **4** formed by electron addition to the monoanion of 4,6-DHP (pK_a(4,6-DHP) = 5.3, Table I) followed by protonation of the electron adduct by water, cf. eq 2. From the small coupling constants found for **4** it is evident that with this radical the unpaired spin is strongly delocalized. The same conclusion may be drawn from the equality of the splittings of the (chemically nonequivalent) N(1) and N(3).

When e^-_{aq} was converted to $CO_2^{-}\cdot$ (via OH) by saturating the solution with N_2O, radical **4** was not formed which shows that $CO_2^{-}\cdot$ does not reduce the monoanion of 4,6-DHP, in contrast to the neutral molecule (see section 1.b.1).

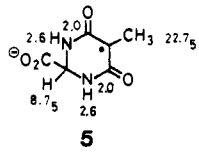


(2)

4

The occurrence of the protonation reaction was also shown by conductance experiments. Reaction 2B yields 1 equiv of OH^- per 4,6-DHP radical dianion. The OH^- neutralizes the H^+ produced in the radiolysis of H_2O. The net conductance change after completion of the neutralization reaction is thus expected to be zero at pH values far below the pK_a of **4** and negative above its pK_a. This was investigated in the pH range 8–12, and it was found that at pH 8.5–9.5 the yield of OH^- from reaction 2B was quantitative, but decreased with increasing pH to about 20–30% at pH 11.6. From this it may be estimated that the pK_a value of **4** is 10–11. Due to the buffering action of 4,6-DHP in the vicinity of its second pK_a (12.0, Table I), it is not easily possible to determine the pK_a of **4** with a higher degree of precision.

2-Me- and 5-Me-4,6-DHP were also reacted with e^-_{aq}, and in both cases ketyl-type electron adducts were observed in the pH range 7–10. Their ESR parameters are shown in Table III. In the case of 5-Me-4,6-DHP an additional radical was seen between pH 3 and 7 when and only when formate was present. The intensity of the lines due to this radical increased on doubling the concentration of $CO_2^{-}\cdot$ by converting e^-_{aq} into $CO_2^{-}\cdot$ with N_2O via the reaction sequence $e^-_{aq} + N_2O + H_2O \rightarrow OH + OH^- + N_2$; $OH + HCO_2^- \rightarrow H_2O + CO_2^{-}\cdot$. The experimentally observed spectrum is characterized by lines from three equivalent protons ($a(H)_{C(5)-CH_3}$ = 22.75 G), a doublet ($a(H)_{C(2)}$ = 8.75 G), a 1:2:1 triplet ($a(H)_N$ = 2.6 G), and a 1:2:3:2:1 quintet ($a(N)_{N(1),N(3)}$ = 2.0 G). It is assigned to **5**, the radical formed by addition of $CO_2^{-}\cdot$



5

g = 2.0033

to C(2) of 5-Me-4,6-DHP. The coupling constants for the methyl protons are similar to β-protons of aliphatic radicals; on this basis, **5** may be considered a localized radical. There is precedence for $CO_2^{-}\cdot$ addition to pyrimidines: with orotic acid addition has been shown[11] to take place at C(6) with the in situ radiolysis ESR method.[12]

1.b.1. Reactions of the Electron Adducts with H^+ and with Phosphate. When the pH of deaerated solutions containing 10–100 mM formate and 1 mM 4,6-DHP or 2-Me-4,6-DHP was decreased below pH 7, the intensity of the lines due to the electron adducts decreased, and lines resulting from the H adducts **1** or **2** showed up. At pH 5 to 4 the conversion of the electron adducts into the H adducts (see Figure 2) was quantitative, and the stationary concentration of **1** or **2** was higher than under conditions of production by reaction with H atoms (see section 1.a). This indicates that the radical $CO_2^{-}\cdot$ contributes to the production of **1** and **2**, obviously via electron transfer to the 4,6-DHP's followed by protonation of the electron adducts. In fact, when e^-_{aq} was scavenged by N_2O and thus—in solutions containing formate—

(11) Neta, P. *Radiat. Res.* **1972**, *49*, 1.
(12) $CO_2^{-}\cdot$ addition at C(2) is also observed with 4,5,6-trihydroxypyrimidine (unpublished results).

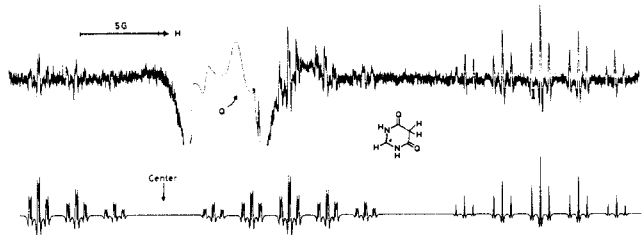
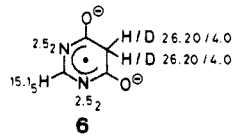


Figure 2. ESR spectrum recorded on reaction of e^-_{aq} and $CO_2^{-}\cdot$ with 1 mM 4,6-DHP in the presence of 0.1 M formate at pH 4.2 and ≈5 °C.

converted to $CO_2^{-}\cdot$, the concentration of **1** and **2** did not decrease, which shows that $CO_2^{-}\cdot$ and e^-_{aq} are equally efficient in reducing 4,6-DHP and 2-Me-4,6-DHP. The conversion of the electron adducts into the H adducts is also seen with *tert*-butyl alcohol as the OH scavenger. This reaction thus does not require formate as a potential catalyst. In the case of 5-Me-4,6-DHP the conversion of the electron adduct to give the C(5)–H adduct **3** is complete already at pH 6. However, this can only be observed in the absence of formate, e.g., with *tert*-butyl alcohol as an OH scavenger, since in the presence of HCO_2^- the spectrum of **3** is masked by that of the $CO_2^{-}\cdot$ adduct **5**.

The transformation of the electron adducts of the 4,6-DHP's into the C(5)–H adducts is catalyzed by phosphate. With, e.g., 4,6-DHP the conversion is quantitative at pH 7 if the solution contains ≥0.01 M phosphate.

1.b.2. OH^- Catalyzed Protonation on C(5). With 4,6-DHP the intensity of the lines of the electron adduct remained approximately constant between pH 7 and 10 but started to decrease above pH 10. At pH ≈11 the lines were very weak but still visible, but at pH ≈12 the electron adduct was not detectible. However, when the OH^- concentration was further increased to 0.4 M, lines from a new radical were seen whose spectrum reflected the presence of two equivalent protons ($a(H)_{C(5)}$ = 26.20 G), a single proton ($a(H)_{C(2)}$ = 15.15 G), and two equivalent nitrogens ($a(N)_{N(1),N(3)}$ = 2.52 G). A corresponding experiment in D_2O instead of H_2O resulted in the replacement of the 26.20 G triplet by a 4.00 G quintet ($a(H)/a(D)$ = 5.55), while the other splittings remained the same. This shows that there are two deuterium atoms at the same site. The proton at C(5) should be exchangeable by deuterium, via the diketo form III in equilibrium with the enol forms. The selective exchange of C(5)–H by D could in fact be demonstrated by NMR experiments: in O-deuterated formic or acetic acid the exchange is complete in ≤10 s. On this basis it must be C(5) and not C(2) which, with the radical, is doubly substituted by D. The observed radical can thus be assigned the structure 6. The mechanism of production of **6** is shown in Scheme II, which also summarizes the results presented in the preceding section.

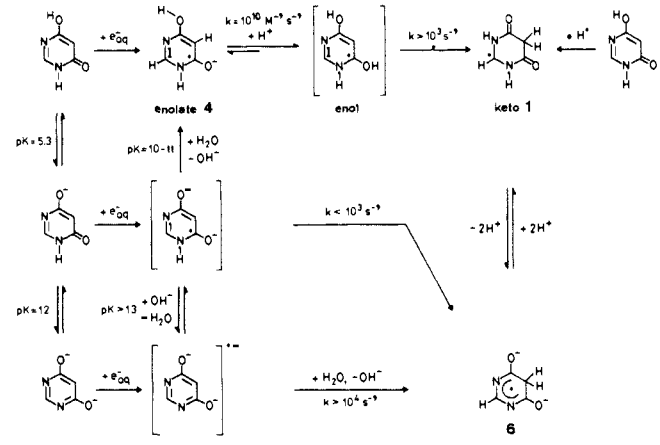


6

g=2.0024

1.b.3. Overall Reaction Scheme. At pH ≥12, reaction of e^-_{aq} with the dianion of 4,6-DHP yields the radical trianion which is rapidly protonated on nitrogen by water to give the radical dianion. This radical was not seen by ESR, possibly due to line broadening resulting from proton exchange at N–H. In competition with the protonation on nitrogen of the trianion is its protonation on C(5). However, this reaction is much slower than the protonation on nitrogen, as can be concluded from the absence of 6 below pH ≈13. A further consequence of this absence is that the rate constant for conversion of the dianion radical into 6 must be $\leq 10^3$ s^{-1}. Since 6 is only seen at $[OH^-] \geq 0.2$ M the pK_a of the dianion radical must be ≥13.5. From the fact that it is not the trianion radical but its protonation product 6 which is seen it can be concluded that the rate of protonation on carbon is $\geq 10^3$–10^4 s^{-1}.

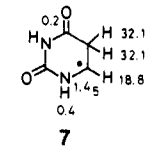
Scheme II. Reactions of 4,6-DHP with e^-_{aq} and H·



Concerning the reactions at pH ≤7, the protonation on C(5) is suggested to proceed via a neutral enol-type radical which undergoes rapid ketonization. This follows from the observation that the "H adduct" **1** from the reaction of 4,6-DHP with e^-_{aq} is fully developed already at pH ≈5. Since the rate constants for protonation on oxygen are typically 10^{10} M^{-1} s^{-1}, whereas those for protonation on carbon are lower by three to five orders of magnitude,[13] the production of **1** via direct reaction of H^+ at C(5) would be too slow at pH 5 to account for the quantitative conversion of the electron adduct.

From the facts that lines assignable to the uncharged enol radical were not seen and that the pH profile for the disappearance of the electron adduct was the same as that for the production of **1** it may be concluded that the rate constant for ketonization of the enol radical is $\geq 10^3$ s^{-1}.

2. The Uracil System. a. Reactions with the Hydrogen Atom. The (strongly-polarized) spectrum observed on irradiation at pH 1–3 of deaerated solutions containing 0.1–0.2 M *tert*-butyl alcohol and 2 mM uracil is interpreted in terms of 5-hydrouracil-6-yl, radical **7**, formed by addition of H to C(5). The distinctive features of the ESR parameters are the coupling constants for the β-protons ($a(H)_{C(5)}$ = 32.1 G(2)), the splitting for the α-proton ($a(H)_{C(6)}$ = 18.8 G), and that for the α-nitrogen ($a(N(1))$ = 1.45 G) besides a splitting of 0.2 G due to N(3) and one of 0.4 G due to a proton (probably that at N(1)). Similar values for the large parameters have previously been measured for the 6-yl radical as produced by photoreduction of uracil in alcoholoic solvents.[14]



7

g=2.0035

The values found for the α- and β-protons are also very similar to the isotropic splittings measured in the solid state for uracil (18.5 and 35.5 G),[15] and for 2′-deoxyuridine (18.4 and 33.1 G),[16] which were also intepreted in terms of the 6-yl radical, i.e., H addition to C(5). This observation by ESR confirms the results of a recent pulse radiolysis study[17] in which the yield of reducing radicals from the reaction of H with uracil at pH 1.6 was determined. From the value obtained it was concluded[17] that 70% of the H atoms add to C(5).

2.b. The Formation of Electron Adducts and Their Conversion into 6-Hydro-5-yl Radicals. Reactions with e^-_{aq} were carried out

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Table IV. Coupling Constants (Gauss) of 6-Dihydrouracil-5-yl Radicals Produced in Aqueous Solution by Protonation of the Electron Adducts. Comparison with Data from Solid State

compound	H(5)	H(6)	N(1)	N(3)	H-N(1)	H-N(3)	ref
		(a) Aqueous Solution[a]					
uracil[b]	19.6	43.4 (2)	0.55	0.55	1.1	2.45	this work
uridine-5′-phosphate	19.6	41.4 (2)		0.6		3.9	this work
2′-deoxyuridine-5′-phosphate	19.5	42.0 (2)		0.6		3.1	this work
thymidine-5′-phosphate	20.4 (CH_3)	36.6 (2)	0.95	0.95		2.8	this work
		(b) Single Crystal (Isotropic Values)					
uracil	18.0	48 (2)					24
2′-deoxyuridine	18.9	43.6 (2)		1		2	25
uridine-5′-phosphate $2Na^+$	18.8	45 (2)					26
thymidine	20.5 (CH_3)	40.5 (2)					32

[a] [Phosphate] = 0.1–0.15 M, pH 7, ≈5 °C. [b] The *g*-factor of the radical is 2.0035.

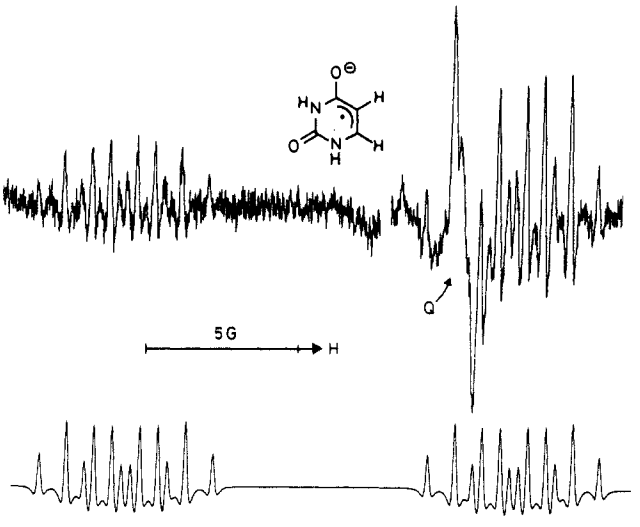
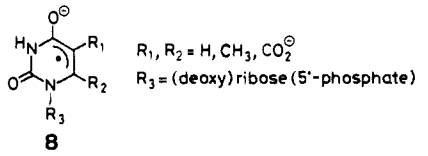


Figure 3. ESR spectrum measured on reaction of e^-_{aq} with 2 mM uracil in the presence of 20 mM formate at pH 11 and ≃5 °C.

in deaerated solutions containing 10–100 mM formate or 0.1–0.5 M *tert*-butyl alcohol. With uracil and a number of the its derivatives (including thymine) spectra were observed in the pH range 7–11 that are all characterized by considerably smaller coupling constants (Table III) than those for the H-adducts. With the spectrum from uracil (see Figure 3), in addition to two nitrogen splittings, there is a small doublet (0.90 G) and a larger doublet (12.65 G). With thymine, the larger doublet (11.80 G) is still there, but the small doublet is replaced by a quartet (0.9 G). With 6-methyluracil the situation is reversed: the small doublet is present (1.25 G) bu the large one is substituted for by a quartet (15.6 G). It is thus evident that with uracil and, by analogy, with the uracil nucleosides and nucleotides (Table III), the large splitting of ≈12.5 G is due to the hydrogen at C(6), whereas the splitting of ≈2 G results from C(5)–H. A radical structure in line with these splittings is the allyl-type ketyl radical **8**, in which



8

the unpaired spin is delocalized between C(4) and C(6). This radical has been called the "π or π^* anion". Its structure in the solid state is very well-established.[3b-e,16,18,19] On the basis of INDO calculations,[16,20,21] which have given results in satisfactory

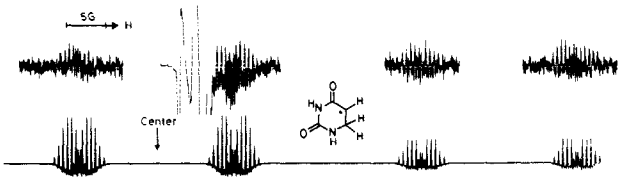


Figure 4. ESR spectrum recorded on reaction of e^-_{aq} with 2 mM uracil in the presence of 0.25 M *tert*-butyl alcohol and 0.15 M phosphate at pH 7 and ≃5 °C. There is some interference from the radical CH_2-$(CH_3)_2COH$.

agreement with the experimental coupling constants, the unpaired spin density is localized mainly on C(6) (55%), C(4) (15%), and O(4) (23%).[21] With uracil, thymine, and 6-methyluracil the electron adducts have also been identified by ESR in aqueous solution,[22] although the spectra were poorly resolved such that only one splitting was seen.

From Table III it is evident that substitution at C(5) or C(6) by methyl or CO_2^- groups or at N(1) by ribose or (deoxy)-ribosephosphate groups has little effect on the distribution of the unpaired spin. The same result was obtained from the ESR parameters measured in the solid state.[3b-e] The assignment of the larger nitrogen splittings to N(1) rather than to N(3) is based on the spin density data[16,20,21] from INDO calculations. The electron adducts of the carboxylated uracils (orotic and isoorotic acid) in aqueous solution have previously been described.[11] The optimum pH for the observation of **8** is ≈10–11. With uracil, at pH ≥12 the lines of **8** ($R_1 = R_2 = R_3 = H$) disappeared without lines from a new radical showing up. Even at 0.5 M OH^- there was no evidence for a base-catalyzed transformation of the electron adduct of the type observed in the case of 4,6-DHP. This was confirmed by performing pulse radiolysis experiments. No change in the optical absorption spectra of the electron adducts of uracil or of uridine was seen between pH 8.5 and 12.9.

In order to investigate whether there exists an H^+ induced conversion of the electron adducts to give H adducts, analogous to the case of electron adducts of 4,6-DHP's (see section 1.b.1), experiments were performed at pH <7, using uracil as a model compound. The intensity of the lines due to the electron adduct decreased with decreasing pH down to pH 4, but no lines assignable to 5- or 6-hydrouracil-5- or 6-yl were detected. From this it follows that the rate of ketonization of the protonated electron adduct ($pK_a = 7.3$)[23] is $\leq 10^3\ s^{-1}$. The same conclusion may be reached from the results of kinetic studies of the electron adduct.[4]

By using pulse radiolysis with optical detection, evidence has recently been presented[4] for formation of 6-hydrouracil-5-yl by phosphate catalyzed protonation on C(6) of the uracil electron adduct. When analogous experiments were performed with 2 mM uracil, 100 mM formate or 0.1–0.5 M *tert*-butyl alcohol, and ≥0.1

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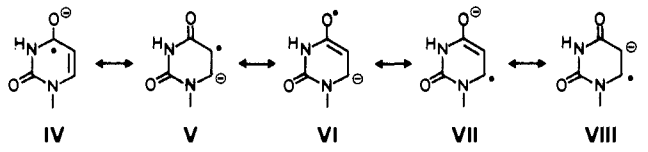
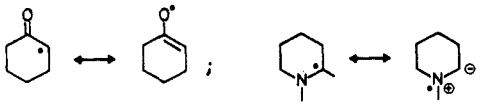
Scheme III



M phosphate, pH 6.5–7.5, the ketyl-type radicals were seen by ESR detection to disappear to yield 5-yl radicals (see Figure 4). The spectra of these radicals (for coupling constants see Table IV) are characterized by splittings of $\simeq$42 G from the β-protons at C(6) and of $\approx$19.5 G from the α-proton at C(5), quite different from those of the 6-yl radical as obtained from the reaction with the H atom.

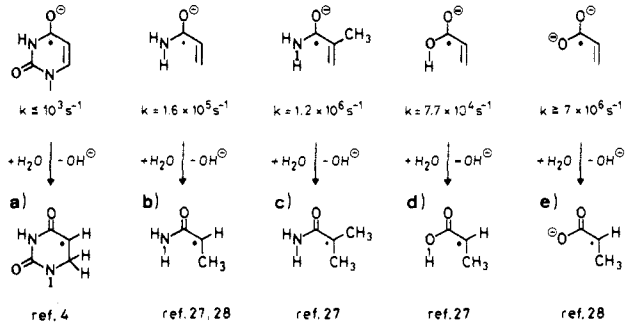
Coupling constants very similar to those found in aqueous solution were found for radicals produced in single crystals of uracil,[24] 2′-deoxyuridine,[25] and the disodium salt of uridine-5′-phosphate.[26] These values, which were interpreted in terms of the 5-yl radicals, are also presented in Table IV. From the similarity of the splittings measured in aqueous solution and in the solid state it is clear that the distribution of the unpaired spin in the radical does not depend to an appreciable degree on its neighborhood, which in solution is quite different from that in a crystal. While this may not be a totally unexpected result, it is certainly surprising that the reactivity of the electron adducts with respect to protons is essentially the same in the solid state and in aqueous solution. This raises the question as to the intrinsic reason for the preference for protonation on C(6) rather than on C(5). This reaction may be thermodynamically or kinetically controlled. In the former case, a prediction as to the favored site of protonation cannot easily be made since in both cases weakly delocalized radicals are formed. If kinetic control of the pro-



tonation reaction is assumed, it is necessary to consider the charge density distribution in the electron adducts. This is symbolized by the mesomeric structures IV–VIII (Scheme III). There are two ways to accommodate the negative charge on C(6) as compared to only one on C(5). In addition, this structure (VIII) should be quite unimportant relative to VII, where the negative charge is on the oxygen. The density of the negative charge is thus probably larger on C(6) than on C(5). Since the rate of protonation should increase with the negative charge on the potential proton acceptor, C(6) is expected to be the preferred site of protonation, in agreement with experiment.

The protonation on C(6) of the uracil electron adducts belongs to the class of protonation on C(γ) of allylic ketyl radicals, as shown below. The reactions b–e in Scheme IV have been studied with the pulse radiolysis method and by ESR.[27,28] From the data presented in Scheme IV it is evident that the rate constants for protonation by water of the 1-oxyallyl type radical anions increase

Scheme IV



as the electron density of the system increases by the effects of substitution (compare reaction b with c or b with d in Scheme IV) or of deprotonation (compare reactions d and e in Scheme IV). The increase in electron density that results from deprotonation[28] is also responsible for the fact that the trianion radical of 4,6-DHP is much more rapidly protonated at C(5) than the dianion radical (see section 1.b.3). With uracil, the rate constant for protonation by water (reaction a, Scheme IV) is much lower than in the other cases. This is suggested to be due to the decrease in electron density induced by the N(1)–C(2) amido group.

Since the phosphate mono- and dianions are stronger proton donors than water, they are able to catalyze the conversion of the anion of uracil[4] into the corresponding β-oxoalkyl type radical (the 5-yl radical). The rate of this reaction also seems to be enhanced by increasing the electron density of the radial anion by, e.g., methyl substitution.[4] Catalysis by phosphate of protonation on carbon has also been observed with the electron adduct of fumaric acid.[27]

3. Summary and Conclusions. The reactions of the reducing radicals, H and e^-_{aq}, with dioxypyrimidines were studied by using in situ radiolysis ESR. The hydrogen atom reacts with 4,6-dihydroxypyrimidines and with uracil by addition to C(5) to give 2-yl and 6-yl radicals, respectively. With these the unpaired spin is essentially not delocalized. In comparison, with the radicals formed by addition of the hydrated electron the unpaired spin is delocalized. The coupling constants of these radicals in aqueous solution are very similar to those measured in the solid state. The electron adducts can be protonated on carbon to give localized radicals. In the case of the 4,6-dihydroxypyrimidines the protonation takes place at C(5), with the uracils C(6) is the preferred site of protonation, probably because of the higher negative charge density at C(6). The same reaction has previously been observed to take place in the solid state.[3] This demonstrates that ESR data relating to the solid state can be of predictive value for the solution chemistry not only from the point of view of structure but also with respect to reactivity.

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