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Spectra and Structure of the 2'-Deoxyuridin-1'-yl Radical

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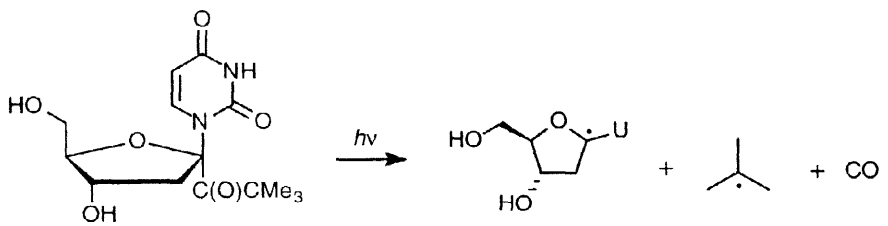
Abstract: The title C-1' radical, obtained by photolysis of the corresponding *tert*-butyl ketone in water, was studied spectroscopically by EPR and laser flash photolysis methods and computationally.

A number of agents react with DNA or RNA to generate macromolecular radical species[1] in processes that are of considerable importance since they can ultimately lead to base modification or strand scissions. As research progresses in the area of the mechanism of attack of oxidative cleavers of DNA and RNA, it is increasingly evident that hydrogen abstraction from the C-1' position of the sugars is involved in many cases.[2-6] To our knowledge, there are only limited spectroscopic studies of C-1' radicals in the literature,[7] and theoretical work involving C-1' radicals is mainly limited to models with an amino group substituted for the base.[8] Evidence for the existence of C-1' radicals is found in product studies.[9-11] In particular, under anoxic conditions, C-1' radicals in model nucleosides abstract hydrogen from thiols leading to a mixture of α- and β-anomers,[9] and, under aerobic conditions, C-1' radicals afford 2-deoxyribonolactone[9] through a number of currently disputed pathways.[1,2] Furthermore, based on the β-(acyloxy)alkyl radical rearrangement of a C-2' radical, it was suggested that C-1' radicals are stabilized substantially by the presence of the base and that the degree of stabilization is similar for purine and pyrimidine moieties.[11] We report herein EPR and UV spectra of a selectively generated sugar-centered radical in a nucleoside, specifically the 2'-deoxyuridin-1'-yl radical (**2**), and computational studies of this species.



1 **2**

The radical precursor **1** was previously reported by Goodman and Greenberg.[9] A slight modification of their procedure was employed here to give **1** with spectroscopic and analytical data identical to those reported.[9] Precursor **1** also was available from a new synthetic route, the details of which will be reported elsewhere. Photolysis of **1** ultimately gives radical **2**, the *tert*-butyl radical, and carbon monoxide.[9]

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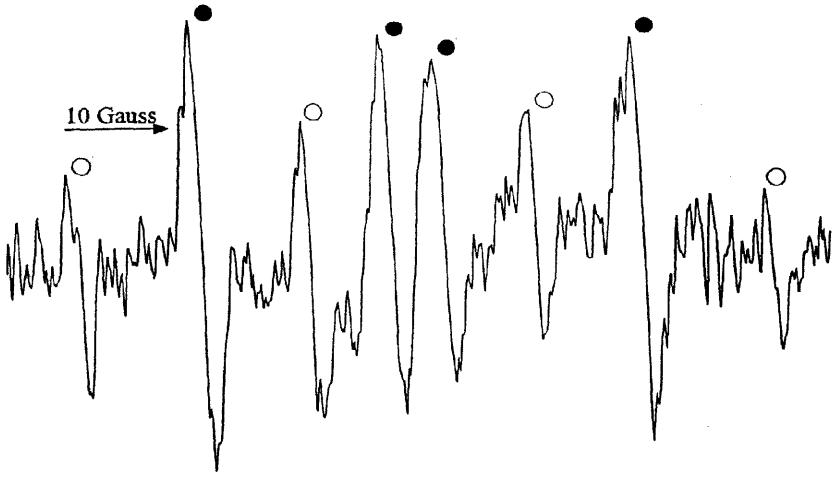
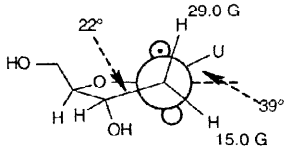


Figure 1. EPR spectrum obtained by photolyzing a 0.15 M solution of **1** in water at room temperature. The spectrum was recorded in a flat quartz cell using the following parameters: microwave power 2.0 mW, modulation amplitude 2.0 G, time constants 163 ms, scan time 163 s. The central four lines of the *tert*-butyl radical are indicated by open circles while signals from radical **2** are labeled with filled circles.

Photolysis of **1** in aqueous solution in a flat quartz cell gave the EPR spectrum shown in Figure 1. Due to the production of CO during the photolysis and the resulting change in the cavity Q, only single scans could be recorded. The signal-to-noise ratio was improved by summing spectra of different samples. The spectrum in Figure 1 is due to the superimposition of signals from two radicals, one identified as the *tert*-butyl radical (a_H = 22.72 G, g = 2.0026).[12] The other radical (g = 2.00296) has couplings of the unpaired electron with two non-equivalent hydrogens (a_H = 19.24 G, a_H = 24.44 G) and a linewidth of 2.8 G, larger than that of the *tert*-butyl radical signal, indicating unresolved hyperfine structure. We assign structure **2** to the new radical. The observed couplings are similar to those assigned to C-2' hydrogen couplings of C-1' radicals from ENDOR spectra of mutiple radicals produced by X-irradiation of crystals at low temperatures.[7]



3

Theoretical calculations were performed to determine the structure of **2** and to assign the observed EPR hyperfine couplings. The preferred conformation determined with *ab initio* calculations at the UHF/6-31G* level is given in structure **3**. Hyperfine couplings (hfs) were computed for the optimized structure with the density functional method at the B3LYP/6-311** level which was found to give reliable results.[13] The calculations indicate couplings of 15.0 and 29.0 G for the C-2' β-hydrogens as shown in **3**, and the agreement of the average experimental hfs (21.84 G) and computed hfs (22.0 G) is excellent. Previous *ab initio* calculations of C-1' radical models gave slightly larger average hfs.[8] The differences between the hfs values observed Figure 1 and those computed probably are due to the fact that vibrational effects are not taken into account in the calculations. For example, the prototype tetrahydrofuranyl radical[14,15] has β-hydrogen couplings in polycrystalline solution at 113 K[14] of 18.5 and 37.0 G, but the hfs are equivalent (28.2 G) at temperatures greater than 148 K[15] due to the averaging over the vibrational states.

Interestingly, the calculations indicate that the coupling constant of the α-nitrogen is small (1.5 G) because a negative spin density contribution due to spin polarization is counterbalanced by the positive contribution arising from the slight pyramidalization at the radical center. Coupling constants of the γ-hydrogens are also predicted to be small (-0.4 G at H-3' and 0.2 G at H-4') because the hydrogens adopt equatorial positions.[16] These small couplings might contribute to the large experimental linewidths observed in the EPR spectrum of **2**.

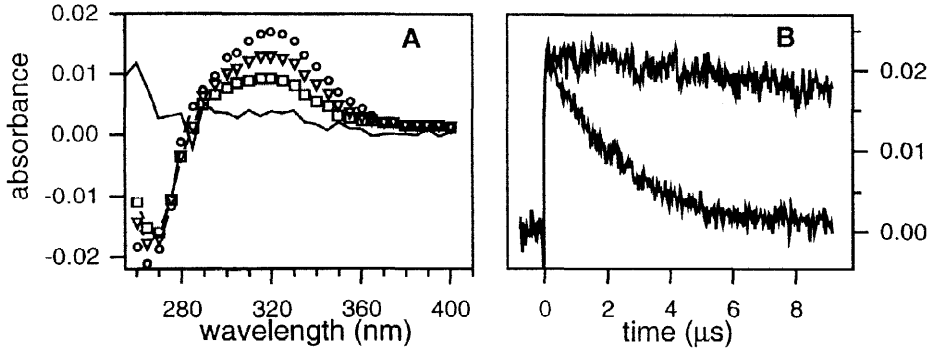


Figure 2. LFP results (Nd-YAG, 266 nm, ca. 40 mJ, r.t., flowing sample with 0.4 absorbance at 266 nm). (A) Time-resolved spectra from irradiation of **1** in non-sparged water solution. The spectra are at 0.1 (circles), 0.9 (triangles), and 1.9 (squares) μs after irradiation; the spectrum observed 0.9 μs following irradiation of di-*tert*-butyl ketone in He-sparged water--acetonitrile is shown as a solid line. (B) Observed signals at 320 nm from irradiation of **1** in He-sparged (top) and non-sparged (bottom) water solutions.

Laser flash photolysis (LFP) of **1** in water gave spectra such as those in Figure 2A. The spectra are superimpositions of those of all products, but we ascribe the signal with λ_{max} at 320 nm mainly to radical **2**. The *tert*-butyl radical has a weak absorbance centered at 315 nm and absorbs more strongly at 260 nm and shorter wavelengths,[17] and photolysis of di-*tert*-butyl ketone in He-sparged water/acetonitrile gave the spectrum shown as a solid line in Figure 2A. The triplet excited state of uracil in water, produced by 266 nm irradiation of a concentrated solution, displays a weak signal with λ_{max} at about 360 nm,[18] but we observed no signals (<0.002 AU) in the range 300-400 nm following 266 nm irradiation of a water solution of uracil with 0.4 absorbance at 266 nm. The fact that the bleached region of the spectrum (λ_{max} = 264 nm for **1**) obtained in the presence of O_2 does not return to baseline as the signal in the region about 320 nm decays further demonstrates that a triplet state of **1** was not observed. Taken in total, the results indicate that the major features observed upon photolysis of **1** are due to radical **2**.

The kinetic traces in Figure 2B show signal intensities at 320 nm for both He-sparged and non-sparged solutions. The rate of decay in He-sparged solution was 1-2 × 10^5 s^{-1} while that in the non-sparged solution was 4-5 × 10^5 s^{-1}. The oxygen concentration in water[19] is ca. 3 × 10^{-4} M which gives a second order rate constant for reaction of **2** with oxygen of 1 × 10^9 M^{-1} s^{-1}, similar to that observed with a variety of α-heteroatom substituted radicals in water.[20] The persistence of the signal in Figure 2B in the absence of oxygen suggests that LFP kinetic studies of **2** can be accomplished readily.

The EPR and UV spectra of the 2'-deoxyuridin-1'-yl radical obtained in this work confirm the identity of this species, previously implicated from product studies.[9] Other DNA and RNA C-1' radicals should have similar spectra, and LFP kinetic studies of the reactions of these species are expected to provide rate constants that permit a better mechanistic understanding of this class of radicals. Further work on the structural and chemical properties of C-1' radicals is in progress.

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