is greatly reduced. The results support, therefore, the idea that the tight ion pairs are more reactive than the loose ones.

Acknowledgment. This research was supported by the National Science Foundation and by the Petroleum Research Fund, administered by the American Chemical Society. One of us, A. L., is grateful to Sinclair-Koppers for the fellowship which enabled him to carry out this study. We also thank Dr. A. Rainis for his help in the esr experiments.

Paramagnetic Resonance Study of Liquids during Photolysis. XIII. Uracil and Derivatives[1,2]

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Abstract: Paramagnetic resonance spectra of short-lived free radicals made from uracil, uracil-6-carboxylic acid (orotic acid), uracil-5-carboxylic acid (isoorotic acid), and thymine have been studied upon photolysis with uv light of solutions near room temperature. When these pyrimidines are photolyzed in hydrogen-donating solvents (isopropyl alcohol, ethyl alcohol, and *p*-dioxane), they abstract a hydrogen from the solvent, and two different radicals are observed due to the addition of hydrogen to the pyrimidine and to the loss of hydrogen from the solvent molecule. When the pyrimidines are photolyzed in aqueous solutions containing hydrogen peroxide, ·OH is formed which then produces radicals from the pyrimidines by addition. The spectra and chemical behavior of the radicals in many cases depend upon the pH, and dynamic exchange phenomena are observed. Hyperfine couplings and *g* values are given, and the structure of the radicals is discussed.

We have been studying the paramagnetic resonance spectra of short-lived free radicals made during the course of photolysis of liquids with uv light. In a preceding paper[2] we reported on radicals derived from heterocyclic compounds containing nitrogen including alloxan and parabanic acid. In this paper the work has been extended to solutions of the pyrimidine base uracil and certain derivatives including uracil-6-carboxylic acid (orotic acid), uracil-5-carboxylic acid (isoorotic acid), and thymine.

There have been many paramagnetic resonance studies of free radicals formed from pyrimidine bases and related compounds. Most of these studies have been on solids, where the radical life times are longer than in solution. While most have involved ionizing radiation, a few have used uv light to form the radicals. For example, Pershan and coworkers[4] have identified a radical from thymine obtained by uv irradiation of DNA at low temperatures. There have been essentially no paramagnetic resonance investigations of radicals formed from these substances in solution upon uv photolysis.[5] There has, however, been work on short-lived radicals formed in solutions of pyrimidines using the rapid mixing technique.[6]

In the present work, two general methods have been used to prepare radicals by photolysis; the substances of interest have been photolyzed in solvents that are good hydrogen donors, and the substances have been photolyzed in aqueous solutions containing hydrogen peroxide. In the former case the pyrimidine is excited by the uv light and then abstracts hydrogen from the solvent to form a pair of short-lived radicals. The behavior is parallel to the photoinduced reduction of ketones[7] and of formic acid and its esters[8] in hydrogen-donating solvents. We have used isopropyl alcohol, ethyl alcohol, and *p*-dioxane as solvents. The spectra of the radicals formed by abstraction of hydrogen from these substances are very well known.[7,9] Forming a pair of radicals sometimes has the disadvantage that lines in their spectra overlap, but there is a distinct advantage in seeing both spectra in that it confirms that a primary process is the transfer of a hydrogen from the solvent to the pyrimidine. Photolysis of solutions containing hydrogen peroxide gives ·OH, which in turn may react with the pyrimidine. This approach is similar to the rapid mixing method[6] where the ·OH is generated chemically. This radical may abstract hydrogen or may add to a double bond. The selectivity of ·OH formed photolytically may be different from that of ·OH formed by rapid mixing. This may, in part, be due to the nature of the media. For example, photolytically generated ·OH abstracts hydrogen from allyl alcohol,[9] while ·OH generated in rapid mixing experiments adds to the double bond.[10]

In our earlier studies of photolytically generated radicals from simple organic substances, we have usually been able to easily and unambiguously identify the radicals. Members of a given class of compounds, say,

(1) Research sponsored by the U. S. Atomic Energy Commission under contract with Union Carbide Corp.
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(3) Fellowship support is gratefully acknowledged to the Deutsche Forschungsgemeinschaft.
(4) P. S. Pershan, R. G. Shulman, B. J. Wyluda, and J. Eisinger, *Physics (Long Island City, N. Y.)*, **1**, 163 (1964).
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(6) Cl. Nicolau, M. McMillan, and R. O. C. Norman, *Biochim. Biophys. Acta*, **174**, 413 (1969).
(7) H. Zeldes and R. Livingston, *J. Chem. Phys.*, **45**, 1946 (1966).
(8) H. Hefter and H. Fischer, *Ber. Bunsenges. Phys. Chem.*, **74**, 493 (1970).
(9) R. Livingston and H. Zeldes, *J. Chem. Phys.*, **44**, 1245 (1966).
(10) W. T. Dixon and R. O. C. Norman, *J. Chem. Soc.*, 3119 (1963).

Table I. Parameters for Radicals Made in Hydrogen-Donating Solvents

Solution	*g* value	Couplings, G	Radical[a]
7 m*M* uracil and 9.8 m*M* HCl in isopropyl alcohol at 34°	2.0031	$a(H_\beta) = 31.3$,[b] $a(H_\alpha) = 18.1$, $a(N) \approx a(H) \approx 0.7$	**1** or **2**[c]
8 m*M* uracil in ethyl alcohol at 33°		$a(H_\beta) = 30.6$,[b] $a(H_\alpha) = 17.5$	**1** or **2**[c]
5 m*M* orotic acid in *p*-dioxane at 33.5°	2.00352	$a(H_5) = 24.51$,[b] $a(N_1) = 1.05$, $a(H_1) = 2.53$, $a(H_{3,4}) = 0.23$, $a(H_{COOH}) = 0.88$	**3** or **4**
Orotic acid in isopropyl alcohol at 33°		$a(H_5) = 25.1$,[b] $a(N_1) = 0.91$, $a(H_1) = 2.35$, $a(H_{3,4}) = 0.22$	**3** or **4**
2.9 m*M* orotic acid and 3.3 m*M* NaOH in ethyl alcohol	2.00345	$a(H_5) = 27.9$,[b] $a(N_1) = 0.80$, $a(H_1) = 2.26$	**3**$^-$ or **4**$^-$
6.4 m*M* isoorotic acid in *p*-dioxane at 33.5°	2.00378	$a(H_6) = 14.15$, $a(N) = 1.91$, $a'(N) = 0.65$, $a(H) \approx a'(H) \approx 0.90$, $a''(H) = 0.09$	**5**
6.4 m*M* isoorotic acid and 0.55 *M* HCl in isopropyl alcohol at 35.7°	2.00367	$a(H_6) = 14.1$, $a(N) = 2.80$, $a'(N) = 0.62$, $a(H) \approx a'(H) \approx 0.95$	**5**
6.4 m*M* isoorotic acid in ethyl alcohol		$a(H_6) = 14.2$	**5**

[a] Structural formulas are given in the text. [b] Two equivalent hydrogens. [c] Preferred structure; see text.

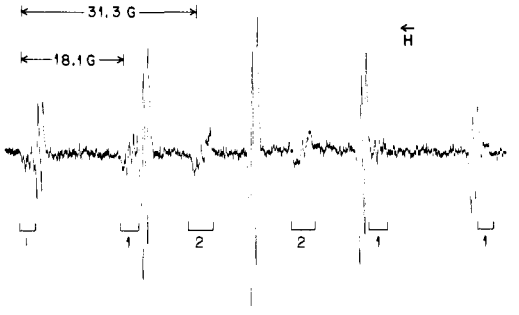


Figure 1. Spectra during photolysis at 34° of *ca.* 7 m*M* uracil and 9.8 m*M* hydrochloric acid in isopropyl alcohol. The brackets locate six groups of lines from a radical from uracil. The sequence of strong doublets is from $(CH_3)_2\dot{C}OH$.

aliphatic alcohols,[9] all behave pretty much alike. We find the behavior of the pyrimidines complex; the behavior of one compound does not necessarily set a pattern for the others.

Experimental Section

The spectrometer, which operated at a nominal frequency of 9.5 GHz and used 100-kHz field modulation, the sample handling operations, and the technique for making measurements of hyperfine couplings and *g* values have already been described.[9,11] The solutions were freed of dissolved oxygen by purging with an inert gas (helium or argon) and were photolyzed near room temperature as they slowly flowed through a flat silica cell positioned inside the cavity of the spectrometer. The uv source was a high-pressure mercury arc, Philips Type SP500W. Measurements of pH were made with a Beckman Model G pH-meter equipped with a glass electrode (Coleman 3-479).

Uracil, thymine, and orotic acid monohydrate (synthetic) from Mann Research Laboratories, isoorotic acid from Aldrich Chemical Co., and 1,3-dimethyluracil from Pfaltz and Bauer were used without additional purification. They are not very soluble in the solvents used. Essentially saturated solutions were prepared using hot solvent to speed the rate of solution and then cooling quickly to room temperature with ice water. A nitrogen atmosphere was used with *p*-dioxane to minimize peroxide formation. Any undissolved pyrimidine was removed by filtration. Acidities were adjusted with either sodium hydroxide or hydrochloric acid. Hydrogen peroxide was added as 98% H_2O_2 (FMC Corp.), and its concentration is given by volume.

(11) R. Livingston and H. Zeldes, *J. Chem. Phys.*, **47**, 4173 (1967).

Results and Discussion for Hydrogen-Donating Solvents

Uracil. Upon photolysis of a saturated solution of uracil in isopropyl alcohol containing 9.8 m*M* HCl, the spectra of two radicals are obtained (Figure 1). The stronger spectrum consisting of a sequence of doublets with relative intensities 1:6:15:20:15:6:1 arises from $(CH_3)_2\dot{C}OH$. The 1-strength lines are not shown in the figure. The presence of this radical clearly shows that a hydrogen was abstracted from isopropyl alcohol. The weaker spectrum consisting of six groups of lines, indicated in Figure 1 by brackets, arises from a radical derived from uracil by hydrogen addition. Some of the groups are not completely resolved from lines of $(CH_3)_2\dot{C}OH$, and the higher field groups are relatively too strong, but this kind of intensity anomaly is frequenly observed in spectra of photolytically produced radicals.[12] The two spectra were also seen in the absence of hydrochloric acid, their strength being somewhat smaller. The addition of 1 m*M* sodium hydroxide or of water (3% or more) decreased the intensity of the weak spectrum below detectability.

The spacings of the groups of lines, Figure 1, arise from hyperfine interactions with two equivalent hydrogens and a unique hydrogen with nominal intensities as indicated in the figure of 1:1:2:2:1:1:1. The components within a group were neither strong enough nor well enough resolved to allow an analysis with great certainty, but the 1-strength groups appeared to contain four components with spacings of approximately 0.7 G which we tentatively interpret as coming from one nitrogen and one hydrogen, giving relative intensities of 1:2:2:1. The 2-strength groups are less suited for analysis, since they contain unresolved second-order splittings[13] of 0.29 G (calculated). The couplings and *g* values are given in Table I.

The spectrum from uracil arises from addition of a hydrogen to the 5,6 double bond, the two possibilities being **1** and **2**. With either structure, we interpret the 18.1-G value (Table I) as an α-coupling constant which gives a π-electron spin density on the α carbon, ρ, of 0.79, where use was made of McConnell's rela-

(12) R. Livingston and H. Zeldes, *ibid.*, **53**, 1406 (1970).
(13) R. W. Fessenden, *ibid.*, **37**, 747 (1962).

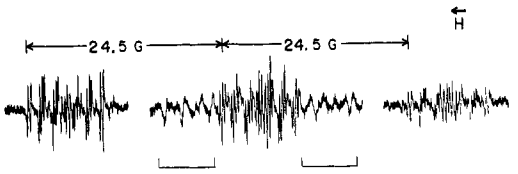
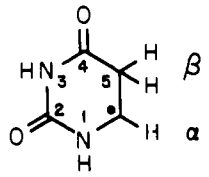


Figure 2. Spectra during photolysis at 33.5° of *ca.* 5 m*M* orotic acid monohydrate in *p*-dioxane. Lines indicated by brackets are described in the text.



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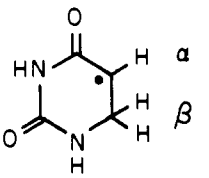

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tion[14] and a Q value of 23 G. With this spin density a prediction of the β-coupling constant can be made from the relation[15] $a(H_\beta) = B_0 + B_1\rho \cos^2 \theta$, where B_0 is a small constant (which we neglect) compared to B_1 = 59 G,[16] and θ is the azimuthal angle between the axis of the orbital of the π electron and the bond C···H_β. Finding a pair of equivalent β hydrogens indicates that θ has the same value for both, and since the carbon to which they are attached should be tetrahedral, the value of θ should be 30°. The predicted value of $a(H_\beta)$ from this equation is 35.0 G. The agreement with the measured value of 31.3 G for the pair of equivalent hydrogens (Table I) is reasonable.

The assignment of the structure is not unequivocal; however, we prefer structure **2** based on the measured g value of 2.0031. As shown later, the analogs of **1** and **2** have been prepared by adding ·OH rather than hydrogen to the double bond, and the g values are 2.0028 for the analog of **1** and between 2.0028 and 2.0033 for the analog of **2**. Similarly, other workers[6] have prepared thymine analogs of **1** and **2** and report g values of 2.0029 and 2.0032, respectively. The small couplings of 0.7 G for one hydrogen and one nitrogen likely come from the 3 position, since couplings are often enhanced across a carbonyl group.

The radical from uracil (and the complementary radical, $CH_3\dot{C}HOH$) was also observed with uracil in ethyl alcohol, and these results are also given in Table I. In this case structure within the groups of lines could not be resolved. The spectrum was not seen with *p*-dioxane as a solvent, in contrast to the findings for orotic and isoorotic acids.

Herak and Gordy[17] observed a spectrum after bombarding a sample of powdered uracil at room temperature with thermal hydrogen atoms. They reported couplings of 33 G from two equivalent hydrogens and 18.5 G from a unique hydrogen, which are in close agreement with our values. These authors felt the more probable radical is **2** based on a consideration of resonance stabilization.

(14) H. M. McConnell and D. B. Chesnut, *J. Chem. Phys.*, **28**, 107 (1958).
(15) C. Heller and H. M. McConnell, *ibid.*, **32**, 1535 (1960).
(16) R. W. Fessenden and R. H. Schuler, *ibid.*, **39**, 2147 (1963).
(17) J. N. Herak and W. Gordy, *Proc. Nat. Acad. Sci. U. S.*, **54**, 1287 (1965).

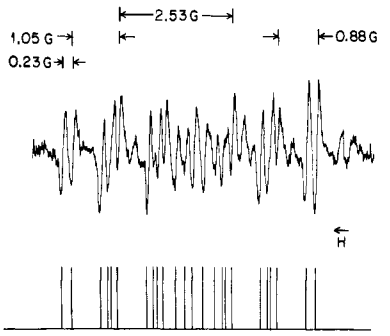
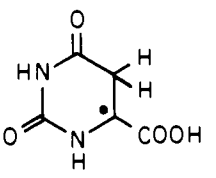


Figure 3. The high-field group of lines of the spectrum of Figure 2. The stick spectrum locates lines from one radical from orotic acid.

1,3-Dimethyluracil. We were unable to detect radicals from photolysis of solutions of 1,3-dimethyluracil in isopropyl alcohol, ethyl alcohol, or *p*-dioxane.

Orotic Acid. Spectra of several radicals were obtained upon photolysis of a saturated solution of orotic acid (about 5 m*M*) in *p*-dioxane. Two of these came from the solvent and could be formed by photolysis of *p*-dioxane alone. The spectrum of one radical formed by abstraction of hydrogen from *p*-dioxane is well known.[7] The other radical from the solvent was of unknown identity and gave a spectrum consisting of a (1:2:1)(1:2:1) pattern from two sets of two equivalent protons with coupling constants of 16.6 and 2.0 G. In addition to radicals from the solvent, there were two spectra arising from orotic acid. One of these gave many sharp lines (Figure 2) and was studied in detail. The other, which was not studied, gave weaker lines, some of which are indicated by brackets in the figure. The highest field group of sharp lines is much stronger than the lowest field group which is typical of the intensity anomaly previously mentioned.[12] In preparing Figure 2, portions of the entire spectrum were omitted, but only a few weak lines from the solvent appeared in these regions.

The spacings of the three groups of lines in Figure 2 arise from the hyperfine coupling of two equivalent hydrogens with $a(H_5)$ = 24.51 G and a g value of 2.00352 at 33.5°. The central group of lines contains resolved second-order splittings of 0.17 G (calculated).[13] The only reasonable way to have a strongly coupled pair of equivalent hydrogens is addition of hydrogen at the 5 position of orotic acid. This gives structure **3**; however, as explained later, there is evidence suggesting that the radical is present in the rearranged form **4**. The value of $a(H_5)$, a β coupling constant, is



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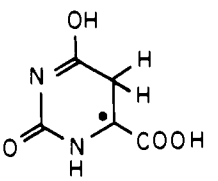

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considerably smaller than the value of 31.3 G found in the radical from uracil (Table I), which suggests greater delocalization of the spin density, probably caused by some of the density appearing in the carboxyl group. A relatively large g value compared with radicals from thymine[6] and uracil is consistent with this view.

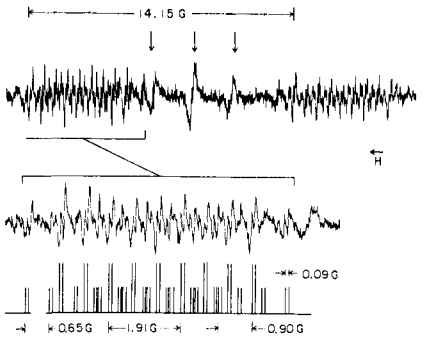


Figure 4. Top: spectrum during photolysis at 33.5° of 6.4 mM isoorotic acid in p-dioxane. The arrows indicate lines from an unidentified radical from p-dioxane. Bottom: expanded view and stick spectrum of the high-field group of lines.

An expanded view of the high-field group of lines in Figure 2 is shown in Figure 3 along with a stick spectrum which locates those components coming from the above radical. There are small splittings from one nitrogen and three inequivalent protons. The parameters are given in Table I. The nitrogen coupling is assigned to the 1 position because it is closer than the other nitrogen to the region of high spin density. Resolved hyperfine splittings are seen from all hydrogens of the radical. One coupling must belong to the carboxyl hydrogen. This is quite unusual, since rapid dynamic exchange usually causes the splitting from acid hydrogen to vanish. The assignment of the weak hydrogen couplings will be discussed later.

Upon adding concentrated hydrochloric acid to solutions of orotic acid in p-dioxane, rapid dynamic exchange effects were seen. Broadening of the lines of the spectrum occurred, and at 0.13 M hydrochloric acid the splittings of 0.88 and 0.23 G (Table I) could not be recognized. The hyperfine pattern within each group of lines (Figures 2 and 3) was made up of five lines arising from the nitrogen splitting of 1.1 G and the proton splitting of 2.5 G. Because of line broadening, a five- rather than six-line pattern was obtained. Unfortunately, enough acid to cause a resharpening of the spectrum could not be added because the orotic acid precipitated and two liquid phases developed.

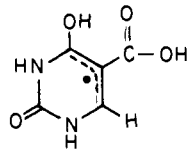
Isopropyl alcohol was also used as a hydrogen-donating solvent, in which case the spectrum of the complementary radical $(CH_3)_2\dot{C}OH$ was also seen. Parameters of the radical from orotic acid in isopropyl alcohol given in Table I are close to those with p-dioxane as the solvent. The 0.88-G splitting was absent. The carboxyl hydrogen should be the most susceptible to exchange of all the hydrogens. The absence of the 0.88-G splitting in isopropyl alcohol and also finding this to be one of the two splittings readily undergoing rapid exchange upon adding acid to the p-dioxane system are the bases for assigning this coupling to the carboxyl hydrogen. Of the remaining couplings $a(H_1)$ and $a(H_{3,4})$, the larger value is assigned to the 1 position because of its closer proximity to the center of high spin density. The smaller value is assigned to the 3 position of structure **3** or the 4 position if the rearranged form **4** is adopted.

Upon successive additions of concentrated hydrochloric acid to the isopropyl alcohol solution of orotic acid, the components of the doublets separated by 0.22 G, $a(H_{3,4})$, broadened, drew together, merged to a single broad line, and resharpened. This behavior is typical of an acid-catalyzed proton exchange.[18] The two components merge when the mean lifetime of the radical between chemical exchanges, τ, has the value $(\sqrt{2}\pi\Delta\nu)^{-1}$ where $\Delta\nu$ is the hyperfine splitting in hertz. The merging occurred at 0.18 M hydrochloric acid, and for this concentration τ^{-1} has the value of 2.75×10^6 sec^{-1} at 33°. If we assume that the acid is completely dissociated and the exchange is first order in $[H^+]$ and in radical concentration, then the second-order rate constant, k, is $k = 1/\tau[H^+] = 2.0 \times 10^7$ l. mol^{-1} sec^{-1}. This value is very similar to the values[7] found for the exchange of hydroxyl hydrogen in $\dot{C}H_2OH$ and $(CH_3)_2\dot{C}OH$ and for exchange for alcohols in water.[19] The exchange of NH protons is not acid catalyzed.[20] Even though the pyrimidines, in general, are in the lactam form in acid solution[21] we propose structure **4** to account for the ready ability of the hydrogen with a coupling of 0.22 G to undergo acid-catalyzed exchange. The nitrogen and hydrogen couplings assigned to the 1 position are essentially unaffected by the addition of acid.

The radical from orotic acid was also prepared upon photolysis of 2.9 mM orotic acid in ethyl alcohol. The three main groups of lines were present (Figure 2) with $a(H_5) = 27.9$ G. The complementary radical $CH_3\dot{C}HOH$ was also present. The components within each group were very complex and could not be analyzed in terms of a single radical. A much simpler spectrum was singled out and analyzed after the addition of sodium hydroxide (3.3 mM). Unfortunately, this solution was unstable and a white precipitate formed during the experiment. Higher concentrations of sodium hydroxide led to serious loss of signal strength. Measured parameters are given in Table I. The sodium hydroxide concentration was large enough to form the carboxylate ion of orotic acid and perhaps large enough to give partial dissociation of one of the NH protons The radical is presumably in the anion form. The g value, however, is smaller than that for the neutral radical in p-dioxane, which is opposite to the trend expected.[2]

Isoorotic Acid. Figure 4 shows the spectrum obtained during photolysis of 6.4 mM isoorotic acid in p-dioxane. The three broad lines indicated by arrows near the center of the spectrum are from an unidentified radical derived from p-dioxane, as discussed earlier. The second radical from p-dioxane formed by loss of a hydrogen was also present. The two groups of sharp lines came from a radical derived from isoorotic acid with measured parameters as given in Table I. The couplings cannot reasonably be accounted for by addition of hydrogen to the 5,6 double bond of isoorotic acid, for then we would expect two strongly coupled hydrogens. Radical **5** does not have this problem and more properly accounts for the observed values. The coupling of 14.15 G assigned to the hydrogen at the 6 position is very similar to the couplings for the terminal hydrogens of the allyl radical,[16] $CH_2{=}CH{-}\dot{C}H_2$,

(18) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High-resolution Nuclear Magnetic Resonance," McGraw-Hill, New York, N. Y., 1959, pp 218–224.
(19) Z. Luz, D. Gill, and S. Meiboom, *J. Chem. Phys.*, **30**, 1540 (1959).
(20) (a) E. Grunwald, A. Loewenstein, and S. Meiboom, *ibid.*, **27**, 630 (1957); (b) A. Loewenstein and S. Meiboom, *ibid.*, **27**, 1067 (1957).
(21) D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952).



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which are equivalent in pairs with values of 13.93 and 14.83 G. It is also similar to the values for the terminal hydrogens of a radical[9] from allyl alcohol, CH_2=CH—ĊHOH, which range from 13.15 to 14.23 G. The structure given for radical **5** implies that much of the spin density is divided between the 4 and 6 positions. We would expect to see hyperfine interactions for both nitrogens, which was the case, and a resolved coupling with the hydroxyl hydrogen is reasonable. We are unable to assign the two nitrogen couplings and the three weak hydrogen couplings to specific positions of radical **5**, but it is likely that the acid hydrogen of the carboxyl group is not one of the hydrogens. Factors that may contribute to the stability of radical **5** in addition to resonance stabilization of the allyl structure are conjugation between this structure and the double bond of the carboxyl group and hydrogen bonding between the hydroxyl proton and the carbonyl oxygen of the carboxyl group. Radical **5** was also seen upon photolysis of 6.4 m*M* isoorotic acid in isopropyl alcohol, but the spectrum was too weak to analyze except for the strong proton coupling, $a(H_6)$, found to be 14.1 G. The spectrum was more intense when the solution was 0.55 *M* in hydrochloric acid and higher flow rates were used. The lines were somewhat broader than in *p*-dioxane, and the 0.09-G splitting was not seen. Measured parameters are given in Table I. Except for one nitrogen coupling, these values are similar to those found in *p*-dioxane. The failure to find the splitting of 0.09 G may mean that this hydrogen was undergoing rapid exchange in the acid medium, in which case this coupling would be assigned to the hydroxyl hydrogen of **5**. The couplings given in Table I accounted for the positions of the lines in the two groups reasonably well; however, there were intensity variations that did not match between the two groups, which suggests that extraneous, unresolved lines from another radical were present.

In our photolytic studies the solution slowly flows through a fused silica cell located in the microwave cavity. Occasionally the strength of a spectrum depends upon the rate of flow This was true for the radical from isoorotic acid for all solvents tried. Flow rates from 0.4 to 3.1 ml/min were used in experiments with isopropyl alcohol, and over this range the signal strength was proportional to the rate of flow. The spectrum of $(CH_3)_2$ĊOH which was present in these experiments showed very little intensity change with rate of flow.

Photolysis of 6.4 m*M* isoorotic acid in ethyl alcohol gave spectra of CH_3ĊHOH and radical **5** with $a(H_6)$ = 14.2 G. The lines within each of the two groups of lines were more complex than in *p*-dioxane and isopropyl alcohol. A complete analysis could not be made.

Thymine. Photolysis of a saturated solution of thymine (about 5.5 m*M*) in isopropyl alcohol made

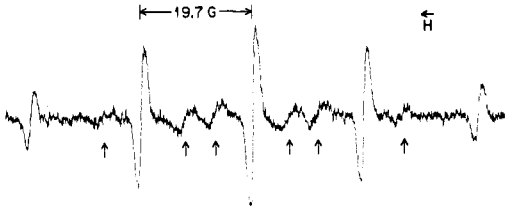


Figure 5. Spectra during photolysis at 36° of 5.5 m*M* thymine in isopropyl alcohol containing 25.5 ml of concentrated hydrochloric acid per liter. A part of the spectrum from $(CH_3)_2$ĊOH is shown. Arrows indicate lines from an unidentified radical from thymine.

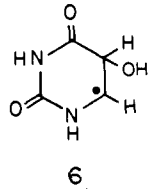
0.31 *M* in hydrochloric acid gave spectra from $(CH_3)_2$ĊOH and from a radical from thymine. The spectrum from thymine indicated by arrows in Figure 5 consists of six broad lines with approximate relative intensities of 1:2:2:2:2:1. Splittings due to the hydroxyl hydrogen interaction of $(CH_3)_2$ĊOH are not present because there was sufficient acid to give rapid exchange.[7] The lines from this radical are also broadened by the high modulation used to favor the broad lines from the thymine radical. The strength of the spectra of both radicals became stronger with decreasing rate of flow of solution through the microwave cavity.

The presence of a strong spectrum of $(CH_3)_2$ĊOH (Figure 5) indicates that a hydrogen atom from isopropyl alcohol transfers to thymine. However, we cannot account for the spectrum arising from thymine by the radicals that would be formed in either of the two ways hydrogen might be added to the 5,6 double bond or by addition to a carbonyl oxygen.

With concentrations of hydrochloric acid other than 0.3 *M*, the outermost lines were weaker, and at some concentrations they could not be seen. This suggests that there are dynamic processes causing line broadening, and we may not be able to see all of the lines of the spectrum. Increasing signal intensity with decreasing rate of flow also suggests complex behavior; there is the possibility of secondary reactions to give radicals different from those expected by the simple addition of hydrogen to thymine. Radicals are not seen upon photolysis of solutions of thymine in ethyl alcohol and in *p*-dioxane.

Results and Discussion for Aqueous Solutions Containing Hydrogen Peroxide

Uracil. Radicals were obtained at various values of pH by photolyzing aqueous uracil (about 27 m*M*) containing 1% hydrogen peroxide. The radicals were formed by addition of ·OH to the 5,6 double bond. Over a pH range of 1–7 radical **6** was formed, along with a much smaller amount of **7**. The strongest spectra were obtained near a pH of 3 as shown in Figure 6. The hydrogens at the 5 and 6 positions are



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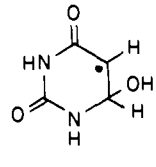

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strongly coupled and give four groups of lines, the center two not being completely separated. All of the

Table II. Parameters for Radicals Made in Aqueous Solutions Containing 1% Hydrogen Peroxide and at Approximately 33°

Solution	pH	*g* value	Couplings, G	Radical[a]
27 m*M* uracil	1–7	2.0028	$a(H_5) = 21.43$, $a(H_6) = 18.32$, $a(N) = 0.88$, $a'(N) = 0.88$	**6**
		>2.0028 ≤2.0033		**7**
	8–10	2.00478	$a(H_6) = 4.95$, $a(N) = 2.20$, $a'(N) = 1.10$, $a(H) = 0.18$[b]	**9**
6 m*M* orotic acid	4–9	2.00493	$a(N_1) = a(N_3) = 0.44$, $a(H_1) = a(H_3) = 0.44$	**10**
		2.00334	$a(H_5) = 12.33$, $a(H) = 1.79$, $a'(H) = 0.10$, $a(N_1) \approx a(N_3) \approx 0.28$	**11** or **12**[c]
	1.7–3.5	2.00332	$a(H) = 12.1$[d]	
	8.5–9.5	2.00486	$a(N) = 2.06$, $a'(N) = 1.03$, $a(H) = 0.16$[b]	**13**
19 m*M* isoorotic acid	2.56	2.0029	$a(H_6) = 17.8$, $a(H_1) = 1.1$, $a(N_1) = 0.8$	**14**
		≈2.0033	$a(H_6) \approx 19.5$	**15**
32 m*M* thymine	1.5–9	2.00280	$a(H_5) = 34.0$, $a(H_6) = 18.3$, $a(N_1) = 1.35$	**16**[e]

[a] Structural formulas are given in the text. [b] Value is pH dependent. Measured at a pH of 8.50. [c] Preferred structure. [d] A complet analysis of hyperfine structure was not made. The radical is unidentified. [e] Tentative assignment; see text.

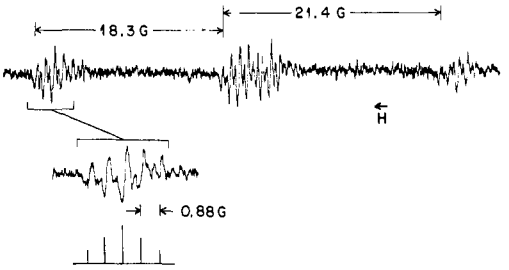


Figure 6. Spectra during photolysis at 34.5° and a pH of 3.0 of aqueous 27 m*M* uracil containing 1% H_2O_2. An expanded view and stick spectrum of the high-field group of lines is also shown. Splittings in the stick spectrum are given for the more abundant radical described in the text.

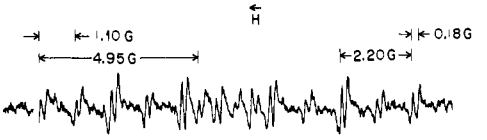


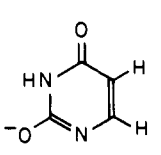
Figure 7. Spectrum during photolysis at 32° and a pH of 8.50 of aqueous 27 m*M* uracil containing 1% H_2O_2.

strong lines come from a single radical. Mixed with each group are weaker lines from the second radical. The highest field group is shown in more detail, and lines from the more abundant radical, **6**, are indicated by the stick spectrum. The strongest lines of each group consist of five components spaced by 0.88 G. These are interpreted as coming from two nitrogens with couplings of 0.88 G. This would give relative intensities of 1:2:3:2:1, which is consistent with the observed spectrum. Although this interpretation is preferred, an alternate interpretation[6] is that the components arise from one nitrogen and two hydrogens, all with couplings of 0.88 G. This would give an intensity distribution of 1:3:4:3:1. The signal-to-noise ratio was not adequate to unequivocally dismiss this possibility. The *g* value of 2.0028 and the hyperfine couplings for this radical are listed in Table II. The weaker spectrum from **7** could not be analyzed in detail, but the *g* value was found to be clearly larger than 2.0028 but not larger than 2.0033. The assignment of which spectrum comes from **6** and which comes from **7** was based on the *g* values; radical **7** should have the larger value.

Nicolau and coworkers,[6] using the rapid mixing technique, formed a radical by the addition of ·OH to uracil which they assigned to **6**. The spectrum had poorer resolution than that reported here. Their *g* value of 2.0029 and hydrogen couplings of 18.1 and 21.2 G assigned to the 6 and 5 positions, respectively, and our values (Table II) are in good agreement. They observed weak couplings of 0.8 G which they assigned to one nitrogen and two hydrogens. We found a value of 0.88 G for these weak couplings (Table II), but preferred to assign them to two nitrogens.

Aqueous solutions of uracil at higher values of pH, between 8 and 10, gave an entirely different spectrum, Figure 7. No spectrum was obtained at pH values greater than 10, where there was marked decomposition of the hydrogen peroxide. Parameters for the radical of Figure 7 are given in Table II. All of the couplings are relatively small. The largest, 4.95 G, is assigned to the 6 position. This assignment stems from a result described later, where a similar radical was made from orotic acid (uracil-6-carboxylic acid) with parameters almost identical with those given for uracil in Table II except that the coupling of 4.95 G was absent. The smallest splitting of 0.18 G depends upon pH and has this value at a pH of 8.50. The hydrogen responsible for this splitting shows typical exchange effects. Upon increasing the pH, each pair of lines with this splitting (Figure 7) broadens, draws together, and merges into a broad line at a pH of 9.43. Further increase in pH causes a sharpening of the broad line.

Aqueous uracil converts[21] to an anion, **8**, in the pH range 8.6–13. This suggests that the radical is **9** where ·OH addition has taken place at the 5 position, the position found most susceptible to addition in acid media. Although there would be substantial spin density at the 1 and 6 positions, the small coupling for



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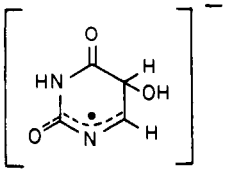

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the 6 position and the high *g* value (Table II) suggest that the greater part is in the region of the 1 position, with some density appearing in oxygen orbitals. The ni-

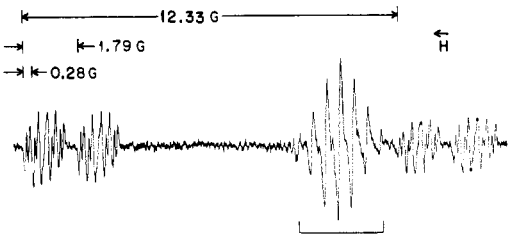
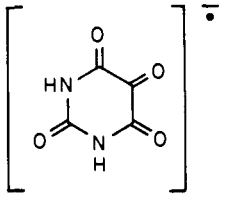


Figure 8. Spectra during photolysis at 34° and a pH of 6.40 of aqueous 6 m*M* orotic acid containing 1% H_2O_2. The bracket indicates a spectrum of a radical derived from alloxan.

trogen couplings have not been assigned to specific positions, but it is reasonable from **9** that both should couple sufficiently to give resolved splittings. The hydrogen with a coupling of 0.18 G that exchanges at an increasing rate with increasing pH could be the hydrogen at the 2 position, although the hydroxyl hydrogen is not ruled out. Spin density at the 6 position could give a noticeable β coupling to the hydrogen in the 5 position, which was not observed. If the hydrogen in the 5 position were sufficiently bent into the plane of the ring, its coupling would become very small.

1,3-Dimethyluracil. Aqueous solutions of 1,3-dimethyluracil containing 1% hydrogen peroxide were photolyzed over a broad range of pH values. No spectra were observed.

Orotic Acid. The spectra obtained upon photolysis of aqueous solutions of orotic acid (about 6 m*M*) containing 1% hydrogen peroxide also depended upon pH. Some of the radicals were similar to those formed with uracil where ·OH adds to the 5,6 double bond; however, an additional spectrum identified as radical **10** was always present. This spectrum, which consists of seven lines spaced by 0.44 G (Table II), may be readily formed from solutions containing alloxan and



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has already been studied.[2] Radical **10** apparently forms from a reaction product of orotic acid and hydrogen peroxide. It was found, for example, that boiling a solution of orotic acid containing 1% hydrogen peroxide prior to photolysis gave an enhanced yield of **10** while the yield of other radicals decreased.

With the pH adjusted with sodium hydroxide to the range of 4–9, spectra like that of Figure 8 were obtained. The seven lines from radical **10** are indicated by a bracket. Mixed with them are much weaker lines from another unidentified radical. The remaining four groups of lines come from a single radical with parameters given in Table II. The nitrogen couplings are slightly nonequivalent, with an average value of 0.28 G. The slight nonequivalence causes the central line of each of the four groups (Figure 8) to appear as a broad line, with an improvement in resolution in progressing to the outermost lines. This system was

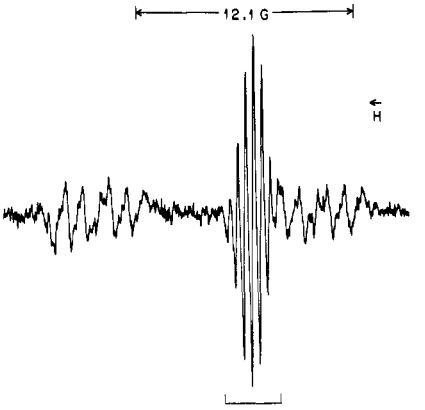
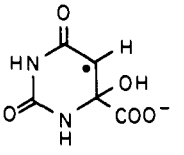


Figure 9. Spectra during photolysis at 33° and a pH of 2.93 of aqueous 6 m*M* orotic acid containing 1% H_2O_2. The bracket indicates a spectrum of a radical derived from alloxan.

also studied with D_2O as the solvent, in which case the hydrogens having couplings of 1.79 and 0.1 G were replaced by deuterium. Since orotic acid has a pK[22] of 2.40, it should be predominantly in the anion form in these experiments. Addition of ·OH to the 5,6 double bond would give **11** or **12**. In either case the coupling of 12.33 G which did not exchange with D_2O is assigned to the 5 position. We prefer structure **12** by analogy with the uracil system where ·OH preferentially adds at the 5 position to give **6**. The higher g value of **12** compared to that of **6** (Table II) probably reflects delocalization of spin density into the carboxyl group and may partly account for the smallness of the hydrogen coupling at the 5 position. If this hydrogen were tilted toward the plane of the ring, its coupling also would become small. The analogy to uracil goes further. At higher pH values the radical from uracil converts to a different form, **9**. As shown later, the radical from orotic acid also converts to a different form with parameters strikingly like those from **9**.



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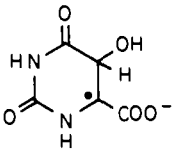

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Upon decreasing the pH the spectra of Figure 8 transformed to those of Figure 9 over the very narrow range of 4–3.5. There were no further changes in decreasing the pH to 1.7. The spectrum from radical **10** indicated by a bracket in Figure 9 remained essentially unchanged. The four groups of lines from radical **12** (Figure 8) have transformed to two groups, each containing five lines, with the central line showing additional structure. Only the strong hydrogen coupling and g value were determined (Table II). They are essentially unchanged from the values for radical **12**. The radical which remains unidentified is probably similar to **12**, but the change in structure is certainly more profound than protonation at the carboxyl group of **12** to form a neutral radical.

The aqueous orotic acid system was explored up to a pH of 9.5. At a pH of 8.5, lines of a new radical

(22) M. Bachstez, *Ber.*, B63, 1000 (1930).

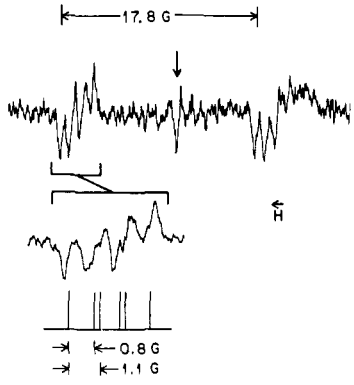
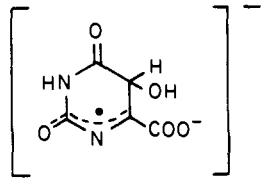


Figure 10. Spectra during photolysis at 33° and a pH of 2.56 of aqueous 19 m*M* isoorotic acid containing 1% H_2O_2. The high-field group of lines and a stick spectrum are shown with higher resolution.

appeared in addition to those shown in Figure 8. With increasing pH the spectra of Figure 8 became weaker, with the four groups of lines from radical **12** vanishing by the time a pH of 9.3 was reached. The lines from the new radical were partly obscured by those of radical **10**, but a complete analysis was possible. The parameters are given in Table II. They are essentially the same as those of radical **9** from uracil except that the hydrogen in the 6 position is absent. The 0.16-G splitting depended upon pH, with the lines broadening with increasing pH and fusing together at a pH of 9.32. The proposed radical is **13**, with its formation and properties analogous to those of radical **9** of the uracil system.



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Isoorotic Acid. The spectra of Figure 10 were obtained near room temperature upon photolysis of aqueous 19 m*M* isoorotic acid containing 1% hydrogen peroxide at a pH of 2.56. In addition to the central unidentified line (arrow), the spectra of two radicals are present. One of these consists of two groups of sharp lines spaced by 17.8 G. One of the groups of lines with a stick spectrum is shown at higher resolution. The multiplicity arises from the weak coupling of one nitrogen and one hydrogen. Parameters are given in Table II. The spectrum of the remaining radical of Figure 10 consists of two very broad lines, where the higher field broad line is almost perfectly superimposed on the higher field group of sharp lines. The lower field line is noticeably displaced to lower field than the corresponding group of sharp lines. Approximate parameters for this radical are given in Table II.

Radicals **14** and **15** are obtained by adding ·OH to the double bond of isoorotic acid. We assign the radical with the lower *g* value to **14** and the other to **15**, with couplings assigned as given in Table II. We found aqueous solutions of isoorotic acid containing hydrogen peroxide to be unstable. Shortly after

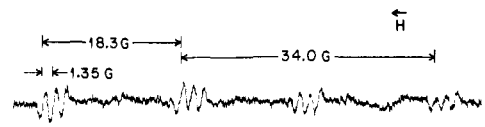
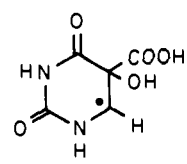


Figure 11. Spectrum during photolysis at 35° and a pH of 3.20 of aqueous 32 m*M* thymine containing 1% H_2O_2.



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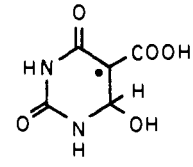

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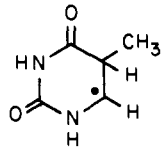
adding the peroxide, the solution develops a yellow color, and within a few hours a yellow, unidentified precipitate forms. No spectrum was seen from the solution after precipitation was complete.

Nicolau and coworkers[6] prepared radical **14** by the rapid mixing technique and reported a hydrogen coupling of 17.8 G and a *g* value of 2.0028. Our results are in excellent agreement with theirs. They did not resolve as much detail within the groups of lines and did not report additional couplings. Neither did they report a spectrum from radical **15**, but they did see two lines with large *g* values that we have not observed.

Thymine. A spectrum of rather broad lines (Figure 11) was obtained at 35° upon photolysis of aqueous 32 m*M* thymine containing 1% hydrogen peroxide and enough hydrochloric acid to give a pH of 3.20. The lines are accounted for by hyperfine interactions, with two hydrogens with couplings of 18.3 and 34.0 G and a nitrogen coupling of 1.35 G. The *g* value is 2.00280. The spectrum of Figure 11 was not seen outside the pH range of 1.5–9. A pH of 3 was optimum. A waviness in the base line suggests the presence of additional extremely broad lines. They do not sharpen with a change of pH.

We cannot account for this spectrum by the addition of ·OH to the 5,6 double bond of thymine[6] nor by the simple abstraction of a hydrogen. The hydrogen couplings of 18.3 and 34.0 G are typical of α- and β-coupling constants, respectively. A radical that could properly account for these couplings and the *g* value, one that we would expect but were unsuccessful in preparing in a hydrogen donating solvent, is **16**. The



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coupling of 1.35 G for nitrogen could arise from the 1 position. We would not expect this radical in a system with hydrogen peroxide and do not know by what mechanism it might form. As with the other pyrimidines, spectra were not obtained from aqueous solutions in the absence of hydrogen peroxide.