Structure of radicals derived from hydroxypyrimidines in aqueous solution

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EPR spectra of radicals derived from the three isomeric trihydroxypyrimidines and from 2-hydroxypyrimidine were obtained either in reduction or oxidation conditions at various pH values. Additionally, three other pyrimidine derivatives, 2-thiobarbituric acid, 2-amino-4,6-dihydroxypyrimidine and 2-methyl-4,5,6-trihydroxypyrimidine, were studied in the same conditions. Reduced radicals were produced by reaction of the pyrimidines with the hydrated electron, the hydrogen atom and with the $CO_2^{\bullet -}$ radical. In these conditions, the electron adducts as well as $CO_2^{\bullet -}$ adducts were identified in most cases. Oxidized radicals were obtained by reaction of the substrates with $OH^{\bullet}$, $O^{\bullet -}$, $SO_4^{\bullet -}$, and $Br_2^{\bullet -}$ radicals. The radicals detected and studied were either of the OH-adduct type or radicals derived from its dehydration. The radicals were investigated by using *in situ* radiolysis and photolysis EPR techniques.

Introduction

The mutagenic and lethal effects of ionizing radiation on living systems are to a large extent due to the chemical transformations induced in the DNA of the cell nucleus.[1] Of the DNA constituents, the purines and pyrimidines are more sensitive than the deoxyribose phosphate moiety, particularly with respect to reaction with the electrons ejected on ionization of a molecule. Numerous studies have been performed with the aim of understanding the sequence of events that lead from the primary act of ionization of a purine or pyrimidine base to a chemically recognizable species,[1,2] the major tools applied being electron paramagnetic resonance (EPR),[3] pulse radiolysis,[2,4] and product analysis.[5] From EPR of irradiated crystals of DNA constituents and of DNA itself,[3] it has long been known that the radicals formed by addition of one electron tend to undergo protonation reactions, which may occur on a heteroatom or on a carbon atom.[3] This type of reaction was later observed to take place also in aqueous solution.[2,6,7] The independence of this reaction of environmental conditions indicates that it has a high degree of intrinsic driving force. The question thus arises whether this reaction is possibly a general one for electron adducts of pyrimidines. Therefore it was considered meaningful to extend the previous studies to the three isomeric trihydroxypyrimidines, *i.e.* pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione or 2,4,6-trihydroxypyrimidine (barbituric acid), dihydropyrimidine-2,4,5(3*H*)-trione or 2,4,5-trihydroxypyrimidine (isobarbituric acid) and dihydropyrimidine-4,5,6(1*H*)-trione or 4,5,6-trihydroxypyrimidine. For comparison purposes, three other pyrimidines, 2-thiobarbituric acid, 2-amino-4,6-dihydroxypyrimidine and 2-methyl-4,5,6-trihydroxypyrimidine, were also studied.

Also relevant are one-electron oxidations, to which hydroxypyrimidines are usually equally sensitive. For this reason, the reactions with the powerful oxidants $OH^{\bullet}$, $Br_2^{\bullet -}$ and $SO_4^{\bullet -}$ were additionally investigated.

Experimental

4,5,6-Trihydroxypyrimidine was synthesized by reaction of formamidine hydrochloride with diethyl hydroxymalonate, following a technique identical to that described for 4,6-dihydroxy-5-methylpyrimidine.[8] 2-Methyl-4,5,6-trihydroxypyrimidine was prepared similarly by using acetamide hydrochloride. The hydroxymalonate ester was obtained through azeotropic esterification of tartronic acid (Fluka) with ethanol and chloroform catalyzed with Amberlite resin IR-120 in the acid form. 2-Amino-4,6-dihydroxypyrimidine was synthesized by reaction of diethyl malonate with guanidine. All the other substances were commercially available from Aldrich, Merck or Fluka and were used without further purification.

The deoxygenated solutions, prepared by bubbling with argon for about 30 minutes, typically contained either 10–100 mM formate (to scavenge H and OH radicals) or 0.1–0.5 M *tert*-butyl alcohol (to scavenge OH radicals) and 1–2 mM of the pyrimidine to react with e^-_{aq} or H atoms. Alternatively, the studies in oxidative media were carried out by bubbling the solutions with N_2O (to scavenge the hydrated electron). In any case the solutions were irradiated with a beam (diameter 1 mm) of 3 MeV electrons from a van de Graaff machine by using the method described by Eiben and Fessenden.[9]

The *in situ* photolysis apparatus was described elsewhere.[10] In this case, reductions were performed in the presence of triethylamine, and oxidations by photolysis of 25 mM potassium persulfate or 2 mM 4-mercaptopyrimidine *N*-oxide.

When possible, pK_a values were estimated from the dependence of the relative concentrations of the acid and basic forms of the radicals on the pH.

Results and discussion

Following our previous studies on radicals produced by one-electron reduction[7] or oxidation[11] of dihydroxypyrimidines like uracil, thymine, 4,6-dihydroxypyrimidine and some of their derivatives in aqueous solution, we have extended these investigations to the radicals derived from the isomeric trihydroxypyrimidines. As before,[7,11] the methods employed involved *in-situ* radiolysis or photolysis of aqueous solutions of the pyrimidines and analysis by EPR of the radicals formed.

On radiolysis of water, the radicals $OH^{\bullet}$, $H^{\bullet}$ and e^-_{aq} are formed with yields per 100 eV of absorbed radiation as indicated in the brackets, *cf.* eqn. (1),

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$$H_2O \longrightarrow OH^{\bullet}\ (2.8),\ H^{\bullet}\ (0.6),\ e^-_{aq}\ (2.7) \qquad (1)$$

The highly reactive OH radical can be scavenged by addition of, *e.g.*, *tert*-butyl alcohol to the solutions, *cf.* eqn. (2),

$$OH^{\bullet} + (CH_3)_3COH \longrightarrow H_2O + {}^{\bullet}CH_2(CH_3)_2COH \qquad (2)$$

leaving only the reducing species, the hydrated electron and the hydrogen atom, to react with the substrates. The resulting radical $^{\bullet}CH_2(CH_3)_2COH$ is not reactive enough to react with the substrates on the ≤ ms timescale.

If it is required to remove also the H atom, propan-2-ol or sodium formate is used instead of *tert*-butyl alcohol (*cf.* eqn. (3)), in which case e^-_{aq} is the main reactive radical remaining.

$$OH^{\bullet}\ (H^{\bullet}) + HCO_2^- \longrightarrow H_2O\ (H_2) + CO_2^{\bullet -} \qquad (3)$$

The $CO_2^{\bullet -}$ radical-anion has strongly reducing properties[12] and in many cases it leads to the same products as does e^-_{aq}. However, it can also add to the pyrimidine system or to other unsaturated molecules.

In order to perform one-electron oxidations, either the OH radical was used, with its yield doubled by converting e^-_{aq} into OH radical by the use of N_2O, *cf.* eqn. (4),

$$e^-_{aq} + H_2O + N_2O \longrightarrow OH^{\bullet} + OH^- + N_2 \qquad (4)$$

or the secondary radicals $Br_2^{\bullet -}$ or $SO_4^{\bullet -}$ were produced *via* $OH^{\bullet}$ (eqn. (5)) or e^-_{aq} (eqn. (6)):

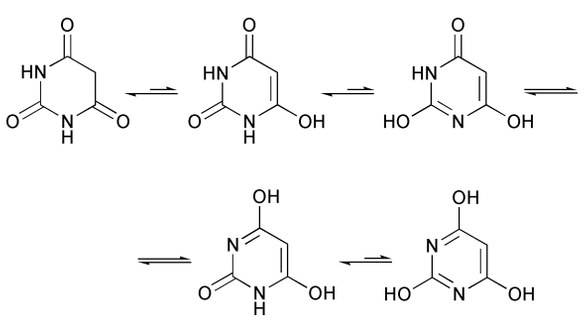
$$OH^{\bullet} + Br^- + H^+ \longrightarrow H_2O + Br^{\bullet} \qquad (5)$$

$$Br^{\bullet} + Br^- \longrightarrow Br_2^{\bullet -}$$

$$e^-_{aq} + S_2O_8^{2-} \longrightarrow SO_4^{2-} + SO_4^{\bullet -} \qquad (6)$$

One-electron oxidations by $OH^{\bullet}$ usually proceed *via* addition–elimination, with the addition step often unselective and the elimination step slow (*e.g.* $k < 10^3\ s^{-1}$).[13] It is for this reason that one-electron oxidation of a system can be achieved more cleanly by the use of $Br_2^{\bullet -}$ or $SO_4^{\bullet -}$, which appear to add more selectively and eliminate (as $2Br^-$ or as SO_4^{2-} respectively) more rapidly than does the $OH^{\bullet}/OH^-$ couple.[14]

The hydroxypyrimidines can in general exist in tautomeric equilibria involving keto and enol forms.[15] In aqueous solution the keto forms are usually favored. An example of this is barbituric acid where, out of the several tautomeric structures that can be considered (*cf.* Scheme 1), the triketo structure largely



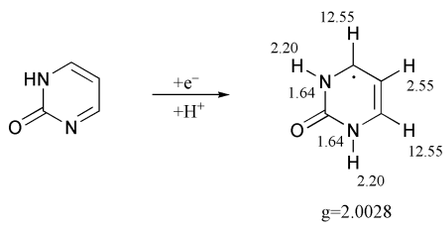
Scheme 1

dominates with only a small contribution (≈5%) of the enol structure.[16]

It is obvious that the keto forms should be easier to reduce and the enol forms more easily oxidized. It is thus to be expected that under reducing conditions the keto forms will dominate the chemistry whereas under oxidizing conditions the products will be mainly determined by the enol forms present in the equilibria. However, the possibility that some minor tautomer may be responsible for the main radical observed cannot be excluded.

Radicals produced under reducing conditions (reactions with e^-_{aq}, $H^{\bullet}$ or $CO_2^{\bullet -}$)

2-Hydroxypyrimidine. This compound was studied as a model compound for trihydroxypyrimidines. On reaction with e^-_{aq} at pH 4–10 in the presence of HCO_2^- (to scavenge $OH^{\bullet}$) only one radical was detected, whose EPR spectrum consisted of a triplet (due to a pair of equivalent protons) with a coupling constant of 12.55 G, another one of 2.20 G, a doublet (due to one proton) of 2.55 G, a quintet (due to two nitrogens) of 1.64 G, and $g = 2.0028$. These parameters were interpreted in terms of the allylic-type radical **1** (Scheme 2), formed by elec-

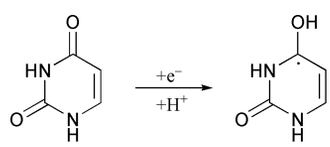


1

Scheme 2

tron addition to the carbonyl group followed by protonation at N1.

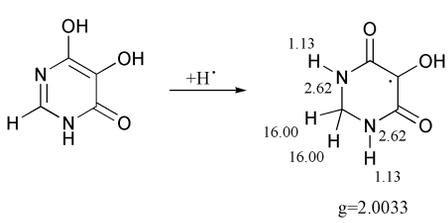
This pattern may be compared to the behavior of the electron adducts of uracil and its derivatives and of thymine, where protonation occurs at O^4 (see Table III of ref. 7). In these cases the protonation at O^4 is favored because not only is its electron affinity higher than that of O^2, but also a delocalized, allyl-type radical is formed (Scheme 3).



Scheme 3

At pH > 10, the radical **1** formed by e^-_{aq} reaction with 2-hydroxypyrimidine disappeared and no new radical was seen. The reason is probably that the aqueous electron does not react with the enolate of 2-hydroxypyrimidine ($pK_a = 9.17$[15]). Radical **1** could also be observed by photolysis of the parent compound in the presence of triethylamine (Fig. 1a).

4,5,6-Trihydroxypyrimidine and 2-methyl-4,5,6-trihydroxypyrimidine. On reduction of 4,5,6-trihydroxypyrimidine at pH = 2.5, a strongly polarized spectrum was seen which was characterized by splittings from two pairs of equivalent hydrogens (16.00 and 1.13 G), two equivalent nitrogens (2.62 G), and $g = 2.0033$. This highly symmetric pattern is interpreted in terms of the radical **2** formed by $H^{\bullet}$ attack at C2 of the enolic form and tautomerization, *cf.* Scheme 4:



2

Scheme 4

Table 1 Coupling constants (G) of pyrimidine–$CO_2^{\bullet-}$ adducts in aqueous solution

Radical	N(1)/N(3)	H(2)	H(5)	H(6)	(N)–H	(O)–H
3	1.50[d]	4.98	—	—	1.2[e]	—
4	0.15[d]	1.55[c]	—	—	1.41[e]	0.18
5-Me-4,6-DHP[a]	2.00[d]	8.75	22.75[c]	—	2.60[e]	—
6	0.41/0.29	—	—	12.9	1.39	0.11
Orotic acid[b]	0.68	—	14.48	—	1.84	—

[a] 5-Methyl-4,6-dihydroxypyrimidine, adduct at C2, ref. 7. [b] Adduct at C6, ref. 18. [c] CH_3. [d] 2N. [e] 2H.

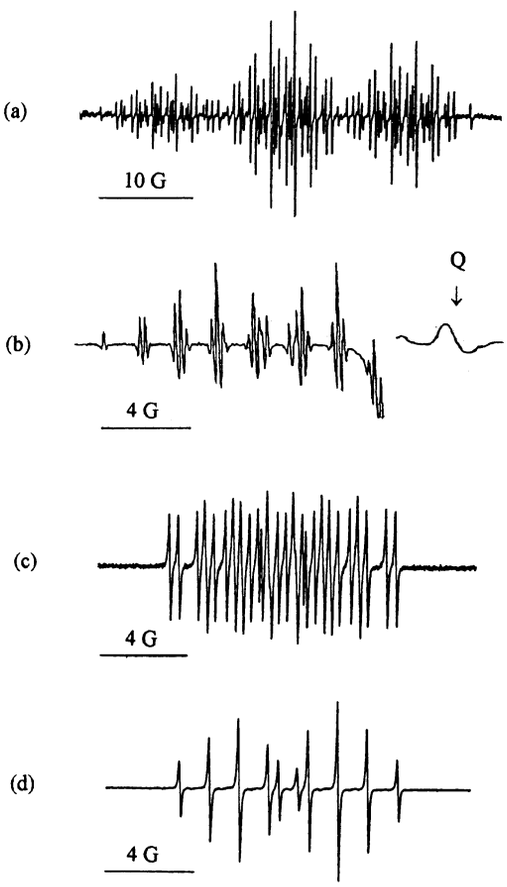
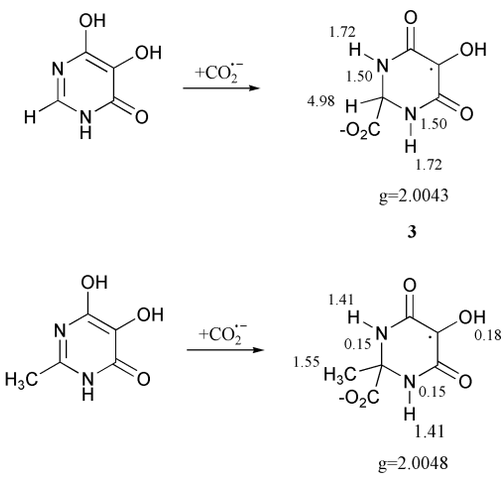


Fig. 1 (a) EPR spectrum obtained upon photolysis of 2-hydroxypyrimidine in the presence of triethylamine (radical **1**); (b) EPR spectrum obtained on electron irradiation of 4,5,6-trihydroxypyrimidine in the presence of sodium formate (radical **3**). Q denotes the quartz signal; (c) EPR spectrum obtained upon photolysis of 4,5,6-trihydroxypyrimidine with potassium persulfate at pH = 8.3 (radical **14**); and (d) pH = 12.1 (radical **15**).

The di-keto structure for radical **2** is suggested on the basis of the relatively large coupling constant for the (N)–H protons and by analogy with the very similar methylene splitting of the H-adduct of 4,6-dihydroxypyrimidine.[7] The electron adduct is assumed to be protonated because the EPR parameters are independent of pH down to pH 2.2. Below this value the solution turned colloidal.

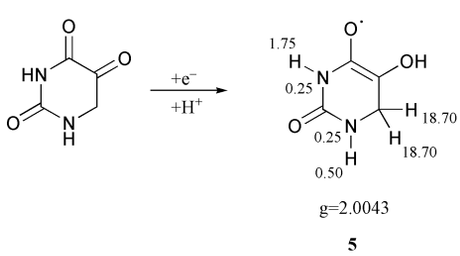
When the reduction of the same compound was performed in the presence of sodium formate at pH values between 5.9 and 7.2, an EPR signal, persistent even after bubbling the solution with N_2O, was observed. Radical **3** was identified as the $CO_2^{\bullet-}$ adduct at C2. Similarly, a $CO_2^{\bullet-}$ adduct, radical **4**, was obtained with 2-methyl-4,5,6-trihydroxypyrimidine at pH 5–7 with the hyperfine coupling constants shown and $g = 2.0048$ (Scheme 5). It is to be noted that no other radical could be observed from these compounds under reductive conditions. Table 1 collects the known $CO_2^{\bullet-}$ adducts of the hydroxypyrimidine system.



Scheme 5

2,4,5-Trihydroxypyrimidine (isobarbituric acid). This compound (pK_a = 8.11 and 11.48)[15] on reaction with e^-_{aq} at pH = 7–8, yielded an EPR spectrum characterized by a triplet (due to 2 equivalent protons) of 18.70 G, two doublets of 1.75 and 0.50 G, a quintet (due to 2 equivalent nitrogens) of 0.25 G, and g = 2.0043. These parameters were interpreted in terms of radical **5**, formed by addition of one electron to C5 followed by protonation at O^5.

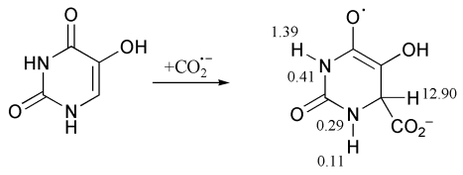
This electron addition is reasonable since the carbonyl group at position 5 is expected to be the one with most electron-affinity. The low nitrogen coupling is probably indicative of an essentially exocyclic oxygen centred radical. The assignment of the two (N)–H couplings shown in Scheme 6 can possibly be



Scheme 6

reversed. At pH values above 10 no other radicals formed by electron addition to this compound were observed. The probable reason is that the anion of isobarbituric acid has too low a reactivity towards the electron.

When the reduction is performed with sodium formate between pH 6.9 and 8.5, an EPR signal can be seen that persists with the same or even increased intensity upon bubbling the solution with N_2O for about 30 minutes. This radical **6** is assigned to a $CO_2^{\bullet-}$ adduct, as shown in Scheme 7. However, it must be emphasized that the assignments of both N and H couplings shown are not unambiguous and can be reversed; also, the small coupling of 0.11 can conversely be assigned to the hydroxy hydrogen.

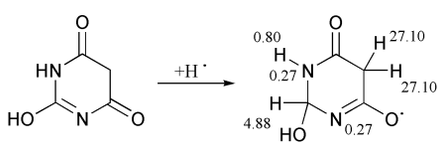


g=2.0044

6

Scheme 7

2,4,6-Trihydroxypyrimidine (barbituric acid). This compound (pK_a = 3.9 and 12.5)[15] exists in aqueous solution mainly in the tri-keto form in equilibrium with a small concentration of the enolic form[16] (Scheme 1). On electron irradiation of an aqueous solution in the presence of 0.1 M *tert*-butyl alcohol (to scavenge OH•) at pH = 2.2, radical **7** was observed (Scheme 8), whose EPR spectrum consisted of a triplet of



g=2.0032

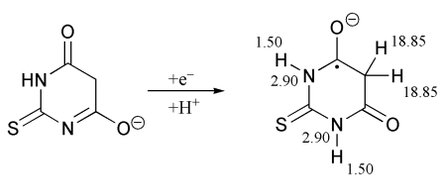
7

Scheme 8

27.10 G, two proton doublets of 4.88 and 0.80 G, a 0.27 G quintet and g = 2.0032. The observed radical must be derived from the attack of the hydrogen atom on position 2 of the enolic form.

A similarly large triplet due to two hydrogens bonded at C5 is seen in a radical derived from uracil (a_H = 32.1 G)[7] and the coupling of 4.88 G can be assigned to the hydrogen at C2, which is of the same magnitude as, for example, the proton coupling at the same position of radical **3**.

2-Mercapto-4,6-dihydroxypyrimidine (2-thiobarbituric acid). When the hydrated electron was reacted with 2-thiobarbituric acid (pK_a = 3.7 and 7.9) an EPR signal was detected between pH 5.5 and 9.5 (radical **8**). On the basis of the pH profile of its persistence, the radical must be derived from the reaction of e^-_{aq} with 2-thiobarbiturate mono-anion. In this case it is expected that the hydrated electron adds to one of the carbonyl groups at the 4 and 6 positions, because the oxygen has more electron-affinity than sulfur. The symmetry pattern of the coupling parameters indicates that the primary product of electron addition to the mono-anion (*i.e.* the radical di-anion) protonates to give radical-anion **8** (Scheme 9). It is to be noted



g=2.0043

8

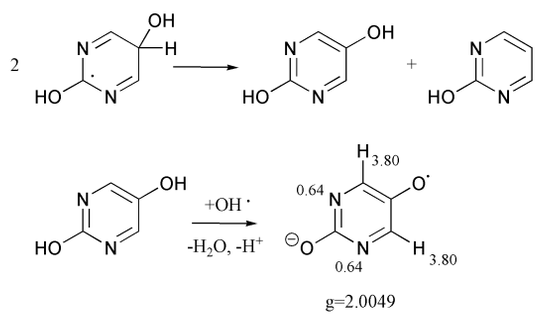
Scheme 9

that there must be a rapid electron transfer between the two carbonyl groups responsible for the symmetry of the radical-anion.

Table 2 shows the known data for the electron and hydrogen adducts of several hydroxypyrimidines.

Radicals formed under oxidizing conditions (reactions with OH•, $SO_4^{\bullet-}$, $Br_2^{\bullet-}$)

2-Hydroxypyrimidine. Radicals derived from 2-hydroxypyrimidine by irradiation of aqueous solutions saturated with N_2O (reaction with OH radical) could only be detected at pH > 9. At this pH a spectrum is observed that is composed of a nitrogen quintet of 0.64 G, a hydrogen triplet of 3.8 G and g = 2.0049. This spectrum, also obtained in the presence of 20 mM KBr, was assigned to the secondary radical **9**. This radical derives from the OH• adduct of 2-hydroxypyrimidine (not observed by EPR) which suffers disproportionation giving, besides the original compound, 2,5-dihydroxypyrimidine. The latter is oxidised to give radical **9** (Scheme 10).

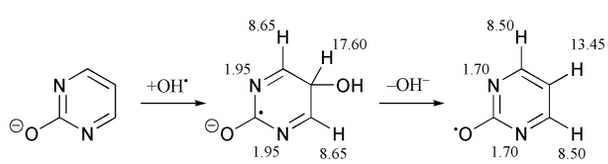


g=2.0049

9

Scheme 10

At pH ≥ 12 two new EPR spectra can be seen. One radical is characterized by one hydrogen doublet of 17.6 G, one hydrogen triplet of 8.65 G and one nitrogen quintet of 1.95 G (radical **10**) and can be assigned to the OH• adduct to the position 5 of the pyrimidine ring. The other EPR signal is due to radical **11**, that must be the product of OH^- elimination from the preceding radical and shows one hydrogen doublet of 13.45 G, one hydrogen triplet of 8.5 G and one nitrogen quintet of 1.7 G (Scheme 11):



10 **11**

Scheme 11

Noticeable is the analogy of the large doublet value of radical **10** with those seen with OH adducts at C5 of other hydroxypyrimidines.[11,17] On the other hand, the structure of radical **11** can be compared with that of the radical-anion obtained from uracil by a similar mechanism.[11,18]

4,5,6-Trihydroxypyrimidine and 2-methyl-4,5,6-trihydroxypyrimidine. On reaction of 4,5,6-trihydroxypyrimidine (4,5,6-THP) with OH• at pH values between 3.5 and 5.5 an EPR spectrum was detected, characterized by two equivalent protons of 0.15 G and one proton with a 15.75 G splitting, two equivalent nitrogens with 2.87 G and g = 2.0034. The radical structure is interpreted as that formed by OH• addition to C2 (radical **12**). The small coupling constant of 0.15 G for the pair of hydrogens is more consistent with the dilactim than with the dilactam structure.

At pH ≥ 5.5 lines of a new radical **14** showed up, which is identified as the deprotonated form of the one-electron-oxidized substrate, produced by elimination of H_2O from the OH-adduct **12**. This radical has previously been described as a secondary radical obtained from 4,6-dihydroxypyrimidine under one-electron-oxidizing conditions.[11] At pH ≈ 10 this

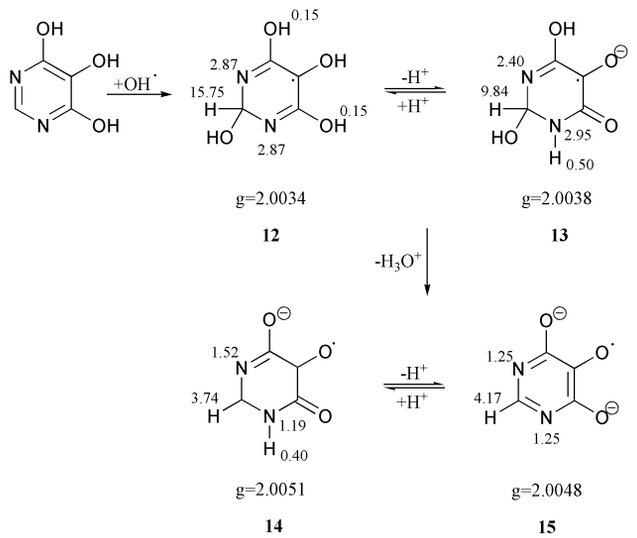
Table 2 Coupling constants (G) of pyrimidine electron or hydrogen adducts in aqueous solution

Radical	N(1)/N(3)	H(2)	H(5)	H(6)	(N)–H
2	2.62[c]	16.00[d]	—	—	1.13[d]
5	0.25[c]	—	—	18.70[d]	1.75/0.50
7	0.27[c]	4.88	27.10[d]	—	0.80
8	2.90[c]	—	18.85[d]	—	1.50[d]
Uracil[a]	1.45/0.2	—	32.10[d]	18.8	0.40
4,6-DHP[a]	2.45[c]	16.10	16.20[d]	—	0.55[d]
2-Me-4,6-DHP[a]	2.70[c]	17.50[b]	17.95[d]	—	0.95[d]
5-Me-4,6-DHP[a]	2.40[c]	16.15	16.15/0.30[b]	—	0.90[d]

[a] Adduct at C5, ref. 7. [b] CH_3. [c] 2N. [d] 2H.

radical begins to disappear to give rise to the corresponding radical dianion **15** which was also obtained as a secondary radical from 4,6-dihydroxypyrimidine.[11]

Under mildly basic conditions (pH 8.5–10) still another radical **13** was seen with proton splittings of 9.84 and 0.50 G and nitrogen splittings of 2.95 and 2.40 G and $g = 2.0038$. The large doublet splitting of 9.84 G can be accounted for in terms of a proton on an sp^3 carbon and on this basis the radical is identified as the ionized OH adduct to C2. Scheme 12 summarizes the formation of the described radicals **12** to **15**.



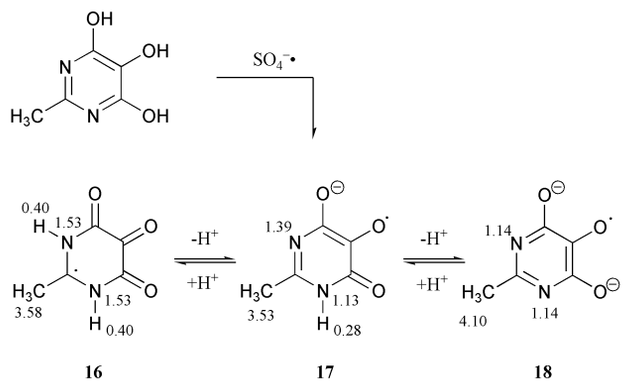
Scheme 12

The one-electron-oxidized radicals **14** and **15** could also be produced from the substrate 4,5,6-THP by one-electron oxidation, using either photochemically produced $SO_4^{\bullet -}$ or radiation-chemically generated $Br_2^{\bullet -}$. The pK_a of radical **14** was estimated to be 10.3.

On oxidation of 2-methyl-4,5,6-trihydroxypyrimidine by photolysis of aqueous solution with $SO_4^{\bullet -}$ and acetone at pH = 3.5 an EPR spectrum could be observed that shows a hydrogen quartet of 3.58 G, a hydrogen triplet of 0.4 G and a nitrogen quintet of 1.53 G. This was assigned to the neutral radical **16** (Scheme 13). At pH ≥ 6.5 the monoionized radical **17** with a hydrogen quartet of 3.53 G, a hydrogen doublet of 0.28 G and two nitrogen triplets of 1.39 and 1.13 G can be observed. At pH = 11 the di-ionized radical **18** is already present with a hydrogen quartet of 4.10 G and a nitrogen quintet of 1.14 G.

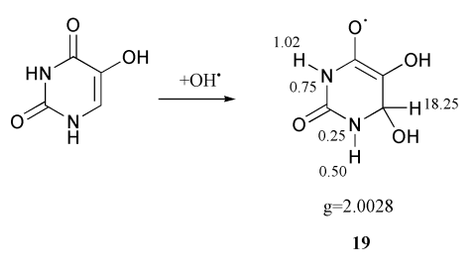
Both these charged radicals **17** and **18** showed very intense EPR signals and were also obtained as secondary radicals from the oxidation of 2-methyl-4,6-dihydroxypyrimidine.[11] pK_a values of **16** and **17** were determined as 4.2 and 11.6, respectively.

2,4,5-Trihydroxypyrimidine (isobarbituric acid). On reaction of this compound with $OH^{\bullet}$ at pH 4–7 an EPR spectrum was obtained with hydrogen constants of 18.25, 1.02, and 0.50 G,



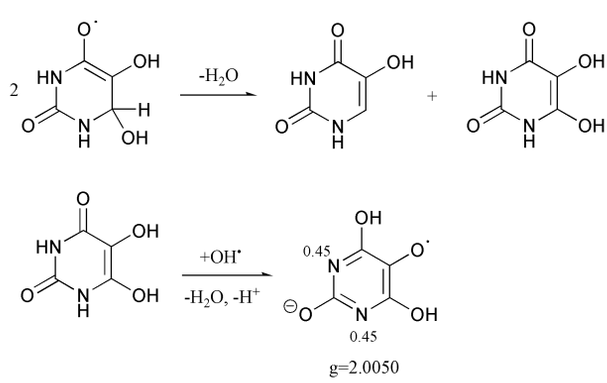
Scheme 13

triplets (due to N) of 0.75 and 0.25 G, and $g = 2.0028$. These parameters were interpreted in terms of formation of the OH adduct to the 6-position of isobarbituric acid, radical **19** (Scheme 14). This radical was also studied by pulse radiolysis with optical detection.[19]



Scheme 14

At low flow rates of the solution, *i.e.* at high conversion, another radical, **20**, with a higher *g*-factor of 2.0050 and having only hyperfine constants corresponding to two equivalent nitrogen splittings of 0.45 G, became apparent (Scheme 15). This radical has also been seen as a secondary product on $OH^{\bullet}$ reaction with barbituric acid.[18] The nature of the radical was verified by the fact that reacting dialuric acid (2,4,5,6-

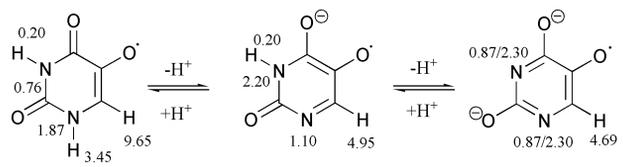


Scheme 15

tetrahydroxypyrimidine) with $OH^{\bullet}$ at pH 4–7 produced the same EPR spectrum. In both cases of barbituric acid and isobarbituric acid, radical **20** is suggested to be formed by disproportionation followed by one-electron oxidation, *e.g. via* $OH^{\bullet}$ addition–H_2O elimination.

One-electron-oxidation of isobarbituric acid was also performed with $SO_4^{\bullet -}$ and with $Br_2^{\bullet -}$. Under these conditions, an EPR spectrum was observed at pH values between 2.3 and 4.0 with doublet splittings of 9.65, 3.45, and 0.20 G, nitrogen splittings of 1.87 and 0.76 G, and $g = 2.0048$. These parameters are interpreted in terms of the neutral radical **21** derived from the enolic form of isobarbituric acid by one-electron-oxidation followed by deprotonation. The same radical was produced by Neta[20] on reaction of $OH^{\bullet}$ with 5-halouracils at pH 2–3.

When the pH was raised above about 5.5 the neutral radical disappeared and lines from a new radical (**22**) appeared instead (Scheme 16). The coupling constants (two hydrogen doublets of



21 **22** **23**

Scheme 16

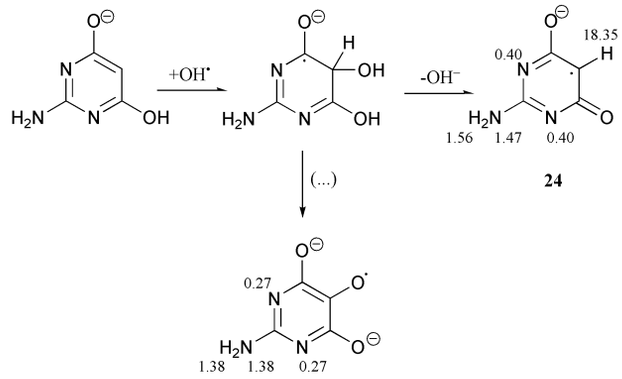
4.95 and 0.20 G and two nitrogen triplets of 2.20 and 1.10 G) show that the spin density has increased on the nitrogens and decreased on C-6, which is evidence for some electronic rearrangement of the molecular system on further deprotonation. Obviously, for deprotonation to be possible one carbonyl group has to enolize and, in the resulting delocalized radical anion, spin density is transferred from the oxygens to the ring nitrogens.

Above pH ≈ 11 the signal of the radical anion disappeared giving rise to a new radical dianion **23** with the C-6 proton splitting of 4.69 G, even smaller than that of the mono-anion, and the difference in the coupling constants of the two nitrogens (2.30 and 0.87 G) is even larger, indicating a further redistribution of spin density in favor of the heteroatoms. These radical mono- and di-anions, **22** and **23**, have previously been described as secondary radicals from the reaction of $OH^{\bullet}$ with uracil,[18] and have also been obtained from 5-halouracils[20] and from 5-nitrouracil.[21]

2,4,6-Trihydroxypyrimidine (barbituric acid). The reaction of this compound with $OH^{\bullet}$ has been studied by Neta[18] and found to lead to formal one-electron-oxidation followed by deprotonation, the mechanism probably being $OH^{\bullet}$ addition–OH^- elimination.

2-Amino-4,6-dihydroxypyrimidine. The reaction of this compound with $OH^{\bullet}$ produced by pulse radiolysis yielded an EPR spectrum with a quintet of lines with 0.40 G, corresponding to two equivalent nitrogens, another single nitrogen coupling constant of 1.47 G, an hydrogen constant of 18.35 and two equivalent hydrogen couplings with 1.56 G. The same spectrum was obtained by photolysis in the presence of persulfate at basic pH values (pH = 9 to 12). Since the $SO_4^{\bullet -}$ radical reacts mostly by one-electron oxidation, the radical observed in both cases should not be the OH-adduct, but instead radical-anion **24**, resulting from OH^- elimination (Scheme 17). It is to be noted that there is some analogy between the observed coupling constants of radical **24** and those of a similar radical obtained from cytosine,[22] 4,6-dihydroxypyrimidine and uracil.[11,18]

A second EPR spectrum could also be observed superimposed on the previous one. This spectrum is consistent with the secondary radical **25**, which is produced from the



24 **25**

Scheme 17

disproportionation of the OH-adduct, as found before for isobarbituric acid (Scheme 15). This radical has some analogy to radical-dianion **15**, obtained by an analogous mechanism from 4,6-dihydroxypyrimidine[11] and by direct oxidation from 4,5,6-trihydroxypyrimidine.

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