Fate of the C-1′ peroxyl radical in the 2′-deoxyuridine system

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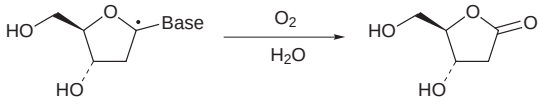
The mechanism of 2-deoxyribonolactone formation from the reaction of photogenerated 2′-deoxyuridin-1′-yl radical with molecular oxygen in water has been investigated.

A number of agents are able to react with DNA to generate macromolecular radical species.[1] These processes are of considerable importance since they can lead to base modification or strand scissions. As research progresses in the area of the mechanism of attack of oxidative DNA cleavers, it becomes evident that hydrogen abstraction from the C-1′ position is involved in many cases.[2,3] Evidence for the existence of these radical species is mainly based on product studies,[4,5] although spectroscopic and theoretical studies have been reported.[6,7] Furthermore, based on the β-(acyloxy)alkyl radical rearrangement of a C-2′ radical, we have 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.[8] Under aerobic conditions, however, C-1′ radicals **1** afford 2-deoxyribonolactone **2** [eqn. (1)] through a number of

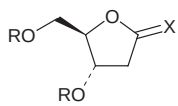


1 **2** (1)

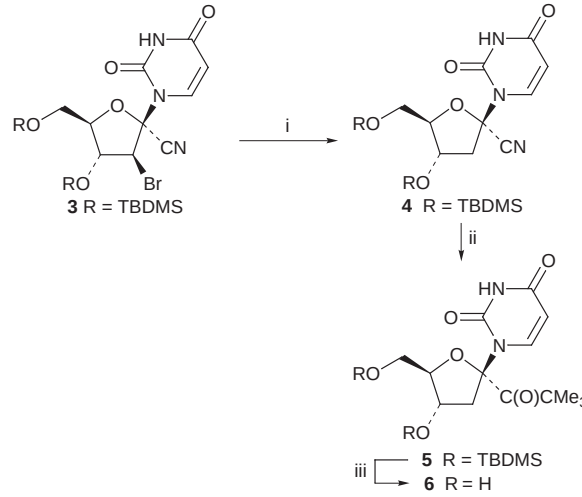
currently disputed pathways.[1,2,5] We report herein an *ex novo* synthesis of *tert*-butyl ketone **6**[9] and the mechanism of the formation of **2** [eqn. (1)].

The recently reported synthesis of **6** has two major drawbacks:[9] (i) a known psiconucleoside triol, prepared by a low yielding, laborious procedure, is used as the precursor,[10] and (ii) a total lack of regioselectivity in the protection step of this triol is observed. For these reasons, we used as our precursor compound **3**, readily available in diastereomerically pure form from the corresponding protected 1′,2′-didehydro-2′-deoxyuridine by a literature procedure.[11] When **3** was treated with $(TMS)_3SiH$[12] under normal free radical conditions, the crystalline cyanide **4** was obtained in excellent yield (94%) (Scheme 1).[13] Short treatment of **4** with excess of Bu^tLi yielded, after silica gel purification, the protected *tert*-butyl ketone **5** as the major product (37% yield, 45% based on recovered starting material).§ Finally, deprotection (NH_4F, MeOH, 60 °C, 24 h, 90%) produced the water soluble adduct **6**.

A quartz tube containing ketone **6** (23 mg; 0.073 mmol) in H_2O (200 μl) was photolyzed with a 500 W high pressure mercury lamp at room temperature. During the photolysis, continuous bubbling of air through the sample ensured the presence of O_2 in the reaction mixture. After 6 h of photolysis, ^1H NMR analysis (CD_3CN) of the lyophilized sample indicated a product ratio of 1 : 0.15 : 0.15 for **6** : **2** : uracil.[14] The products were derivatized with Bu^tMe_2SiCl/imidazole/DMF and isolated by flash chromatography (hexane–ethyl acetate 9:1) to give **7**[14]



7 R = TBDMS, X = ^{16}O
8 R = TBDMS, X = ^{18}O



Scheme 1 *Reagents and conditions:* i, $(TMS)_3SiH$, AIBN, toluene, 80 °C, 2 h, 94%; ii, 3 equiv. Bu^tLi, THF, −78 °C, 5 min, 37%; iii, NH_4F, MeOH, 60 °C, 24 h, 90%

and **5** in 15 and 85% yield, respectively. GC–MS analysis of compound **7** showed an isotopic cluster of $[M - 57]^+$ as reported in Fig. 1(*a*). A control experiment showed that photolysis of the sample under anaerobic conditions gave neither compound **2** nor uracil among the products.

The oxygen source of the carbonyl in the lactone **2** was determined by photolysis of **6** in the presence of ^{16}O–^{16}O and

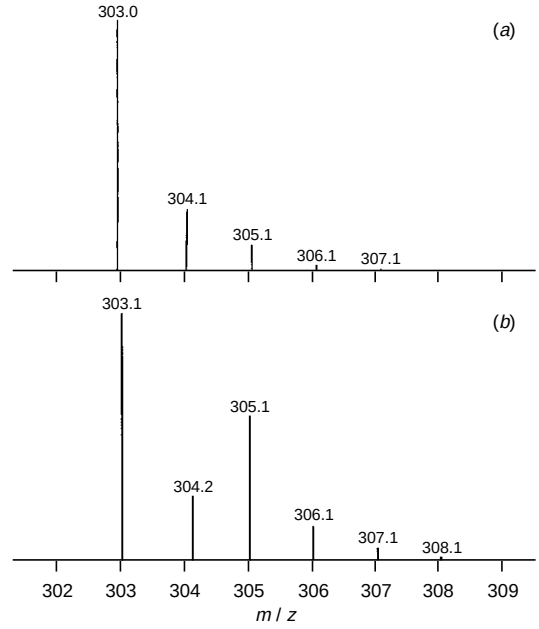
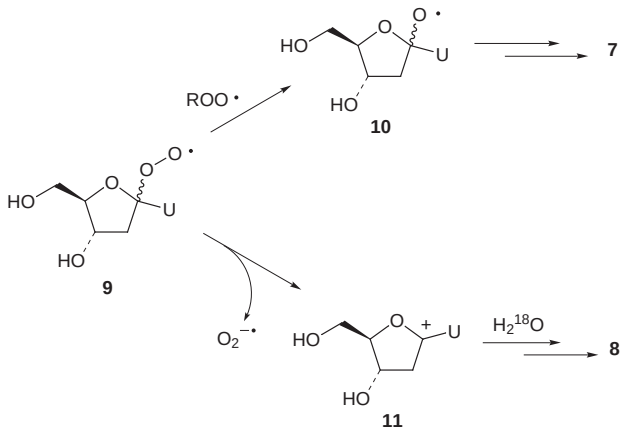


Fig. 1 Isotopic clusters of $[M - 57]^+$ from the electron impact mass spectra of protected 2-deoxyribonolactone obtained by GC–MS analysis. (*a*) Sample isolated from the reaction in $H_2{}^{16}O$ (of natural isotopic distribution). (*b*) Sample isolated from the reaction in $H_2{}^{18}O$ (95 atom% ^{18}O).



Scheme 2 Proposed mechanism for the formation of 2-deoxyribonolactone

$H_2{}^{18}O$. For this reason, the above experiment was performed in $H_2{}^{18}O$ (95 atom% ^{18}O) as solvent. After work-up, the protected 2-deoxyribonolactone was analyzed by GC–MS. Inspection of the mass spectrum of the isotopic cluster of $[M - 57]^+$, shown in Fig. 1(*b*), indicates the presence of coeluting isotopomers **7** and **8**. Analysis of this isotopic cluster revealed that the product of interest contains 65 and 35% oxygen-16 and oxygen-18, respectively. A control experiment showed that the product lactone **2** does not exchange oxygen with the solvent under the conditions employed.

The mechanism we envisage for the formation of the ^{18}O-labelled 2-deoxyribonolactone is outlined in Scheme 2. Reaction of C-1′ radical **1** with O_2 gives the peroxyl radical **9.** Laser flash photolysis studies showed that rate constant for oxygen trapping of the C-1′ radical is about 1×10^9 M^{-1} s^{-1}.[7] The peroxyl radical **9** decays either *via* a bimolecular reaction with another peroxyl radical to generate the alkoxyl radical **10** or *via* a unimolecular path (heterolytic cleavage) to generate the carbocation **11** and superoxide radical anion.[15a] The heterolytic cleavage of peroxides or alternatively the reaction of electron-rich carbon-centered radicals to give superoxide and carbocations is not without precedent.[15] The cationic intermediate **11** was trapped by $H_2{}^{18}O$, thus demonstrating the partition between the two channels.

An important consequence of the mechanism in Scheme 2 is that the C-1′ peroxyl radical generated on DNA, in the absence of good hydrogen donors, should mainly undergo heterolytic cleavage since the probability that two macromolecular peroxyl radicals meet is low. This finding accentuates the different chemical reactivity exhibited by the C-1′ radical species when compared with the one observed in the more studied C-5′ and C-4′ positions,[1] a reactivity which is mainly due to the presence of the two α-heteroatoms present in the anomeric position. Further work on the reactions of compounds **3** and **4** with a variety of electrophiles and on the kinetics of radical reactions associated with the C-1′ position is in progress.

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Notes and References

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§ *Selected data* for **5**: white solid, mp (pentane) 179–181 °C. δ_H(200 MHz; C_6D_6) −0.06, −0.04 (3 H each, s, SiMe), −0.01 (6 H, s, 2 × SiMe), 0.86, 0.91 (9 H each, s, SiBu^t), 1.27 (9 H, s, Bu^t), 2.26 (1 H, dd, *J* 13.8, 7.4, 2′α-H), 3.46 (1 H, dd, *J* 12.3, 2.4, 5′α-H), 3.62 (2 H, m, 4′, 5′β-H), 3.75 (1 H, dd, *J* 13.8, 8.8, 2′β-H), 4.41 (1 H, ddd, *J* 8.8, 7.4, 3′-H), 5.73 (1 H, d, *J* 8.2, 5-H), 8.15 (1 H, d, *J* 8.2, 6-H), 9.29 (1 H, bs, NH); δ_C(50 MHz; C_6D_6) −5.63, −5.57, −5.02, −4.42 (each CH_3), 18.0, 18.4 (each C), 25.78, 25.79, 29.0 (each 3 × CH_3), 42.5 (CH_2), 43.5 (C), 60.2 (CH_2), 68.5, 87.0 (each CH), 97.5 (C), 102.0, 139.0 (each CH), 150.5, 163.2, 201.2 (each C).

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