2,2-Diamino-4-[(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)amino]-5-(2*H*)-oxazolone: A Novel and Predominant Radical Oxidation Product of 3′,5′-Di-*O*-acetyl-2′-deoxyguanosine

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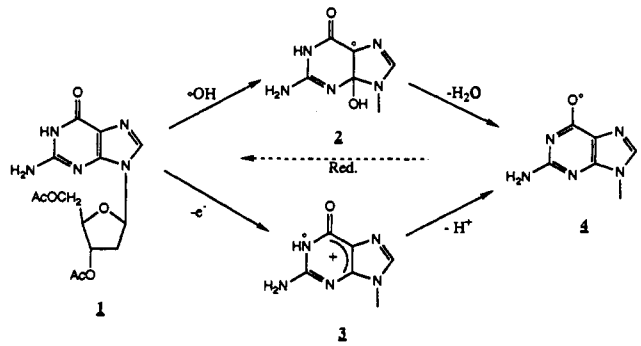
During the past decade significant progress has been made toward a better understanding of the mechanisms of radical oxidation of the base and osidic moieties of DNA model compounds.[4] The structure and redox properties of most of the radical intermediates involved in hydroxyl radical and one-electron oxidation reactions of purine and pyrimidine nucleosides and nucleotides have been determined.[5] While the bulk of the final decomposition products arising from the radical oxidation of thymidine[6] and to a lesser extent of 2′-deoxycytidine[7] have been isolated and characterized, there is still a paucity of structural information concerning the stable radical oxidation products of purine nucleosides, particularly those deriving from 2′-deoxyguanosine (dGuo), the DNA nucleoside which exhibits the lowest ionization potential. Although 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxodGuo) has received considerable attention,[4d] it is important to point out that it is only a minor product of the OH°-induced decomposition of dGuo in aerated aqueous solution.[8] It should be added that such a modified nucleoside is even a least significant product of the ionization of the guanine moiety of dGuo.[9]

We wish to report the isolation and the characterization of the two major radical oxidation products of the base moiety of 3′,5′-di-*O*-acetyl-2′-deoxyguanosine (**1**).[10] Exposure of **1** to OH radicals in aerated aqueous solutions led to the formation of two main modified nucleosides **9** and **10** which were efficiently separated by HPLC on an ODS column.[11] Interestingly, a similar HPLC elution profile is obtained when **1** was exposed in aerated aqueous solutions to photoexcited sensitizers,[12] such as benzophenone and riboflavin, which act predominantly through a type I mechanism.[13] The slowest eluting nucleoside **9** is quantitatively converted into **10** when left in neutral aqueous solution (half-life of 147 min at 37 °C). The structure assignments of both nucleosides **9** and **10** were achieved by extensive spectroscopic measurements.[14] Exact mass measurement of the pseudomolecular ion (*m/z* 331 and 1258) and the aglycon fragment (*m/z* 131 and 0571) of **10** in the positive mode FAB mass spectrum indicates that the oxidized purine moiety has, with respect to **1**, lost two carbons and one nitrogen and gained one oxygen and one hydrogen. It should be noted that there is a gain of one molecule of water in the conversion of **9** (MW = 312) to **10** (MW = 330). The ^{1}H NMR spectra of **9** and **10** in DMSO-d_6 both contain a downfield exchangeable resonance coupled to vicinal anomeric proton indicating that the imidazole ring has opened. The absence of nonexchangeable protons in the aglycon of **9** and **10** is corroborated to the sole presence of three quaternary carbons in the downfield region of the ^{13}C NMR spectra. Taken together, the spectrometric data can be rationalized in terms of a 2,2-diamino-4-[(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)amino]-5-(2*H*)-oxazolone structure[15] for **10**, whereas the precursor **9** was assigned as 2-amino-5-[(3,5-di-*O*-acetyl-2-deoxy-

Scheme 1



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(10) The 3′,5′-di-*O*-acetyl derivative of dGuo was used as the substrate in order to facilitate the HPLC separation of the polar oxidation products of the guanine moiety. In addition, the presence of the acetyl group at C5′ prevents intramolecular cyclization to occur.

(11) Typically 100 mL of a permanently aerated aqueous solution of 1 mM of **1** was exposed to the γ-rays of ^{60}Co (dose rate = 80 Gy/min) for 10 min. The oxidation products of **1** were separated on a homepacked Nucleosil (Macherey-Nagel, Düren, Germany) ODS column (250 × 4.6 mm i.d.) with water (pH 6.0)–methanol (75:25) as the eluent at a flow-rate of 1 mL/min. Detection: differential refractometry. Capacity factors (*k′*): **9** (3.5) and **10** (2.5).

(12) Photosensitization experiments were carried out by exposing 50 mL of 1.0 mM nucleoside **1** in steady-state aerated aqueous solution containing 1 mg of benzophenone to 16 black light lamps (max = 350 nm) of a Rayonet photoreactor.

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(14) 400 MHz ^{1}H NMR data (^2H_2O, TSP): **9** (δ) 5.97 (1H, $J_{1',2'}$ = 6.3, $J_{1',2''}$ = 6.6, H-1′), 5.44 (1H, $J_{3',4'}$ = 3.5, H-3′), 4.48 (1H, $J_{4',5'}$ = 5.5, $J_{4',5''}$ = 3.4, H-4′), 4.36 (1H, $J_{5',5''}$ = −14.5, H-5′), 4.33 (1H, H-5″), 2.67 (1H, $J_{2',2''}$ = −14.5, H-2′), 2.61 (1H, H-2″), 2.20 (3H, CH_3), 2.18 (3H, CH_3); **10** (δ) 5.88 (1H, $J_{1',2'}$ = 6.3, $J_{1',2''}$ = 6.5, H-1′), 5.38 (1H, $J_{3',4'}$ = 3.2, H-3′), 4.40 (1H, $J_{4',5'}$ = 5.4, $J_{4',5''}$ = 4.2, H-4′), 4.32 (1H, $J_{5',5''}$ = −12.1, H-5′), 4.30 (1H, H-5″), 2.54 (1H, $J_{2',2''}$ = −13.8, H-2′), 2.51 (1H, H-2″), 2.21 (3H, CH_3), 2.19 (3H, CH_3). 50.3 MHz ^{13}C NMR data (DMSO-d_6, TMS): **9** (δ) 184.1 (s), 175.3 (s), 170.0 (s, CH_3CO), 169.8 (s, CH_3CO), 165.9 (s), 83.3 (d, C-1′), 80.2 (d, C-4′), 74.7 (d, C-3′), 63.8 (t, C-5′), 34.9 (t, C-2′), 20.3 (q, CH_3CO), 20.1 (q, CH_3CO); **10** (δ) 170.2 (s, CH_3CO), 170.0 (s, CH_3CO), 166.4 (s, C-2), 159.3 (s, C-4), 157.1 (s, C-5), 81.7 (d, C-1′), 80.4 (d, C-4′), 74.8 (d, C-3′), 64.0 (t, C-5′), 35.6 (t, C-2′), 20.8 (q, CH_3CO), 20.6 (q, CH_3CO).

(15) (a) Additional support is provided by the 40.5 MHz ^{15}N NMR features of the nonacetylated derivative of **10** in DMSO-d_6 (with $NaNO_3$ as the internal reference) by using the antigate sequence with the suppression of NOE effects: δ (ppm) 86.9 (2 × NH_2), 117.5 (NH), 191.0 (N=C). It should be added that a brown coloration was observed for **10** after spraying the silica gel TLC plates with the hydroxylamineiron(III) chloride dyeing reagent which is used for the detection of lactones (Whittaker, V. P.; Wijesundera, S. *Biochem. J.* **1952**, *51*, 348–353). (b) However, as suggested by a reviewer we cannot rule out the possibility of the occurrence of an equilibrium between the orthocarbonate **10** and its ring open tautomer which exhibits a free carboxylic acid group.

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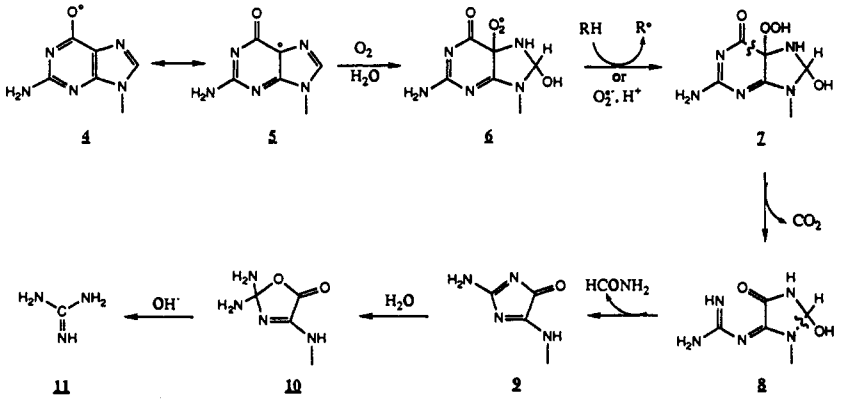
Scheme 2



β-D-*erythro*-pentofuranosyl)amino]-4*H*-imidazol-4-one. An interesting property shared by both compounds is their high alkali lability as **9** and **10** have half-lives estimated at 11.0 and 21.8 min, respectively, in buffered aqueous solutions (pH 10) at 65 °C. In fact, the quantitative breakdown of **10** into guanidine (**11**) upon alkali treatment provides additional indirect support for its structure.

The formation of **9** and **10** upon exposure of **1** to either OH radicals or photoexcited type I photosensitizers is likely to involve common intermediates. One such common intermediate is the oxyl radical **4** which may arise either from fast dehydration of the OH radical adduct **2**[16] at C4 or by efficient deprotonation of the guanine radical cation **3**.[5b,17] Earlier works have shown that the oxidizing radical **4** does not react with molecular oxygen, at least on the millisecond and shorter time scale.[18] However, on a longer time scale we have shown that one molecule of oxygen is able to react with guanine radicals induced by either OH radicals or type I photosensitization. This was inferred from the incorporation of one atom of ^{18}O in **10** as determined by FAB mass spectrometry analyses of the final products of the photosensitization experiments of **1** performed in $^{18}O_2$ saturated solutions.[19] It should be noted that reducing agents such as Fe^{2+} [20] and antioxidants,[20,21] such as serotonin and ascorbate are able to compete with the fixation of O_2 on the tautomeric carbon centered radical **5** by restoring the starting nucleoside **1** (Scheme 1). Conversion of the resulting peroxyl radical **6** into either the corresponding hydroperoxide **7** or the related 1,2-dioxetane[22] would lead, in a subsequent step, to the opening of the pyrimidine ring at the C5–C6 bond (Scheme 2). Hydration likely takes place at the 7,8-C=N double bond of either the resulting decarboxylated derivative or the hydroperoxide precursor as indirectly shown by the formation of (2*S*)-2,5′-anhydro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-guanidinylidene-2-hydroxy-4-oxoimidazolidine[23] when dGuo is exposed to either OH radicals or photoexcited type I photosensitizers.[24] Ring-chain tautomerism of the carbinolamine **8** would lead to the opening of the imidazole ring with subsequent intramolecular cyclization of the guanidine residue.[25] Hydrolysis of the resulting imidazolone **9** gives rise to the oxazolone **10**.

It should be added that the oxazolone **10** and its precursor **9** represent more than 80% of the OH radical and riboflavin-mediated oxidation of the base moiety of **1**. The two latter oxidized guanine modifications are also the predominant base modifications observed when dGuo is substituted for **1**. Preliminary experiments show that 2,2-diamino-4-[(2-deoxy-β-D-*erythro*-pentofuranosyl)-amino]-5-(2*H*)-oxazolone and 8-oxodGuo are the two main stable radical oxidation products of the guanine nucleosides within double-stranded DNA. Work is in progress to establish whether most of the alkali-labile sites observed at guanine residues within oxidized DNA[26] are related to the formation of the oxazolone derivative and its imidazolone precursor.

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Supplementary Material Available: HPLC elution profiles of the benzophenone photosensitized oxidation products of 3′,5′-di-*O*-acetyl-2′-deoxyguanosine, UV absorption spectrum of **9** in water as a function of time, FAB-MS spectra of **9** and **10**, and ^1H NMR spectrum of **10** in DMSO-d_6 (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(22) An alternative pathway would involve the dismutation of the peroxyl radical into the corresponding oxyl radical with subsequent α-cleavage.

(23) Similarly, the 2*R* and 2*S* diastereomer of 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2-methoxy-4,5-imidazolidinedione were generated upon benzophenone photosensitization of 2′-deoxyguanosine in methanol–water solutions (Cadet, J.; Buchko, G. W.; Berger, M.; Morin, B.; Ravanat, J.-L. *Photochem. Photobiol.* **1993**, *57*, 82s). This constitutes a relevant model system for investigating the radiation-induced and photosensitized formation of DNA–protein cross-links.

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