A Novel Nitroimidazole Compound Formed during the Reaction of Peroxynitrite with 2′,3′,5′-Tri-*O*-Acetyl-Guanosine

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Abstract: Peroxynitrite reacts with 2′,3′,5′-tri-*O*-acetyl-guanosine to yield a novel compound identified as 1-(2,3,5-tri-*O*-acetyl-β-D-*erythro*-pentofuranosyl)-5-guanidino-4-nitroimidazole (**6**). This characterization was achieved using a combination of UV/vis spectroscopy and ESI-MS. Additionally, 1-(β-D-*erythro*-pentofuranosyl)-5-guanidino-4-nitroimidazole (**6a**) was synthesized by an independent route, characterized by UV/vis spectroscopy, ESI-MS, and ^{1}H- and ^{13}C NMR, and shown to be identical to deacetylated **6**. This product is extremely stable in aqueous solution at both pH extremes and is formed in significant yields. These characteristics suggest that this lesion may be useful as a specific biomarker of peroxynitrite-induced DNA damage. We also observed formation of 2′,3′,5′-tri-*O*-acetyl-8-nitroguanosine (2′,3′,5′-tri-*O*-acetyl-**8-NO$_2$Guo**), 2-amino-5-[(2,3,5-tri-*O*-acetyl-β-D-*erythro*-pentofuranosyl)amino]-4H-imidazol-4-one (2′,3′,5′-tri-*O*-acetyl-**Iz**), and the peroxynitrite-induced oxidation products of 2′,3′,5′-tri-*O*-acetyl-8-oxoGuo. The formation of **6** and 2′,3′,5′-tri-*O*-acetyl-**8-NO$_2$Guo** was rationalized by a mechanism invoking formation of the guanine radical.

Introduction

Peroxynitrite ($ONOO^-$) is a physiologically relevant and reactive molecule formed by the diffusion-limited reaction of nitric oxide ($^\bullet NO$) with superoxide ($O_2^{\bullet -}$).[1] While $ONOO^-$ is stable in alkaline solutions, its conjugate acid, peroxynitrous acid (ONOOH), is very short-lived ($t_{1/2} \approx 1$ s),[2] giving rise to the $HO^\bullet/^\bullet NO_2$ radical pair in ~33% yield.[3−5] The decomposition of $ONOO^-$ is dramatically enhanced in the presence of carbon dioxide. Through intermediate formation of peroxynitrosocarbonate ($ONOOCO_2^-$), the $CO_3^{\bullet -}$ and $^\bullet NO_2$ free radicals are generated, each in 33% yield.[6−8]

Peroxynitrite causes oxidation and nitration of a variety of substrates, including low molecular weight antioxidants such as ascorbate,[9,10] trolox,[10] and glutathione[10,11] and larger biomolecules such as lipids,[12] proteins,[13−18] and DNA. With DNA, sugar damage results in strand breaks,[19−21] and several base lesions have been identified. Both in DNA and at the nucleoside level, guanine is the most reactive nucleobase.[22,23] Several products of the reaction between 2′-deoxyguanosine (dG) and $ONOO^-$ have been characterized (see Figure 1), including the oxidation products, 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG),[21] 2-amino-5-[(3,5-di-*O*-acetyl-β-D-*erythro*-pentofuranosyl)amino]-4H-imidazol-4-one (**dIz**), the precursor to 2,2-diamino-4-[(2-deoxy-β-D-*erythro*-pentofuranosyl)amino]-5-(2H)-oxazolone (**dZ**) and a spiroiminodihydantoin.[24] The nitration product, 8-nitroguanine, has also been described,[25] as well as an addition product, 4,5-dihydro-5-hydroxy-4-(nitrosooxy)-2′-deoxyguanosine (**nox-dG**).[26]

Despite identification of the various reaction products, a clear mechanism has not emerged to fully account for their formation. In this contribution, we report the characterization of the novel

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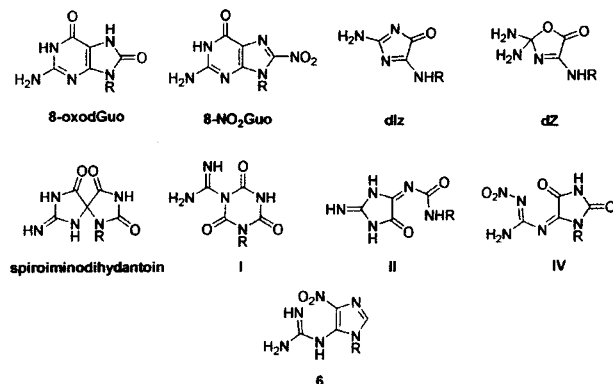

Figure 1. Summary of products we have confirmed to be formed during the reaction of peroxynitrite with 3′,5′-di-*O*-acetyl-dG and 2′,3′,5′-tri-*O*-acetyl-Guo.

compound, **6**, formed during the reaction of 2′,3′,5′-tri-*O*-acetyl-Guo with peroxynitrite. This product, together with 2′,3′,5′-tri-*O*-acetyl-**8-NO$_2$Guo**, gives definitive insights into the mechanism(s) of 2′,3′,5′-tri-*O*-acetyl-Guo oxidation by $ONOO^-$. Thus, we have proposed that the guanine radical is a key intermediate from which **6** and 2′,3′,5′-tri-*O*-acetyl-**8-NO$_2$Guo** are derived.

Furthermore, of potential biological significance is the fact that **6** is a significant and stable product of 2′,3′,5′-tri-*O*-acetyl-Guo oxidation even at low peroxynitrite concentrations and is likely to arise only from the reaction of 2′,3′,5′-tri-*O*-acetyl-Guo with peroxynitrite. Therefore, this compound may be important in understanding peroxynitrite-induced mutagenesis and also may serve as a specific biomarker of peroxynitrite-induced DNA damage.

Experimental Section

General. DMSO-d_6 and D_2O were obtained from Cambridge Isotope Laboratories (Andover, MA); $Na^{15}NO_2$ was from Isotech (Miamisburg, OH). All solvents were HPLC grade.

Instrumentation. UV/vis measurements were made using an HP8452 diode array spectrophotometer (Hewlett-Packard, Palo Alto, CA). ^{1}H NMR spectra were recorded at 500 MHz, and ^{13}C NMR spectra (proton decoupled), at 125 MHz on an Inova 500 spectrometer (Varian). ^{1}H COSY and HETCOR experiments were used to aid in the assignment of sugar and base protons and carbons. High performance liquid chromatography (HPLC) was performed using an HP1100 pump equipped with a 1090 or an 1100 diode array detector (Hewlett-Packard). Electrospray ionization mass spectrometry (ESI-MS) and tandem mass spectrometry (ESI-MS/MS) experiments were carried out using either an HP 5989B (Hewlett-Packard) or TSQ 7000 (Finnigan, San Jose, CA) mass spectrometer, respectively. Unless stated otherwise, ESI-MS and ESI-MS/MS spectra were obtained in negative and positive ion mode using spraying solutions with the composition 78/20 water/2-propanol with 2% ammonium hydroxide or 50/50 water/methanol with 0.05% acetic acid, repsectively.

Peroxynitrite Synthesis. ^{14}N-Peroxynitrite was prepared either by ozonolysis of an alkaline solution of sodium azide (Fisher) solution[27] or by the reaction of isoamyl nitrite (Aldrich, Milwaukee, WI) with hydrogen peroxide (Fisher) at pH 12−14.[28] In the latter case, excess hydrogen peroxide was removed by passing the peroxynitrite solution through a 7.5 cm × 1.5 cm manganese oxide (Aldrich) column. ^{15}N-Peroxynitrite was prepared by reacting 53 μmol of H_2O_2 in 1 M HCl with 53 μmol of $Na^{15}NO_2$ as previously described.[29] The reaction was quenched by rapidly adding 35 μL of 5 M NaOH. Peroxynitrite concentrations were determined by making dilutions in 0.1 M NaOH and measuring the absorbance at $\lambda = 302$ nm ($\epsilon = 1670$ M^{-1} cm^{-1}).[29]

Acetylation of Guo and Synthesis of 2,3,5-Tri-*O*-acetyl-[C8-D]-Guo. Guo (10.2 g, 36 mmol) was acetylated by suspending in a 2:1 v/v pyridine/acetic anhydride mixture until all of the starting material had been converted into the triacetylated product as determined by HPLC and ESI-MS. Conversion of Guo into [C8-D]-Guo was achieved as previously reported.[30] Exchange was confirmed by negative ion ESI-MS and ^{1}H NMR in DMSO-d_6. From the ^{1}H NMR, it was determined that the product was 92% [C8-D]-Guo and 8% [C8-H]-Guo. Acetylation was carried out as described above.

Synthesis of 1-(β-D-*erythro*-Pentofuranosyl)-5-cyanamido-4-nitroimidazole (5). To prepare this compound, 2,4,5-tribromoimidazole (**1**) was reduced to 4-bromoimidazole (**2**)[31] and nitrated to give 5(4)-bromo-4(5)-nitroimidazole (**3**),[32] which was subsequently fused with tetra-*O*-acetyl-ribose to give 1-(2,3,5-tri-*O*-acetyl-β-D-*erythro*-pentofuranosyl)-5-bromo-4-nitroimidazole (**4**).[33] The latter compound (1.0 g, 2.22 mmol) was allowed to react with cyanamide (0.36 g, 6.66 mmol) and sodium methoxide (0.28 g, 6.66 mmol) in anhydrous methanol (45 mL) at room temperature for 2 days, at which time the reaction was complete as determined by UV/vis spectroscopy and HPLC. For characterization, 10 mL of the reaction mixture was evaporated to dryness and taken up in ~15 mL double distilled water and loaded onto a C18, 125 Å, 55−105 μm beads (Waters, Milford, MA) column preequilibrated and washed with a 99/1 water/acetonitrile mixture. The product eluted as a broad band and was collected in 15 mL fractions. The third fraction was dried in vacuo and used as the analytical sample. ^{1}H NMR (D_2O) δ (ppm) 7.45 (s, 1H, H2), 5.58 (d, 1H, H1′), 4.37 (t, 1H, H2′), 4.13 (t, 1H, H3′), 3.96 (m, 1H, H4′), 3.71−3.58 (m, 2H, H5′ and H5″). ^{13}C NMR (DMSO-d_6) δ (ppm) 146.86 (C4), 133.02 (C5), 130.26 (C2), 118.96 (C7), 87.11 (C1′), 85.40 (C4′), 74.70 (C2′), 70.83 (C3′), 62.01 (C5′). ESI-MS: 284 $(M - H)^-$; UV/vis: $\lambda_{max} = 230$ nm, 410 nm. HRMS calcd for $C_9H_{10}N_5O_6$ $[M - H]^-$ 284.0631, found 284.0632.

Synthesis of Authentic 1-(β-D-*erythro*-Pentofuranosyl)-5-guanidino-4-nitroimidazole, 6a. The crude reaction mixture containing **5** (20 mL, 0.44 g, 0.99 mmol) was refluxed with ammonium chloride (1.65 g, 30.8 mmol) for 5 days after which the mixture was cooled and evaporated to dryness in vacuo. The residue was taken up in water and purified by semipreparative HPLC on a 250 mm × 10 mm, 5 μm Nucleosil C18 column (Alltech). Ammonium acetate (50 mM) and acetonitrile were used as solvents A and B, respectively. The column was eluted isocratically with 1% B for 10 min before a gradient from 1 to 20% B over 10 min was initiated. After a 2 min isocratic wash with 20% B, the eluent composition was restored to 1% B over 3 min. A flow rate of 4.0 mL/min was used, and products were monitored simultaneously at 230, 380, and 410 nm. Approximate yield of **6a** was 10−15%. ^{1}H NMR (D_2O) δ (ppm) 7.59 (s, 1H, H2), 5.56 (d, 1H, H1′), 4.37 (t, 1H, H2′), 4.15 (t, 1H, H3′), 3.97 (m, 1H, H4′), 3.72−3.52 (m, 2H, H5′ and H5″). ^{13}C NMR (DMSO-d_6) δ (ppm) 158.07 (C7), 143.64 (C4), 133.16 (C5), 129.99 (C2), 87.08 (C1′), 84.77 (C4′), 74.55 (C2′), 69.90 (C3′), 60.99 (C5′). ESI-MS: 303 $(M + H)^+$. UV/vis: 230 nm, 380 nm. HRMS calcd for $C_9H_{15}N_6O_6$ $[M + H]^+$ 303.1053, found 303.1059.

Reaction of 2′,3′,5′-tri-*O*-Acetyl-guanosine with Peroxynitrite. 2′,3′,5′-Tri-*O*-acetyl-Guo (100 nmol) was reacted with peroxynitrite (5 μmol) in 150 mM KH_2PO_4, 25 mM $NaHCO_3$, pH 7.2 buffer (1 mL). This mixture was purified on a 250 mm × 4.6 mm, 5 μm Columbus C18 column (Phenomenex) using 50 mM ammonium acetate (solvent A) and acetonitrile (solvent B) as mobile phases. The column was eluted isocratically with 5% B for 10 min, followed by a gradient from 5 to 40% B in 20 min, another isocratic wash with 40% B for 5 min, and finally a gradient from 40 to 5% B over 5 min. The flow rate was 1.0 mL/min, and products were monitored simultaneously at 230, 252, and

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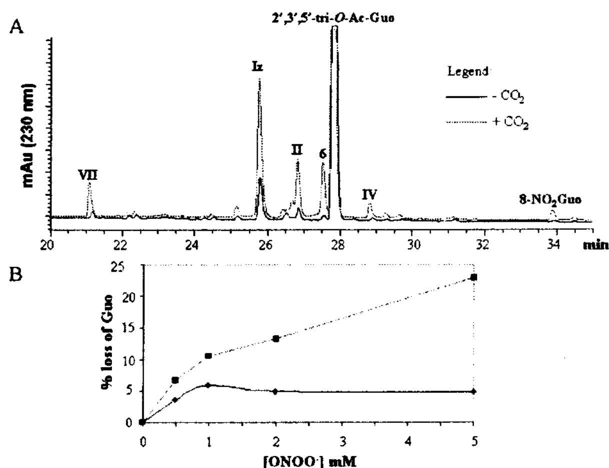


Figure 2. Impact of CO_2 on the relative yields of the various products of peroxynitrite-mediated 2′,3′,5′-tri-*O*-acetyl-Guo oxidation. **VII** (presently uncharacterized) is the precursor to 3-(2,3,5-tri-*O*-acetyl-β-D-*erythro*-pentofuranosyl)-2,4,6-trioxo-[1,3,5]triazinane-1-carboxamidine, **I**.

380 nm. The putative nitroimidazole product, **6**, was collected and dried in vacuo. Deacetylation was effected, as necessary, by dissolving the residue in 0.5 M NaOH for 30−60 min, and the product was isolated with a 1% B isocratic wash using the same solvents and column as above.

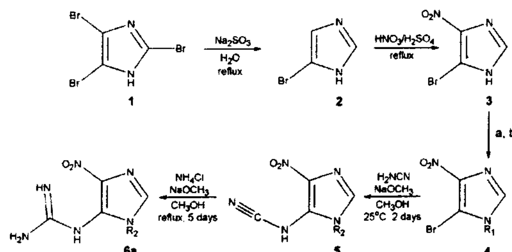
Comparison of 6a and Deacetylated 6. 6a and deacetylated **6** were compared using HPLC, UV/vis, and ESI-MS. For the HPLC studies, a 150 mm × 2.0 mm, 5 μm Columbus C18 column was used along with 50 mM ammonium acetate (solvent A) and acetonitrile (solvent B) as mobile phases. The column was eluted isocratically with 1% B at a flow rate of 0.2 mL/min, and products were monitored simultaneously at 230 and 380 nm.

Results

When we reacted 2′,3′,5′-tri-*O*-acetyl-Guo with peroxynitrite, the previously reported products 2′,3′,5′-tri-*O*-acetyl-**8-NO$_2$Guo** and 2′,3′,5′-tri-*O*-acetyl-**Iz** were detected by both HPLC−UV and ESI-MS (see Figure 2A). No 2′,3′,5′-tri-*O*-acetyl-8-oxoGuo was detected by these methods, but its peroxynitrite-induced oxidation products I, II and IV[34−36] were identified (Figures 1 and 2A). Since 8-oxodG is at least 1000 times more readily oxidized by peroxynitrite than dG,[37] this finding indicated that 2′,3′,5′-tri-*O*-acetyl-8-oxoGuo formed during the oxidation of 2′,3′,5′-tri-*O*-acetyl-Guo was further oxidized. Previously, **nox-dG** was identified as a product arising from the addition of peroxynitrite across the C4−C5 double bond of dG.[26] In our experiments, though, we could not detect any products having a molecular weight corresponding to this compound. However, a novel and stable nitroimidazole compound, **6**, was isolated and characterized as detailed below. The impact of CO_2 on the yield of the various products and on the destruction of 2′,3′,5′-tri-*O*-acetyl-Guo was also investigated. We found that CO_2 enhanced both the oxidation and nitration product yields (Figure 2A), and correspondingly, more 2′,3′,5′-tri-*O*-acetyl-Guo reacted in the presence of CO_2 than in its absence (Figure 2B).

Characterization of 6. The UV/vis spectrum of **6** exhibited maxima at 230 and 380 nm, with no significant absorbance in the range 250−320 nm. This suggested that the purine ring system had been destroyed. ESI-MS studies revealed that this compound had a molecular weight of 428 [$(M - H)^- = 427$]. ESI-MS deuterium exchange studies were carried out to determine the number of exchangeable protons in **6**. To do so, the sample was exchanged into D_2O (99.9%) by repeated lyophilization and resuspension in D_2O. In addition, a spraying solution with composition 80/20 2-propanol/D_2O, 2% ammonium hydroxide was used. In these studies, the $[M - H]^-$ ion was observed at $m/z = 430$. Since one proton (deuteron) is lost to produce the observed negative ion, the $\Delta(m/z) = +3$ when D_2O is used indicates that **6** has four exchangeable protons. Furthermore, we were also able to show that when (a) 2′,3′,5′-tri-*O*-acetyl-[C8-D]-Guo reacted with ^{14}N-peroxynitrite, and (b) 2′,3′,5′-tri-*O*-acetyl-Guo reacted with ^{15}N-peroxynitrite, the molecular weight of **6** increased by 1 amu (data not shown). These results indicated, respectively, that (i) the C8−H of the parent 2′,3′,5′-tri-*O*-acetyl-Guo was retained, and (ii) in conjunction with the molecular weight of **6**, that a peroxynitrite-derived nitro group was incorporated into **6**. Altogether, the above findings were consistent with the base moiety of **6** being 5(4)-guanidino-4(5)-nitroimidazole. To prove this, we synthesized authentic 1-(β-D-*erythro*-pentofuranosyl)-5-guanidino-4-nitroimidazole, **6a**, by an independent route for comparison with deacetylated **6**.

Scheme 1. Synthesis of Authentic 1-(β-D-*erythro*-Pentofuranosyl)-5-guanidino-4-nitroimidazole, **6a**[a]



[a] a) NH_4SO_4, chlorotrimethylsilane, anhydrous hexamethyldisilazane, reflux for 16 h; b) 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose, CH_2Cl_2, $SnCl_4$, stir at rt for 18 h under Ar atm, R_1 = 2,3,5-tri-*O*-acetyl-β-D-*erythro*-pentofuranosyl, and R_2 = β-D-pentofuranosyl.

Synthesis of 6a. The synthesis of **6a** is summarized in Scheme 1. The preparation of compounds **2**, **3**, and **4** has been reported previously by Balaban et al.,[31] Barrio et al.,[32] and Hasan et al.,[33] respectively. In the report by Hasan et al., a mixture of the structural isomers 1-(2,3,5-tri-*O*-acetyl-β-D-*erythro*-pentofuranosyl)-5-bromo-4-nitroimidazole (**4**) and 1-(2,3,5-tri-*O*-acetyl-β-D-*erythro*-pentofuranosyl)-4-bromo-5-nitroimidazole was obtained during the fusion of the ribosyl moiety and the 4(5)-bromo-5(4)-nitroimidazole.[33] However, in our experiments using the same conditions, a single product having UV/vis characteristics of the 1-(2,3,5-tri-*O*-acetyl-β-D-*erythro*-pentofuranosyl)-5-bromo-4-nitroimidazole isomer[38] was isolated. To verify this observation, we reacted this product (0.30 g, 0.67 mmol) with sodium cyanide (0.17 g, 3.5 mmol) and potassium iodide (17.66 mg, 0.11 mmol) in DMSO at ambient temperature. Under these conditions, cyanide is known to displace the bromo group of 1-(2,3,5-tri-*O*-acetyl-β-D-*erythro*-pentofuranosyl)-5-bromo-4-nitroimidazole but not that of its structural isomer.[39,40] Analysis

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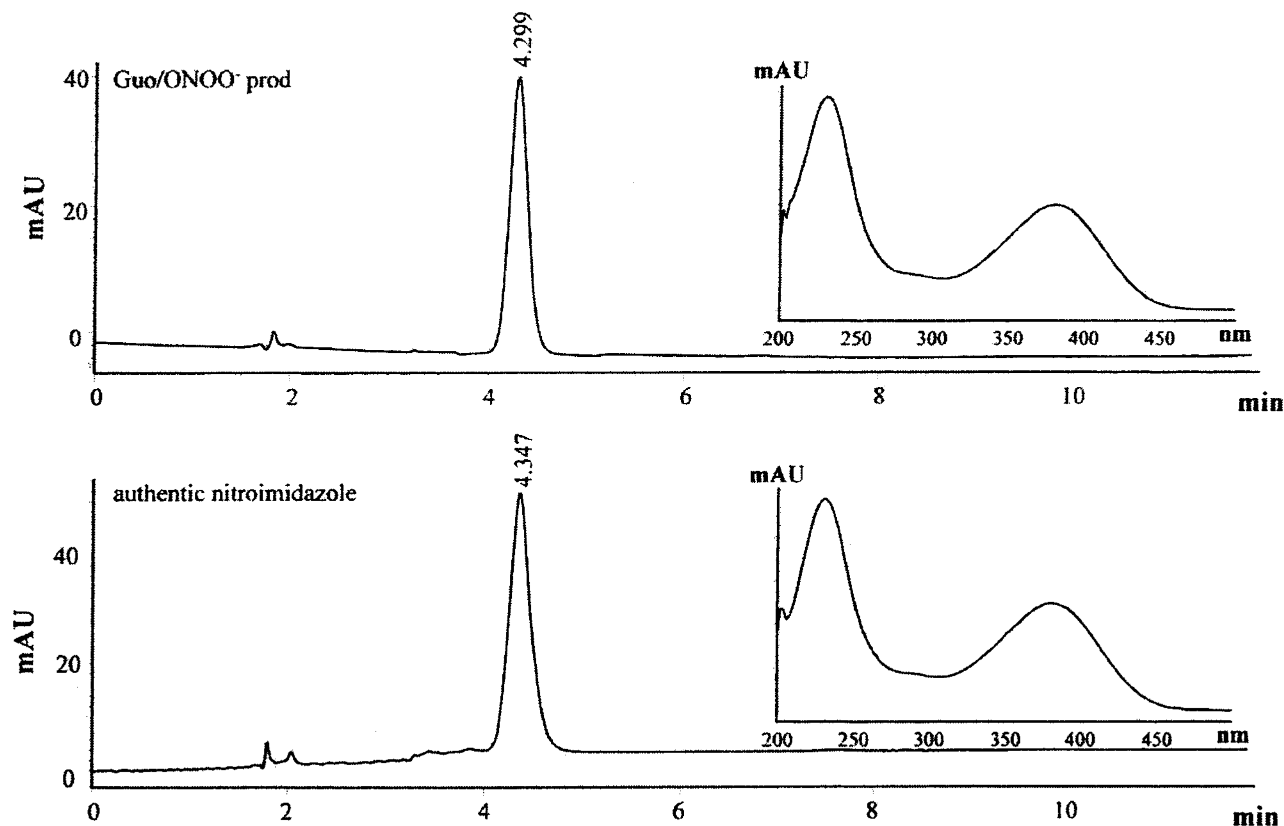

Figure 3. Both deacetylated **6** (above) and authentic 1-(β-D-*erythro*-pentofuranosyl)-5-guanidino-4-nitroimidazole, **6a** (below) have identical HPLC retention times and UV spectra.

of the reaction mixture by HPLC and ESI-MS revealed that the starting material had been quantitatively converted into the cyano derivative.[41] This finding confirmed, therefore, that 1-(2,3,5-tri-*O*-acetyl-β-D-*erythro*-pentofuranosyl)-5-bromo-4-nitroimidazole is the sole product of the glycosylation reaction. The synthesis of **5** has not previously been described and was achieved by displacing the bromo group of **4** with cyanamide in a reaction analogous to the cyanide displacement reaction. No displacement of the nitro group was observed, and this reaction was quantitative (see Experimental Section for characterization). Finally, the cyanamido derivative was converted to **6a** in ~10−15% yield by refluxing with ammonium chloride, purified by HPLC and characterized as described in the Experimental section, confirming its identity as 1-(β-D-*erythro*-pentofuranosyl)-5-guanidino-4-nitroimidazole.

Comparison of 6a with Deacetylated 6. Deacetylated **6** was compared with **6a** using HPLC−UV and ESI-MS. Each compound was analyzed separately and found to have the same retention time (4.3 min) and UV/vis spectrum as shown in Figure 3. When both compounds were mixed, a single peak eluted after 4.3 min. Additionally, we verified that **6a** remained unchanged during deacetylation by subjecting it to the same conditions as **6** followed by HPLC analysis to determine that the retention time and UV spectrum had not changed. Taken together, these data confirmed the identity of **6** as 1-(2,3,5-tri-*O*-acetyl-β-D-*erythro*-pentofuranosyl)-5-guanidino-4-nitroimidazole.

Discussion

In this study, we have examined the impact of CO_2 on the peroxynitrite-mediated oxidation of 2′,3′,5′-tri-*O*-acetyl-Guo, and have shown that oxidation and nitration yields are enhanced in CO_2-containing versus CO_2-free buffers. In addition to confirming earlier reports of 8-oxoGuo, **8-NO$_2$Guo**, and **Iz** formation, we have identified the novel compound, 5-guanidino-4-nitroimidazole, **6**. The formation of both **6** and 2′,3′,5′-tri-*O*-acetyl-**8-NO$_2$Guo** provide important insight into the mechanism by which peroxynitrite oxidizes 2′,3′,5′-tri-*O*-acetyl-Guo.

Mechanism of Peroxynitrite-Mediated Oxidation of 2′,3′,5′-Tri-*O*-acetyl-Guo Leading to Formation of 6 and 2′,3′,5′-Tri-*O*-acetyl-8-NO$_2$Guo. We have proposed the mechanism summarized in Scheme 2 that involves, as a first step, the one-electron oxidation of 2′,3′,5′-tri-*O*-acetyl-Guo to produce the radical cation, 2′,3′,5′-tri-*O*-acetyl-Guo$^{\bullet+}$. Similar one-electron oxidations of Guo by $Br_2^{\bullet-}$ and $SO_4^{\bullet-}$,[42] photoionization,[43] and MnTmPyp/$KHSO_5$[44] have been previously described. The Guo$^{\bullet+}$ (pK_a = 3.9[42,45]) will rapidly deprotonate at pH 7.2−7.4 to yield the neutral radical, Guo$^{\bullet}$. In fact, the species produced from peroxynitrite, namely $CO_3^{\bullet-}$ ($E° = 1.5$ V[46]) and HO$^{\bullet}$ ($E° = 1.9$ V[47]), are sufficiently potent to oxidize Guo ($E° = 1.29$ V[48]). Also, the Guo$^{\bullet}$ has significant unpaired electron density at the O6, C5, and C8 positions.[49] These properties are expected to

(41) During the reaction with cyanide, some diacetylated cyano product is also formed, and the yield increases with reaction time. This most likely results from deacetylation of **5** due to trace amounts of water present, which leads to formation of HBr and acidification of the reaction mixture.

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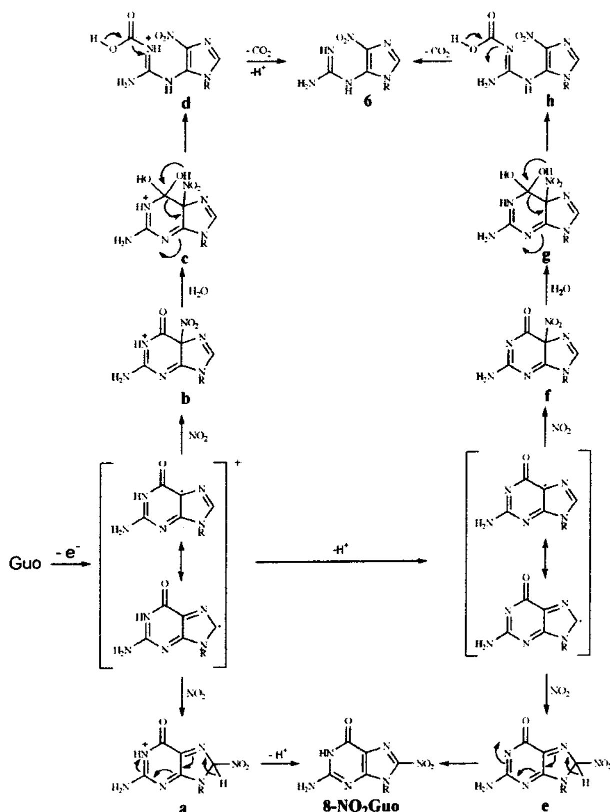
Scheme 2. Proposed Mechanism for the Formation of **6** and 2′,3′,5′-Tri-*O*-acetyl-**8-NO$_2$Guo** during the Peroxynitrite-Induced Oxidation of 2′,3′,5′-Tri-*O*-acetyl-Guo



remain unchanged for 2′,3′,5′-tri-*O*-acetyl-Guo$^{•+}$. Thus, radical combination between $^•NO_2$ and the C8 or C5 positions of 2′,3′,5′-tri-*O*-acetyl-Guo$^•$, respectively, gives the intermediates **e** and **f**, as shown in Scheme 2. In the case of C8 addition, **e** rearomatizes to yield 2′,3′,5′-tri-*O*-acetyl-**8-NO$_2$Guo**. In the case of C5 addition, **f** can be attacked by water at the electrophilic C6 to yield **g**, with subsequent cleavage of the C5−C6 bond to give the carbamate derivative, **h**. Decarboxylation of **h** then leads to formation of the final product **6**. While deprotonation of the 2′,3′,5′-tri-*O*-acetyl-Guo$^{•+}$ is likely to occur concurrently with or rapidly following its formation, it is possible for the 2′,3′,5′-tri-*O*-acetyl-Guo$^{•+}$ to serve as a precursor to the observed products as shown in Scheme 2. The reactions are analogous to those described for the 2′,3′,5′-tri-*O*-acetyl-Guo$^•$, with formation of **a** and **b** resulting from the combination of $^•NO_2$ with the C8 and C5 of the 2′,3′,5′-tri-*O*-acetyl-Guo$^{•+}$, respectively. Deprotonation of **a** then leads to 2′,3′,5′-tri-*O*-acetyl-**8-NO$_2$Guo** formation, while **b** is hydrolyzed to give **c**, which undergoes C5−C6 bond cleavage to yield **d**. This intermediate then undergoes decarboxylation and deprotonation to yield the final product **6**.

It is worth emphasizing that although the reaction of peroxynitrite with dG has been previously studied, this is the first report of nitroimidazole product formation during this reaction. In many of the prior studies, products had been characterized but no mechanistic rationalization provided. Here, we have proposed a radical-based mechanism for the formation of **6**, consistent with the extensively studied mechanism of peroxynitrite decomposition.

Biological Significance

Product **6** is a significant product of peroxynitrite-mediated 2′,3′,5′-tri-*O*-acetyl-Guo oxidation. Importantly, this product is extremely stable and is expected to accumulate in DNA unless it is efficiently repaired. Therefore, this lesion might contribute to the mutagenicity of peroxynitrite, which has been shown to induce predominantly G → T (75%), G → A (14%), and G → C (12%) mutations in the supF gene treated in vitro and transiently transfected into human AD293 cells.[50] The specific lesion(s) causing these mutations remain(s) unknown, and thus, to evaluate the contribution of **6**, understanding both the mutagenic potential and kinds of mutations induced by **6** becomes critical. Additionally, the stability of this lesion implies that it might be useful as a highly specific biomarker of peroxynitrite-induced DNA damage. This is particularly interesting since it can facilitate a comparison of the extent of peroxynitrite-induced DNA damage occurring in inflamed tissues, where peroxynitrite production is expected to be high, relative to normal tissues.

Summary

We have identified the novel compound **6** as a stable and significant product of the reaction between peroxynitrite and 2′,3′,5′-tri-*O*-acetyl-Guo. From the product identity, we have proposed a mechanism that implicates the guanosine radical as a key intermediate in the formation of **6** and 2′,3′,5′-tri-*O*-acetyl-**8-NO$_2$Guo**. However, involvement of the guanosine radical cation cannot be completely excluded. As the product **6** is likely to be a specific peroxynitrite-induced product, it has the potential to serve as a biomarker of peroxynitrite-induced DNA damage and, at the same time, expand our understanding of the toxicity and mutagenicity of peroxynitrite-induced DNA base lesions.

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