

The UV Absorption Spectra of the C(5) and C(6) OH Adduct Radicals of Uracil and Thymine Derivatives. A Pulse Radiolysis Study

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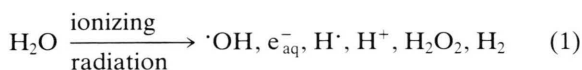
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Dedicated to Professor Dr. Tino Gäumann on the Occasion of His 60th Birthday

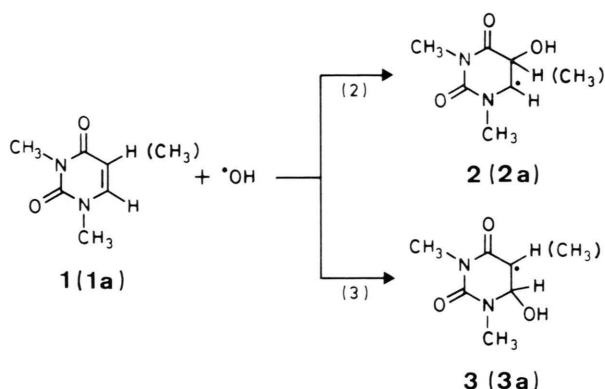
Hydroxyl Radical, Pyrimidine, UV Spectra, Pulse Radiolysis

Using pulse radiolysis the UV spectra of the C(6) OH adduct radicals of 1,3-dimethyluracil and 1,3-dimethylthymine were obtained by reacting the formate radical with the corresponding 5-bromo-5,6-dihydro-6-hydroxy derivatives. In the reaction of the pyrimidines with OH radicals a mixture of C(5) and C(6) OH adduct radicals is formed. From the known ratios in which these two radicals are formed and the spectra of the C(6) OH adduct radicals, the spectra of the C(5) OH adducts were calculated.

Ionising radiation can be harmful to living cells causing mutations and reproductive cell death. It is thought that radiation-induced DNA damage is responsible for these effects. Cells contain about 70% water and as much as 69% of the DNA damage has been estimated [1] to be due to water radicals formed in reaction (1).



Of these radicals it is primarily the OH radicals which produce these deleterious effects, the solvated electrons appearing to contribute very little to DNA inactivation [2]. The OH radicals mainly add to the nucleobases. Pulse radiolysis has provided the spectra of the OH adduct radicals of uracil and some of its methyl-substituted derivatives [3]. In these systems the OH radicals react by adding to the pyrimidine 5, 6 double bond (*cf.* reactions (2) and (3)).



Fujita and Steenken [4] have shown that OH adducts with the radical site at C(6), such as **2 (2a)**, are reducing and their yield can be quantitatively determined by measuring the amount of nitroform anion (NF^-) produced on their reaction with tetranitromethane (TNM). The OH adducts with the uracil site at C(5) (*e.g.* **3, 3a**) have oxidizing properties and their yield can be determined using N,N,N',N'-tetramethylphenylenediamine as the probe.

Using these methods it has been shown that in uracil [4] and in 1,3-dimethyluracil [5] the OH radicals add mainly ($\geq 80\%$) at C(5) (*cf.* reaction (2)). In thymine on the other hand [4] OH addition at this position is only 61% and substantial addition occurs at C(6) (28%). H-abstraction at methyl is of minor importance ($\leq 10\%$). Thus if the UV/VIS spectra of the OH adducts of uracil and 1,3-dimethyluracil reflect the relative amounts of their two component radicals, both should be dominated by the contribution of the C(5) OH adduct. However, in thymine and its 1,3-dimethyl derivative both OH adduct radicals would make significant contributions to the observed spectrum. In our investigations on the H-adducts of uracil derivatives [6, 7] we noticed that there is a considerable difference between the C(5) and the C(6) H adduct spectra and we wondered whether this might also be the case for the OH adducts.

We now report an unambiguous way of obtaining the spectra of the C(6) OH adducts of 1,3-dimethyluracil **1** and 1,3-dimethylthymine **1a**. Then by subtracting the appropriate contributions of these spectra from the spectra of the mixture (produced by OH radical addition), the spectra of the corresponding C(5) OH adducts have been determined.

As shown by reaction (4), 5-bromo-5,6-dihydro-1,3-dimethyl-6-hydroxyuracil **4** or the corresponding

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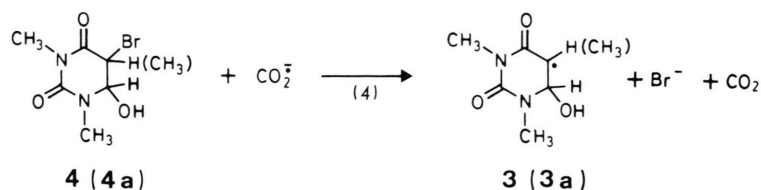


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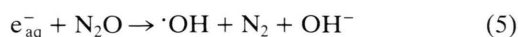
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thymine derivative **4a** can be readily reduced by the formate radical.

The formate radicals are generated in an N_2O -saturated solution containing sodium formate (reactions (5) and (6)).



Under the experimental conditions used (bromo compound **4**, **4a** $10^{-3} \text{ mol dm}^{-3}$, N_2O $2.2 \times 10^{-2} \text{ mol dm}^{-3}$, formate 0.2 mol dm^{-3} , pH 5.2) all primary radicals including the H atoms from reaction (1) are converted into formate radicals. Their radiation chemical yield, $G(\text{CO}_2^-) = 6.8$, has been determined by converting the CO_2^- radicals into NF^- with TNM (NF^- : $\epsilon(350 \text{ nm}) = 13.250 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$). The CO_2^- radicals react rapidly ($t_{1/2} < 0.4 \mu\text{s}$) with the bromo compounds **4**, **4a** and within $< 3 \mu\text{s}$ the spectra of

the C(6) OH adducts can be measured. These are shown in Figs. 1 and 2. In Figs. 3 and 4 the spectra of the mixture of the C(5) and C(6) OH adducts of 1,3-dimethyluracil and 1,3-dimethylthymine are given. These were obtained by irradiating N_2O -saturated solutions of 1,3-dimethyluracil and 1,3-dimethylthymine, respectively. Extinction coefficients were calculated using a G value of 5.5 for OH radicals; the spectra have been corrected for the presence of the H-adducts (spectra already known [7]) assuming a G value of 0.6 for H atoms. The contribution of the C(6) OH adducts to these spectra is about 20% in the case of 1,3-dimethyluracil (*cf.* ref. [5], after a small correction has been made to allow for the presence of reducing H-adducts (*cf.* ref. [8])). Using the technique mentioned above [4] we found that 61% of OH radical addition to 1,3-dimethylthymine occurs at C(5).

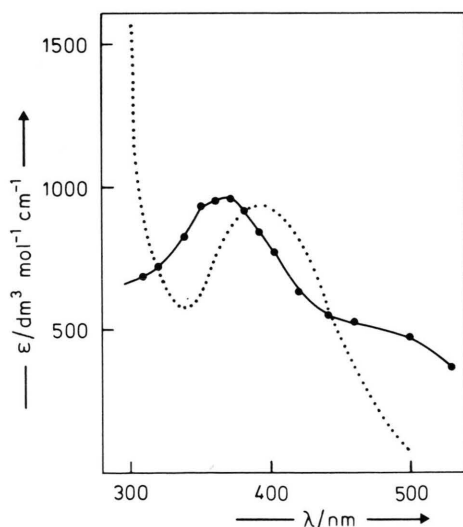


Fig. 1. Absorption spectrum of the C(6) OH adduct radical of 1,3-dimethyluracil obtained by reacting CO_2^- with 5-bromo-5,6-dihydro-1,3-dimethyl-6-hydroxyuracil (for experimental conditions see text). The dotted line is the calculated spectrum of the corresponding C(5) OH adduct radical.

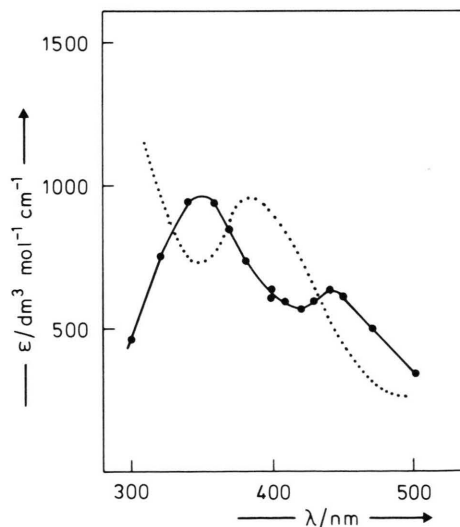


Fig. 2. Absorption spectrum of the C(6) OH adduct radical of 1,3-dimethylthymine obtained by reacting CO_2^- with 5-bromo-5,6-dihydro-1,3-dimethyl-6-hydroxythymine (for experimental conditions see text). The dotted line is the calculated spectrum of the corresponding C(5) OH adduct radical.

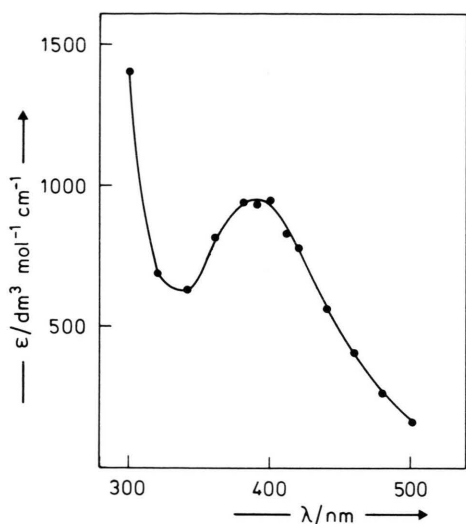


Fig. 3. Absorption spectrum of the mixture of the OH adduct radicals formed by the reaction of OH radicals with 1,3-dimethyluracil. (For experimental conditions see text.)

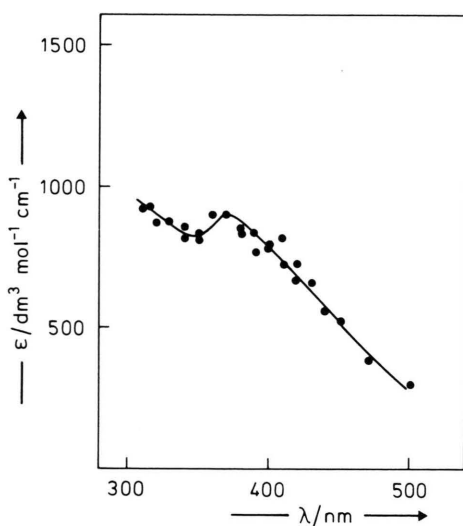


Fig. 4. Absorption spectrum of the mixture of the OH adduct radicals formed by the reaction of OH radicals with 1,3-dimethylthymine. (For experimental conditions see text.)

The pure C(5) OH adduct spectra have been obtained by subtracting the contribution of the C(6) OH adduct spectra, measured using the bromo compounds, from the mixed spectra. In the 1,3-dimethylthymine case no account has been taken of the small contribution of the radical formed by H-abstraction

from the methyl group. Some preliminary data indicate that such radicals have an absorption maximum at around 500 nm. A small additional correction in this wavelength region would even improve the shape of the calculated C(5) OH adduct spectrum.

The calculated C(5) OH adduct spectra thus obtained are shown as dotted lines in Figs. 1 and 2. As can be seen, there are marked differences between the C(5) and C(6) OH adduct spectra. However, the C(6) OH adduct spectra of 1,3-dimethyluracil and 1,3-dimethylthymine are similar as indeed are the corresponding C(5) OH adduct spectra.

Experimental

The brominated compounds **4**, **4a** were prepared by adding bromine water to a solution of 1,3-dimethyluracil or 1,3-dimethylthymine [9]. Any small excess of bromine was purged out with argon. A parallel preparation in D₂O enabled an NMR spectrum to be measured. From this spectrum it was clear that the expected product was produced quantitatively. The NMR spectrum of isolated material (obtained by extraction with chloroform) run in CDCl₃ confirmed the presence of the OH group at C(6) (e.g. 5-bromo-5,6-dihydro-1,3-dimethyl-6-hydroxythymine $\delta(\text{CDCl}_3) = 1.97$ (s, 5-Me), 3.15 and 3.19 (2s, 1-Me and 3-Me), 3.66 (d, 7 Hz, OH), 4.87 (d, 7 Hz, 6-H)).

For pulse radiolysis the brominated products **4**, **4a** were not isolated, because of their possible decomposition during the attempt to crystallize the material [9]. Therefore, a concentrated aqueous solution of sodium formate (pH 5.2) was added to the freshly prepared solution of the bromo compound to give final concentrations of 0.2 mol dm⁻³ formate and 1×10^{-3} mol dm⁻³ bromo compound. Under these conditions the bromo compounds remained stable for > 8 hours at room temperature. The solutions were saturated with N₂O by gassing for 40 minutes, the pulse radiolysis setup has been described elsewhere [10]. Transient absorbance were obtained using 2.2–2.6 Gy pulses of 0.4 μ s duration, 30 shots were taken at each wavelength and computer averaged. The traces exhibited no significant decay in the time range 3–15 μ s following the pulse and the absorbance at 6 μ s has been used to plot the spectra.

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