absorb appreciably at 313 nm, photoisomerization with 254-nm light was investigated. Irradiation of *E*-**3** with 1.0 molar equiv of BF_3·OEt_2 results in >95% conversion to *Z*-**3** by ^1H NMR and GC analysis. Since both *E*- and *Z*-**3** are fully complexed, the photostationary state will be described by eq 3. Moreover, as the BF_3 complex of *Z*-**3** absorbs more strongly at 254 nm than that of E-$_3$, it is evident that $\bar{\Phi}_{EA} \gg \Phi_{ZA}$. A possible explanation for the observation of reversible photoisomerization of noncomplexed *E*- or *Z*-**3** but irreversible $E \rightarrow Z$ isomerization for their BF_3 complexes is provided by the observation of significantly enhanced intramolecular hydrogen bond strength for *Z*-3 upon complexation with BF_3. Increased intramolecular hydrogen bond strength might result in a large thermal barrier for isomerization on the excited state energy surface and thus preclude $Z \rightarrow E$ photoisomerization.

Concluding Remarks. Irradiation of both β-furylacrylic (365 nm) and urocanic acids (254 nm) in the presence of Lewis acids results in nearly quantitative $E \rightarrow Z$ photoisomerization, thereby extending the scope of Lewis acid catalyzed photoisomerization to heterocyclic analogues of cinnamic esters.[1-3] While the effect of Lewis acids on the photoisomerization equilibria of these esters is similar, the mechanistic bases differ significantly. Lewis acid complexation of *E*- or *Z*-**2** occurs on the carbonyl oxygen causing a large red-shift in the absorption spectrum, whereas complexation of *E*- or *Z*-**3** occurs on the imidazole N_3 causing a large blue-shift in the absorption spectrum. Selective $E \rightarrow Z$ photoisomerization of **2** results from a combination of three factors: stronger *E* vs. *Z* complexation, stronger absorption of light by the *E* vs. *Z* complex, and a higher quantum yield for $E \rightarrow Z$ vs. $Z \rightarrow E$ photoisomerization. Selective isomerization of **3** is solely a consequence of the photostability of *Z*-**3**. These results serve to illustrate the importance of the site of complexation in determining the spectroscopic properties and photochemical behavior of Lewis acid complexes.

Experimental Section

General Methods. NMR spectra were obtained on a Varian CFT20 or a Varian EM390 spectrometer. Ultraviolet absorption spectra were recorded on a GCA/McPherson EU 700 spectrophotometer, infrared spectra on a Perkin-Elmer 283 spectrophotometer, and mass spectra on a Hewlett-Packard 5986 GC/MS. Irradiated solutions were analyzed on a Hewlett-Packard 5750 or Varian 3700 flame ionization gas chromatograph with a calibrated 6 ft × $^1/_8$ in. column containing 5% SF-96 on Chromosorb G (*E*,*Z*-**2**) or 3% SP 2100 on Supelcoport (*E*,*Z*-**3**).

Irradiations were conducted with a Rayonet reactor with 254-nm lamps or a Hanovia 450 W medium pressure mercury lamp in a water-cooled Pyrex lamp well. Corning glass filters 7-54 and 0-52 were used to isolate 365-nm light, and monochromatic 313-nm irradiation was obtained with use of a potassium chromate filter solution with the Hanovia lamp. Quantum yield and conversion vs. time measurements were carried out on a merry-go-round apparatus with use of potassium ferrioxalate (254 nm), *trans*-stilbene[21] (313 nm), or Amberchrome 540[22] (365 nm) solution actinometers run in triplicate. Solutions of ester in dichloromethane contained in 13 mm o.d. quartz or Pyrex test tubes equipped with serum caps were bubbled with dry N_2. Lewis acid solutions were added by syringe under a N_2 atmosphere in a Kewaunee Scientific Products drybox.

Acid catalyzed isomerization reactions were conducted by refluxing toluene solutions of 3.3×10^{-4} M *E*-**1**-**3** and *p*-toluenesulfonic acid. Solutions were monitored by GC until equilibrium was established.

Materials. Dichloromethane (Aldrich gold label) was distilled from phosphorus pentoxide, refluxed over calcium hydride, and distilled immediately by prior to use. (*E*)-β-Furylacrylic acid (Aldrich) was esterified via the acid chloride and distilled under reduced pressure (92-95 °C, 5 Torr). UV, Table I; ^1H NMR, Table II. (*E*)-Urocanic acid (Aldrich) was esterified by refluxing its hydrochloride salt in methanol. The hydrochloride salt of the ester was liberated as a colorless oil prior to use. UV, Table I; ^1H NMR, Table II.

Methyl (*Z*)-β-Furylacrylate (*Z*-2). Methyl (*E*)-β-furylacrylate (0.91 g, 6.0×10^{-3} mol) and BF_3·OEt_2 (300 mL, 0.36 g, 0.0026 mol) were dissolved in 0.2 L of dichloromethane under a dry nitrogen atmosphere. The solution was transferred to a Pyrex annulus, sealed with a septum, and irradiated for 3 h in a Rayonet reactor with broad-band 350-nm light. The solution was extracted with water, dried over magnesium sulfate, concentrated, and subjected to flash vacuum chromatography on silica gel (200 g) with 1% ethyl acetate/hexane. Pure *Z*-**2** (0.77 g, 85%) was obtained free of *E*-**2**. UV, Table I; ^1H NMR, Table II.

Methyl (*Z*)-Urocanate (*Z*-3). Methyl (*E*)-urocanate (0.3 g, 2.0×10^{-3} mol) and BF_3·OEt_2 (1.0 molar equiv) in 0.1 L of dichloromethane were irradiated under dry N_2 in a Vycor reaction flask with 254-nm light. The solution was extracted with water, dried over magnesium sulfate, concentrated, and subjected to evaporation distillation (110 °C, 1 Torr) to yield *Z*-**3** as a viscous, colorless oil (0.19 g, 63%). UV, Table I; ^1H NMR, Table II.

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Mechanism of OH Radical Reactions with Thymine and Uracil Derivatives†

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Abstract: Yields of reducing, 6-yl, oxidizing, 5-yl, and substituted methyl radicals, generated by OH radical reaction with uracil (U), thymine (T), and 1-methyluracil (1-MeU), were determined by pulse radiolysis in aqueous solutions at pH 7. The absolute yields and ratio of 5-yl/6-yl radicals in uracil (20% 5-yl-U, 80% 6-yl-U) are altered in methyl-substituted derivatives of uracil. For thymine one finds 35% of 5-yl-T and 57% of 6-yl-T and for 1-methyluracil 20% of 5-yl-1-MeU and 65% of 6-yl-1-MeU radicals. The yields of pyrimidine glycols in the presence of an oxidizing agent (G = 3.2 for thymine glycols, 4.3 for uracil glycols, 3.7 for 1-methyluracil glycol), as measured by HPLC, were shown to be equal to the yields of 5-OH-6-yl-Py reducing radicals of thymine (56% OH), uracil (80% OH), and 1-methyluracil (65% OH). It is suggested that 5-OH-6-yl radicals are the exclusive precursors of glycols. Mechanisms of glycol formation in the presence and absence of oxidizing agents are proposed and discussed.

Measurement of thymine glycol (5,6-dihydroxythymine), $T(OH)_2$, in urine has been suggested as a dosimeter of oxidative damage of DNA in humans and animals.[1] The suggestion is based on the rather high yield of $T(OH)_2$ (as thymine glycol and thymidine glycol) in human urine (32 nmol/day) and the 15 times

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higher yield per body-weight unit in rat urine. Because the rat has a higher metabolic rate than the human and because a higher metabolic rate is expected to lead to increased generation of oxyradicals,[2-4] it has been argued[1] that $T(OH)_2$ yield is in fact a measure of the DNA damage rate. Release of thymine glycol and thymidine glycol from damaged DNA already has been shown to take place via DNA repair by $T(OH)_2$ glycosilase[5] and excision repair,[6] respectively. The attractive hypothesis that $T(OH)_2$ reflects damage to DNA offers a nonevasive dosimetry of exposure to various deleterious agents, including ionizing radiations.

Thymine glycol can be generated either by strong oxidants such as OsO_4[7] and permanganate[8] or by OH radicals.[9] These two strong oxidants are not expected to be present in physiological systems. On the other hand, OH radicals have been shown to be generated in aqueous media by ionizing radiations.[10] Since biosystems have a high water content, OH radical reactions with DNA should abound in irradiated organisms, including humans. OH radical also can be generated by Haber–Weiss–Fenton-type reactions from hydrogen peroxide,[11,12] which is a product of autoxidation processes.[2-4,10] Generation of OH radicals by autoxidation and endogenous Haber–Weiss–Fenton processes in living organisms, however, has not been demonstrated unequivocally.[13,14]

Despite numerous kinetic studies[16,17] and product measurements,[18-20] mechanisms of thymine glycol formation from OH radical either in DNA model systems or in DNA[15] are not fully understood.

In this paper some novel aspects of $T(OH)_2$ formation mechanisms are proposed. The mechanisms are based on the reactions of OH radicals with various uracil derivatives (including thymine). These investigations were conducted by pulse radiolysis and the measurements of glycol yields.

Materials and Methods

Chemicals were of the highest purity available, which was sufficient for the experiments, and were used without further purification. Thymine, uracil, and ascorbate were obtained from Sigma (mention of commercial products does not imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the products identified are necessarily the best available for the purpose); 1-methyluracil was from Chemical Dynamics Corp.; *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD) was from Aldrich; and ferricyanide was from Fisher. Pyrimidine glycols were produced by the oxidation of a pyrimidine with OsO_4. Water was purified by a Millipore Milli-Q system, and solutions were freshly prepared before each experiment. The pH was adjusted by 0.2 mM phosphate buffer.

The pulse-radiolysis experiments were conducted on the Febetron 705 pulse-radiolysis setup,[21] which allows single-pulse transient absorption spectra measurements and simultaneous absorbance vs. time readings at a fixed wavelength with the lowest time resolution of 1 μs. A suprasil quartz cell with 2-cm optical path length was used in all experiments. Doses were in the range of 2–50 Gy, as determined by thiocyanate dosimetry, using $G[(SCN)_2^{\cdot-}] = 6.0$ in 0.1 M KSCN and $\epsilon_{480} = 7600\ M^{-1}\ cm^{-1}$.

The pulse-radiolytic measurements of the yields of either oxidizing or reducing pyrimidine radicals were performed in N_2O-saturated aqueous solution of 1 mM pyrimidine and 0.02–0.1 mM of an oxidant or a reductant. Under these conditions, e_{aq}^- is converted to ·OH,[22] which predominantly reacts with a pyrimidine to give pyrimidine radicals. The yields of reduction of *p*-benzoquinone and ferricyanide by pyrimidine radicals were compared with the yield of $CO_2^{\cdot-}$ reaction with these oxidants measured at pH 7 and 0.1 M HCOONa, which was taken as 100%. Similarly, the yield of $Br_2^{\cdot-}$-induced oxidation of TMPD and ascorbate obtained at pH 7 and 0.1 M KBr was utilized for the determination of relative yields of oxidation of these reductants by pyrimidine radicals. The dose used in the determinations of redox properties of pyrimidine radicals was 5 Gy, which is equal to 3.4 μM of total radicals produced (based on[22] $G_{OH} + G_H = 6.6$).

Prior to irradiation, aqueous solutions of pyrimidine derivatives were saturated with N_2O (Matheson). Irradiation of solutions for steady-state investigations was carried out at 20 °C with a ^{60}Co source, Gammacell 220, Atomic Energy of Canada Ltd., with a nominal activity of 20.8 kCi. The dose rate used in all experiments was 50 Gy/min as determined by Fricke dosimetry. The conversion of uracil derivatives was ~25% for the highest dose used in experiments, based on $G_{OH} = 5.6$ in N_2O-saturated aqueous solutions.[22]

High-performance liquid chromatography (HPLC) was used for separation and characterization of stable radiolytic products. Irradiated solutions of uracil derivatives studied were subjected to HPLC analysis immediately (within approximately 5 min) after completion of irradiation to minimize possible chemical alterations of samples. The liquid chromatograph used in HPLC separations was the Hewlett-Packard liquid chromatography, type 1084 B, with automated injection and variable-wavelength UV detector. Up to 30 μL of irradiated solution was injected and separated on a reversed-phase LC-18 DB column (Supelco), 15-cm length and 3-μm particle size, which was presaturated with 0.1% trifluoroacetic acid in water. Millipore water was used as an eluent. Isocratic elution of stable radiolytic products at a flow rate of 0.25 mL/min was monitored at 210 vs. 430 nm. Under these conditions, glycols or uracil derivatives appeared as follows: uracil glycol (cis), 7 min; uracil glycol (trans), 9.7 min; 1-methyluracil glycol (cis), 10.5 min; thymine glycol (cis), 13.75 min; thymine glycol (trans), 15.5 min. The assignment of glycol peaks in HPLC chromatograms was done on the basis of retention times for the glycol standards, and their UV spectra were recorded under stop-flow conditions. Quantitative analysis was performed, assuming similar response of the UV detector for pyrimidine glycols and 5,6-dihydrouracil. The *G* values of pyrimidine glycols were determined from six-point yield–dose plots.

Results and Discussion

Formation of Uracylyl Transients. In principle, OH radical reacts with uracil derivatives by two basic mechanisms at neutral pH. The more favored reaction is addition of OH to the double bond.[9] The less favored reaction is abstraction of H from the methyl group.[10] Consequently, when OH reacts with uracil derivatives, the transient free radicals that would be expected are shown in eq 1a–c. As shown, in the OH radical reaction with methylpyrimidines, e.g. thymine and 1-methyluracil, in addition to two types of OH adducts (radicals A and B), two types of methyl radicals may be formed as a consequence of the abstraction of H atom from the *C*-methyl and *N*-methyl groups.

Although the kinetics of reactions 1a–c and the absorption spectra of the transients have been investigated previously by pulse radiolysis,[16,17] the absorption bands of each particular transient (A–D) could not be resolved. The transients were found to absorb in the 300–500-nm region.[16,17] These absorption spectra might be ascribed to radicals type A and B, because radicals C and D are allylic radicals whose absorption maxima should be around 270 nm with high ε values.[23] However, these spectral properties of uracylyl transients do not provide a meaningful basis for direct determination of their yields by pulse radiolysis. Differentiation and quantitation of these radicals, therefore, had to be approached from the measurements of their redox properties and product analysis.

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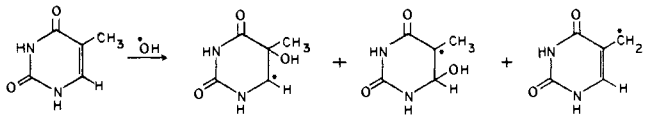
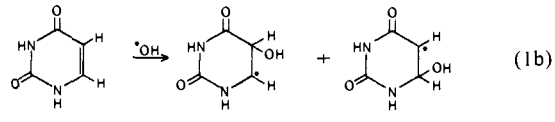
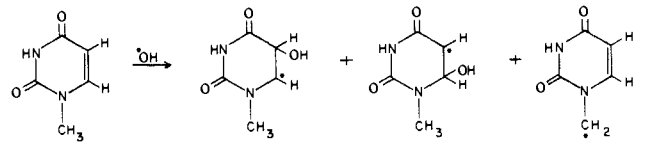


(1a)

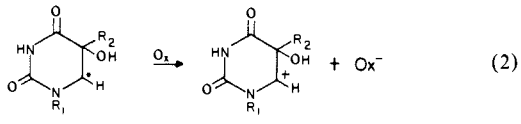


(1b)



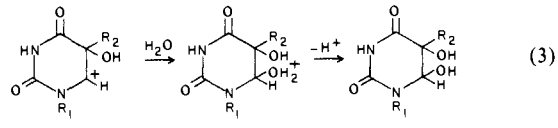
(1c)

Redox Properties of Pyrimidine Radicals. Radical A is an α amino radical and as such is a good reductant. Oxidizability of this radical was demonstrated initially by using *p*-benzoquinone as an oxidant.[24] The yields of reducing pyrimidine radicals obtained in this study using *p*-benzoquinone as an oxidant (Table I) are in good agreement with the values previously reported when tetranitromethane was used as an oxidant.[25] Hence, in general



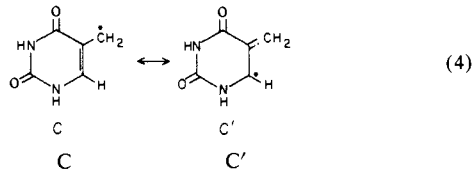
(2)

Reaction 2 is expected to be followed by rapid solvolysis and formation of a glycol,[15] as seen in eq 3.



(3)

The measured yields of radicals with reducing properties (Table I) may not pertain to transient A only. The 1-methyluracil radical D should also be a reductant since it is an α amino radical. In addition, thymine radical C may also act as reducing radical, as indicated by the resonant forms in eq 4. However, radical C



(4)

should behave as weaker reductant due to resonance stabilization. An attempt to discriminate between radicals A and C in the case of thymine transients and A and D in the case of 1-methyluracil transients was made using ferricyanide as an oxidant. It may be seen from Table I that the yields of reduction of ferricyanide and formation of semiquinone are different from thymine and 1-methyluracil radicals, whereas the yield of uracil-reducing radicals remains the same, irrespective of the oxidant used in its determination. Consequently, it might be suggested that weaker reductants, radicals C and D, do not reduce ferricyanide, whereas a stronger reductant, radical A, does. These conclusions are also

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Table I. Oxidation Yield of Pyrimidine Radicals Produced by OH Radical Reaction with Some Uracil Derivatives by Various Oxidants at 20 °C, pH 7, As Determined by Pulse Radiolysis

deriv	oxidant	k,[a] M^{-1} s^{-1}	yield, % [OH]
thymine	$Fe(CN)_6^{3-}$	1.7×10^9	56[a]
	p-benzoquinone	1.8×10^9	65[b]
	tetranitromethane	1.5×10^9[c]	61[c]
uracil	$Fe(CN)_6^{3-}$	2.6×10^9	75
	p-benzoquinone	1.9×10^9	80
	tetranitromethane	1.9×10^9[c]	83[c]
1-methyluracil	$Fe(CN)_6^{3-}$	3.1×10^9	64
	p-benzoquinone	1.9×10^9	80

[a] Estimated to be accurate to ±10%. [b] The yields of semiquinone production are estimated to be accurate to ±5%. [c] From ref 25.

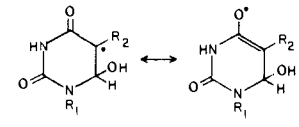
Table II. Reduction of Pyrimidine Radicals Produced by OH Radical Reaction with Some Uracil Derivatives at 20 °C, pH 7.0, As Determined by Pulse Radiolysis

deriv	reductant	k,[a] M^{-1} s^{-1}	yield,[b] % [OH]
thymine	TMPD	6×10^8	35
	ascorbate	2×10^7	35
uracil	TMPD	1×10^9	20
	ascorbate	2×10^7	20
1-methyluracil	TMPD	1×10^9	20
	ascorbate	1.9×10^7	20

[a] Estimated to be accurate to ±10%. [b] Estimated to be accurate to ±5%.

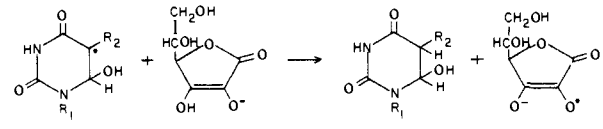
in fair agreement with the expected yields based on k(OH + C-CH_3) $\sim 6 \times 10^8$ and k(OH + N-CH_3) $\sim 1.6 \times 10^9$ M^{-1} s^{-1}.[26] From these values, taking k(OH + Py) $= 6 \times 10^9$ M^{-1} s^{-1}, one can calculate the concentration of 5′-methylthymine (C) and 1′-methyluracil (D) radicals as 10% and 25% of total OH radicals, respectively.

Radical B is an oxidizing radical since its mesomeric form is an oxyradical (eq 5). Reducing agents such as ascorbate and



(5)

TMPD, two well-known electron donors, can be utilized to measure the yield of an oxidizing radical. Reduction of radical B with ascorbate is shown in reaction 6. Pulse-radiolytic measurements



(6)

indicate that radical B is formed in uracil with a 20% yield and in thymine with a 35% yield (see Table II), in agreement with the previously determined value with TMPD.[25] A 20% yield of radical B was also determined for 1-methyluracil.

Reactions of Pyrimidine Radicals with One Another. Pyrimidine radicals generated by the reactions of OH radical with the pyrimidine derivaties studied, reactions 1a–c, react with one another to give various stable radiolytic products. The decay rate constants are close to the diffusion-controlled value, $2k_{app} \sim 10^9$ M^{-1} s^{-1}. The derived experimental values are based on the measured apparent ϵ values and kinetic data. The actual $2k$(decay) values might be somewhat higher (by a factor not larger than ~2) if the true ϵ value could be derived.

Reactions of each particular radical from thymine were not discriminated previously,[15] and the radicals were assumed to

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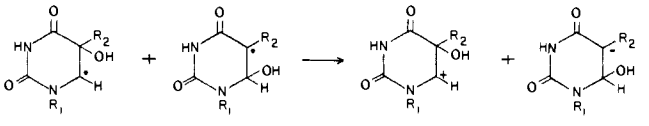
Table III. Yield of Pyrimidine Glycols Measured by HPLC Analysis of Aqueous Solutions of Pyrimidine Derivatives and Solutes Irradiated under Steady-State Conditions

pyrimidine deriv	additn solute, S	redox prop of S	G[Py-(OH)$_2$][a]	yield,[b] % [OH]
thymine	none		0.6	11
	$Fe(CN)_6^{3-}$	oxidant	3.2	57
	p-benzoquinone	oxidant	3.2	57
	ascorbate	reductant	0.3	5
uracil	none		0.33	6
	$Fe(CN)_6^{3-}$	oxidant	4.3	77
1-methyluracil	none		0.32	6
	$Fe(CN)_6^{3-}$	oxidant	3.7	66

[a] Estimated to be accurate to ±10%. [b] Based on $G_{OH} = 5.6$.

disproportionate or to dimerize. Since redox properties of pyrimidine radicals are different, as indicated above, we propose that each particular type of radical may follow a different reaction pattern. In order to establish the mechanism of OH radical induced decomposition of pyrimidines, the identification and quantitative analysis of stable radiolytic products were undertaken by the HPLC technique. The results are summarized in Table III.

In pyrimidine systems where A- and B-type radicals are generated in the absence of any redox solutes, radical A may be oxidized by radical B to a carbocation[15] (eq 7). Reaction 7 is



(7)

followed by rapid solvolysis of the resulting carbocation and the formation of a glycol, as indicated by reaction 3. A similar reaction to reaction 7 was observed in the presence of various oxidizing radicals.[27] The yield of glycol is rather low under these conditions, $G = 0.3$–0.6 (Table III). Since the yield of reducing radical A is considerably higher than the measured yield of glycols and also is higher than that of oxidizing radical B, it may be suggested that radical A does not disproportionate to give carbocation and pyrimidine glycol. Nor should the yield of pyrimidine glycol be equal to that of radical B, since some of the B radicals are "wasted" in the B + B reaction.

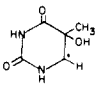
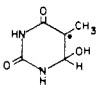
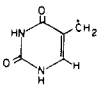
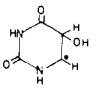
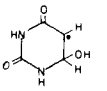
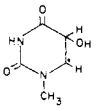
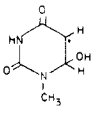
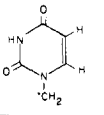
The reaction of reducing pyrimidine radical A with an oxidant (reaction 2) should result in the formation of a glycol in a yield equal to that of radical A, providing that the concentration of oxidant is higher than the radical concentration and the rate of conversion is sufficiently high. It may be seen from Table I that both of these conditions are met for reactions that involve reducing pyrimidine radicals and the oxidants investigated. Complete agreement between the yields of radical A, obtained by pulse radiolysis and the measurements of pyrimidine glycols, as summarized in Table IV, supports our hypothesis that glycols are formed exclusively by oxidation of reducing pyrimidine radical A.

Oxidizing pyrimidine radical B may react not only with radical A (reaction 7) but also with reducing radicals C or D to produce 6-hydroxy-5*H*-pyrimidine. Similarly, the yield of radical B should match the yield of 6-hydroxy-5*H*-pyrimidine in the presence of a reducing agent, whereas the production of glycols via reaction 7 would be diminished. It may be seen from Table III that the yield of thymine glycol decreases by 50% in the presence of ascorbate.

Pyrimidine radicals also dimerize, as previously shown for thymine,[28] cytosine,[29] and uracil.[30] However, it appears from

(27) Dizdaroglu, M., Simic, M. G., in preparation.
(28) Dizdaroglu, M.; Simic, M. G. *Int. J. Radiat. Biol.* **1984**, 46, 241–246.
(29) Dizdaroglu, M.; Simic, M. G. *Radiat. Res.* **1984**, *100*, 41–46.
(30) Idriss Ali, K. M. *J. Radiat. Res.* **1979**, *20*, 84–94.

Table IV. Redox Properties of Pyrimidine Radicals at pH 7

deriv	radical	struct formula	designation	yield, % [OH] P.R.[a]	yield, % [OH] P.A.[b]
thymine	A		reducing	56	57
	B		oxidizing	35	
	C		weakly reducing	9	
uracil	A		reducing	80	77
	B		oxidizing	20	
1-methyl-	A		reducing	64	66
uracil	B		oxidizing	20	
	D		weakly reducing	16	

[a] As determined by pulse radiolysis. [b] From product analysis.

the measured yields of glycols that only reactions of two identical radicals may produce dimers. Hypothesizing that in the case of uracil radicals only reactions 8–10 take place and that reaction

$$A + B \rightarrow U(OH)_2 + 6\text{-OH-}(5H)\text{-U} \quad (8)$$

$$B + B \rightarrow \text{dimer B} \quad (9)$$

$$C + C \rightarrow \text{dimer C} \quad (10)$$

rate constants are similar, which is most likely, the yield of uracil glycols is expected to be equal to $^1/_3$ of the radical B yield. The measured yield of uracil glycols, $G[U(OH)_2] = 0.33$, compares well with the calculated yield of $^1/_3G(B) = {}^1/_3(1.12) = 0.37$.

Similarly, in the case of thymine and 1-methyluracil radicals (eq 11–15), the yield of pyrimidine glycols may be calculated as

$$A + B \rightarrow \text{glycols} + 6\text{-OH-}(6H)\text{-Py} \quad (11)$$

$$A + A \rightarrow \text{dimer A} \quad (12)$$

$$B + B \rightarrow \text{dimer B} \quad (13)$$

$$B + C \text{ or } D \rightarrow 6\text{-OH-}(6H)\text{-Py} + P_{CorD} \quad (14)$$

$$C \text{ or } D + C \text{ or } D \rightarrow \text{dimer C or D} \quad (15)$$

G(pyrimidine glycols = $^1/_3[G(B) - {}^1/_3G(C \text{ or } D)]$. The calculated G values for thymine glycol, $G[T(OH)_2] = 0.6$, and for 1-methyluracil glycols, $G[1\text{-MeU}(OH)_2] = 0.29$, correlate well with the measured values of 0.6 and 0.32, respectively.

Conclusions

(1) The addition of OH radical to the 5,6 double bond of pyrimidines gives a reducing radical (5-OH-6-yl-Py) and an oxidizing radical (6-OH-5-yl-Py), as previously indicated. (2) The yield of 5-OH-6-yl-Py radical in uracil (5-OH-6-yl-U) is 80% of the total OH yield. (3) The 5-OH-6-yl-Py/6-OH-5-yl-Py ratio

is affected by substitution: uracil, 4; thymine, 1.6; 1-methyluracil, 3.2. Substitution at the C5 position has a more pronounced effect than at the N1 position. (4) The oxidation of the 5-OH-6-yl-Py radical leads to the formation of pyrimidine glycols, 5,6-Py$(OH)_2$. (5) The yield of pyrimidine glycol in the presence of oxidizing agents at pH 7, as measured by HPLC, and the yield of 5-OH-6-yl-Py radical, as measured by pulse radiolysis, are identical for the pyrimidines studied. (6) In the absence of oxidizing agents, the much lower yields of 5,6-Py$(OH)_2$ (Table III) indicate oxidation of reducing radical (6-yl) by the oxidizing radical (5-yl) only and the absence of disproportionation reaction between identical radicals. (7) On the basis of presented evidence it is concluded that pyrimidine glycols are formed exclusively on oxidation of 5-OH-6-yl-Py radicals.

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Registry No. T, 65-71-4; T (radical A), 23402-95-1; T (radical B), 23402-94-0; T (radical C), 80857-92-7; U, 66-22-8; U (radical A), 23402-99-5; U (radical B), 14836-21-6; 1-MeU, 615-77-0; 1-MeU (radical A), 103478-73-5; 1-MeU (radical B), 103478-74-6; 1-MeU (radical D), 50656-54-7; *cis*-T$(OH)_2$, 1124-84-1; *trans*-T$(OH)_2$, 1431-06-7; *cis*-U$(OH)_2$, 3683-34-9; *trans*-U$(OH)_2$, 3952-56-5; *cis*-1-MeU-$(OH)_2$, 64629-87-4; $OH^{\bullet}$, 3352-57-6.

Possible Model Reaction for Some Amine Oxidases. Kinetics and Mechanism of the Copper(II)-Catalyzed Autoxidation of Some Diaminouracils[1a,b]

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Abstract: It was found that the autoxidation of various 6-N-substituted 5,6-diaminouracils is strongly catalyzed by Cu(II). Since this reaction is believed to be similar to one of the steps in the reaction catalyzed by the copper-containing amine oxidases, it has been studied in some detail. Evidence is presented that the catalyzed reaction involves the reduction of O_2 to H_2O_2 and the oxidation of the diaminouracil by two electrons to 5,6-diiminouracil or its tautomer. However, this initial product is unstable and hydrolyzes eventually to alloxan and NH_3 (an amine is also formed when a 6-N-substituted derivative is autoxidized). Under all conditions examined, the reaction is first order in O_2. With diaminouracil in excess the reaction is first order in Cu(II), and with Cu(II) in excess it is first order in diaminouracil. However, at higher concentrations the rate approaches zero order in each reactant at a constant concentration of the other. Among other observations are the following: the reaction proceeds more rapidly in H_2O than in D_2O by a factor of 2.2–4.7, superoxide dismutase has no effect on the rate, and Cu(II) does not oxidize the diaminouracil in the absence of O_2. The evidence is most consistent with a mechanism involving a ternary complex of the diaminouracil—Cu(II) and O_2 reacting by an ionic two-electron mechanism to give products. In such a mechanism the Cu(II) is believed to have two important functions: by binding to both reactants it provides a pathway for transferring electrons from one to the other, and by having an unpaired electron it allows the two-electron reduction of O_2 to occur in one step.

The Cu(II)-containing amine oxidases[2a] catalyze the reaction shown in eq 1, where the reactant can be a mono-, di-, or polyamine depending on the specificity of the individual enzymes.

$$RCH_2NH_2 + O_2 + H_2O \rightarrow RCHO + H_2O_2 + NH_3 \quad (1)$$

These enzymes as a class are of considerable biological interest because they are involved in several important metabolic pathways in animals. As examples, some are responsible for the degradation of the biogenic amines (histamine, tyramine, norepinephrine, putrescine, spermine, etc.) while others participate in the biosynthesis of collagen.

In addition to the metal ion, these enzymes possess a covalently bound organic prosthetic group that contains a reactive carbonyl. Although it was originally thought that this group is a pyridoxal derivative, that now seems unlikely. Recently, G.A.H. proposed[2a] that the reactive carbonyl compound is an oxidized aminohydroxyuracil derivative (**1**), formed by hydrolytic cleavage of a flavin covalently bound to the enzyme.[2b] Furthermore, an overall mechanism for the reaction was proposed, and this is illustrated in eq 2.

More recently, Duine and his co-workers[3] have reported evidence that the cofactor is pyrroloquinoline quinone (**6**), presumably covalently bound to the enzyme through one of its carboxyl groups. Because **6** is expected to have reactivity characteristics similar to those of **1**, a mechanism related to that in eq 2, but which involves **6** as the reactant, can be written,[4] and that is illustrated in eq 3. In either case **1** or **6** may not be a true catalytic cycle intermediate because a simple trans-Schiffization of **5** or **10** would lead directly to **2** or **7**.

(1) (a) This work was supported by a research grant (AM 13448) from the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, Public Health Science. (b) Taken from: Al-Arab, M. M. Ph.D. Thesis, The Pennsylvania State University, 1983. (c) Present address: Department of Chemistry, Yarmouk University, Irbid, Jordan.

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