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Oxidation of a Biomarker for Phenol Carcinogen Exposure: Expanding the Redox Chemistry of 2′-Deoxyguanosine

Jennifer L. Weishar, Christopher K. McLaughlin, Michael Baker, Wojciech Gabryelski,* and Richard A. Manderville*

Departments of Chemistry and Toxicology, University of Guelph, Guelph, Ontario, Canada N1G 2W1

rmanderv@uoguelph.ca

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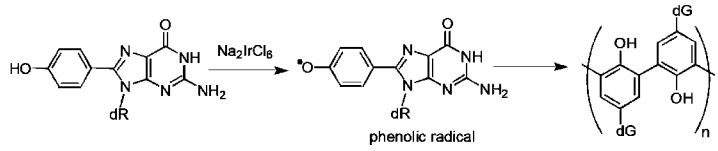
ABSTRACT



A biomarker for phenolic carcinogen exposure, 8-(4″-hydroxyphenyl)-2′-deoxyguanosine, has been found to undergo oxidative coupling in the presence of Na_2IrCl_6 to afford ortho−ortho C−C-coupled polyphenols through the intermediacy of a phenoxyl radical. One can envision using such unique chemistry to oxidatively couple strands of DNA for the generation of new biomaterials. Our results also demonstrate the potential for phenolic adducts of DNA to undergo further oxidation reactions that may contribute to phenol-mediated cytotoxicity and genotoxicity.

Covalent attachment of electrophiles to DNA forms DNA adducts that may induce mutations and carcinogenesis.[1] The DNA adduct can clarify the nature of the activated intermediate which reacted with DNA and can serve as a biomarker for exposure to a particular carcinogen. In this regard, 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxo-dG, Figure 1 is the primary oxidation product of dG and is a biomarker of cellular oxidative damage.[2] The C8-site of dG is also targeted by arylamines that undergo bioactivation to form nitrogen (*N*)-bound C8-arylamine adducts that initiate mutagenicity.[3]Heteroatom attachment to the C8-site of dG lowers the oxidation potential relative to dG,[4,5] and further oxidation of 8-oxo-dG[6] and C8-arylamine adducts[5,7] can produce spiroiminodihydantoin (Sp) products following attachment of H_2O to the oxidized purine ring system. The detection of Sp lesions in vivo[8] combined with their mutagenicity[9] has stimulated much interest into their mechanisms of formation and biological implications.[10]

Our interest in modified nucleobases stems from research on DNA adduction by phenolic carcinogens that form C8-dG adducts via radical intermediates.[11] A representative member is 8-(4″-hydroxyphenyl)-dG **1** (X = OH, Figure 1),[12] which is structurally related to other C8-aryl adducts formed by aryl hydrazines,[13] polycyclic aromatic hydrocarbons,[14] and estrogen derivatives.[15] Like 8-oxo-dG[4] and C8-

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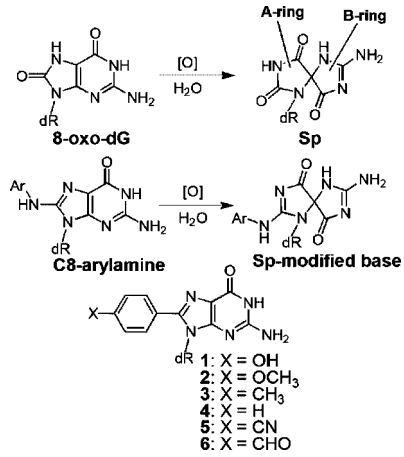


Figure 1. Oxidation of C8-dG adducts and structures of C8-aryl adducts **1**−**6**.

arylamine adducts,[5] we have shown that adduct **1** also undergoes oxidation more readily than dG.[16] We now report structure−activity relationships for the electrochemical oxidation of substituted phenyl adducts **1**−**6** and a product analysis from the chemical oxidation of adduct **1**. Our results show that the C8-aryl group can expand the redox properties of dG to afford new reactive intermediates that may yield useful biomaterials and enhance the biological impact of C8-aryl adducts.

Adducts **1**−**6** (Figure 1) were synthesized from 4-substituted phenylboronic acids and 8-Br-dG using a palladium-catalyzed Suzuki cross-coupling reaction (see the Supporting Information for details). The redox properties of **1**−**6** were studied by cyclic voltammetry in anhydrous DMF containing 0.1 M tetrabutylammonium hexafluorophosphate (TBAF) using a standard three-electrode cell with a glassy carbon (diameter 2 mm) working electrode (υ = 0.2 V/s).[16] The adducts showed irreversible one-electron oxidation peaks with half-peak potentials ($E_{p/2}$) ranging from 0.85 V/SCE for **1** up to 1.11 V/SCE for **6** (Table 1). All adducts were

Table 1. Electrochemical Data for Adducts **1**−**6** and dG[a]

adduct	X	E_p (V/SCE)	$E_{p/2}$ (V/SCE)
1	OH	0.97	0.85
2	OCH_3	1.08	0.99
3	CH_3	1.12	1.04
4	H	1.14	1.06
5	CN	1.19	1.09
6	CHO	1.20	1.11
dG		1.23	1.14

[a] Recorded in DMF containing 0.1 M TBAF and 0.5 equiv of 9,10-anthraquinone as an internal standard.

oxidized more readily than dG, which gave $E_{p/2}$ = 1.14 V/SCE under our experimental conditions.

For other aryl-substituted systems, oxidation potentials correlate with Hammett σ^+ values.[17] Figure 2 shows plots

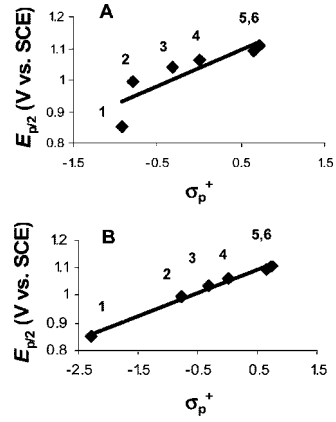
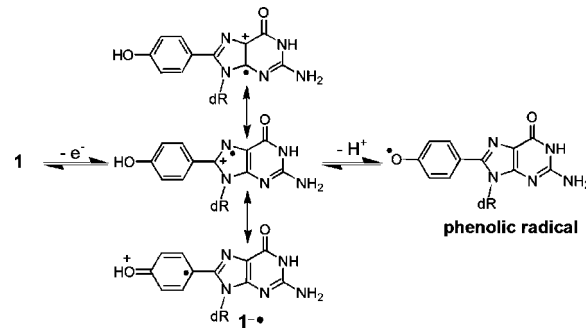


Figure 2. Plots of Hammett σ^+ versus $E_{p/2}$ (V vs SCE) for adducts **1**−**6** using the (A) σ^+ value for OH (−0.92) for **1** or (B) σ^+ value for O^- (−2.30) for **1**.

of Hammett σ^+ versus $E_{p/2}$ for adducts **1**−**6**. Interestingly, the regression deviated from linearity using the OH (−0.92) σ^+ value for **1** (Figure 1a, r^2 = 0.75), while using the O^- (−2.30[18]) σ^+ value improved the regression to almost unity (Figure 1b, r^2 = 0.99). This suggested that oxidation of **1** may be coupled with phenol deprotonation. As shown in Scheme 1, resonance structures of $\mathbf{1}^{\bullet+}$ involving the phenolic

Scheme 1. Proposed Pathway for the Oxidation of Adduct **1**



group create a para-substituted phenol radical cation that have negative pK_a values (pK_a ∼ −2 for phenol radical cation[19]).

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Phenol radical cations deprotonate rapidly in the presence of H_2O ($(0.6-6) \times 10^8\ M^{-1}\ s^{-1}$)[20] to yield neutral phenolic radicals. In DMF solvent used for electrochemical measurements, a purine N-7 adduct atom or adventitious H_2O in the solvent could serve as base to facilitate phenolic radical production. Such an event for **1** would change the oxidative chemistry of dG and form products derived from the phenolic radical intermediate.

To identify oxidation products of **1**, the adduct was treated with Na_2IrCl_6 using conditions developed by Burrows[6] and recently utilized by Rizzo to study the oxidation of C8-arylamine adducts.[5] Interestingly, the oxidation of **1** gave rise to three new products observable by HPLC (Figure 3)

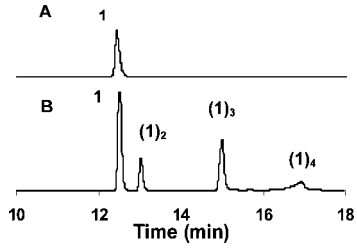

Figure 3. HPLC traces of (A) adduct **1** and (B) product mixture following oxidation of **1** with Na_2IrCl_6 at pH 7, rt.

that eluted after the starting material **1**. Analysis using negative ionization electrospray mass spectrometry (ESI^-) showed that the peak labeled $(\mathbf{1})_2$ had a parent ion at $[M - H]^- = 715$ for dimerization of **1** (359 Da) with loss of two protons (Figure 4A). The daughter ions at *m/z* 599 and 483

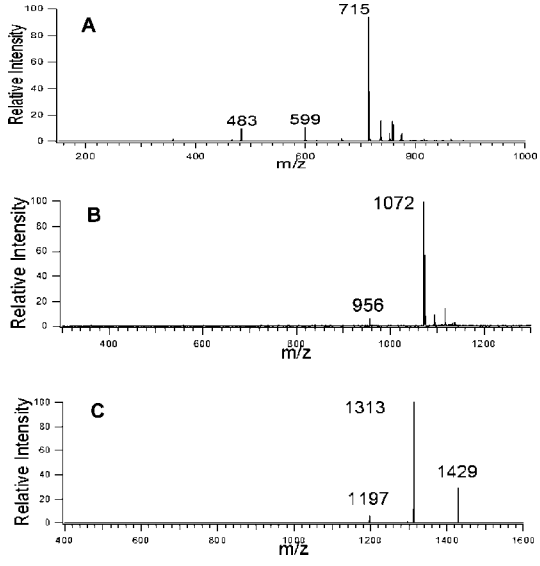

Figure 4. ESI^- spectra of (A) $(\mathbf{1})_2$, (B) $(\mathbf{1})_3$, and (C) $(\mathbf{1})_4$.

represent loss of two deoxyribose (dR, 116 Da) groups. The major product $(\mathbf{1})_3$ was 357 mass units heavier than $(\mathbf{1})_2$ ($[M - H]^- = 1072$) and showed a fragment ion at *m/z* 956 for loss of dR (Figure 4B). Additional fragment ions at 840 and 724 for the loss of two more dR groups was observed in the MS/MS spectrum of $(\mathbf{1})_3$. The broad peak for $(\mathbf{1})_4$ had $[M - H]^- = 1429$ with fragment ions at *m/z* 1313, 1197 (Figure 4C), and 1081, 965 in the MS/MS spectra for the total loss of four dR groups. The same species were also formed by treating adduct **1** with horseradish peroxidase (HRP, type VI, 25 units/mL)/H_2O_2 using conditions optimized for the polymerization of 4-hydroxybenzoic acid derivatives.[21] These results were completely consistent with oxidative coupling of **1** to form polyphenol materials through the intermediacy of the phenolic radical,[21,22] as predicted from the electrochemical experiments.

Oxidative coupling of para-substituted phenols can give rise to a range of products: O−C vs C−C.[21,22] The structure of $(\mathbf{1})_3$ was determined by NMR spectroscopy in DMSO-d_6. At room temperature, the ^1H resonances of $(\mathbf{1})_3$ were broad and unresolved. However, upon warming to 33 °C, the peaks sharpened (Figure 5) and confirmed ortho−ortho C−C coupling of **1** during oxidation by Na_2IrCl_6. The aromatic peaks of $(\mathbf{1})_3$ were found at 6.90 (H_a, 2H, d, $J = 8.0$), 7.42 (H_b, 2H, dd, $J = 2.3, 8.0$), 7.63 (H_d, 2H, s), and 7.69 (H_c, 2H, d, $J = 2.3$). These peaks were accompanied by a broad phenolic OH peak at 10.48 (3H) and a single set of dG peaks at 10.70 (NH, 3H, br s), 6.32 (NH_2, 6H, s), 6.13 (H1′, 3H, m), 4.34 (H3′, 3H, m), 3.84 (H4′, 3H, m), 3.62, 3.52 (H5′,H5″, 6H, m), 3.22,1.99 (H2′,H2″, 6H, m).

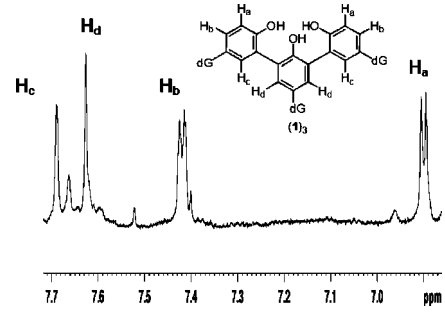

Figure 5. Aromatic region (6.9−7.7 ppm) of the 800 MHz ^1H NMR spectrum of $(\mathbf{1})_3$ recorded in DMSO-d_6 at 33 °C.

In contrast to the oxidative behavior of **1**, treatment of the methoxy adduct **2** with Na_2IrCl_6 gave rise to two new peaks (i, ii, Figure 6) that eluted before the starting material. Analysis of peak i by ESI^+ showed the presence of two products with $[M + H]^+ = 390$ and $[M + H]^+ = 408$ that are 16 (M + 16) and 34 (M + 34) mass units heavier than adduct **2** (Figure S1, Supporting Information). The M + 16

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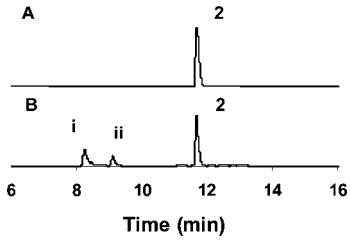
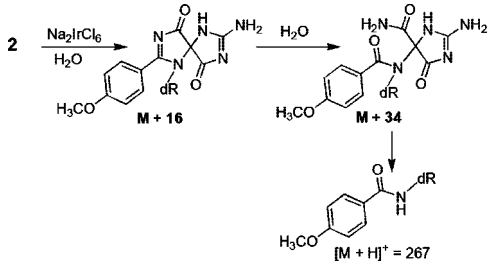


Figure 6. HPLC traces of (A) adduct **2** and (B) product mixture following oxidation of **2** with Na_2IrCl_6 at pH 7, rt.

species showed fragment ions at *m*/*z* 274 (loss of dR) and *m*/*z* 189 for loss of 85 Da from the 274 fragment. The M + 34 lesion showed fragment ions at *m*/*z* 323 for initial loss of 85 Da and *m*/*z* 292 for loss of dR. These results were consistent with spirocyclic formation to generate the M + 16 species; loss of 85 Da represents the Sp B-ring (see Figure 1).[6] The M + 34 lesion is consistent with hydrolytic opening of the Sp A-ring, as observed for the oxidation of G following treatment with dimethyl-dioxirane.[23] Peak ii had $[M + H]^+ =$ 267 and a fragment ion at *m*/*z* 151 for loss of dR (Figure S2, Supporting Information). This species was ascribed to decomposition of the M + 34 product. The transformations for the oxidation of adduct **2** are outlined in Scheme 2 and show that replacement of the OH in **1** with OCH_3 restores the well-established oxidative chemistry of the purine nucleoside.[6,23]

Scheme 2. Proposed Pathway for the Oxidation of Adduct **2**



In summary, adduct **1** undergoes oxidation more readily than dG and should represent a preferential target for further oxidation within duplex DNA. However, unlike other C8-dG adducts that undergo oxidation to form mutagenic Sp lesions through H_2O attachment to the purine radical cation, the phenolic ring of **1** is conjugated with the purine radical cation and a neutral phenolic radical intermediate is produced. Phenolic radicals undergo oxidative coupling reactions to generate polyphenol materials. Hence, the oxidation of **1** produces ortho−ortho C−C oligomeric cross-linked adducts. One can envision using such chemistry to oxidatively couple strands of DNA for the generation of new biomaterials consisting of DNAs covalently tethered by conducting polymers. Extention of this chemistry to include C8-pyrrole and C8-thiophene substituents that also undergo oxidative coupling reactions should be feasible. In addition, other nucleoside radicals are known to generate interstrand cross-links (ISCs) when formed in duplex DNA.[24] Given that ISCs are often responsible for the cytotoxic effects of DNA-damaging agents,[24,25] our efforts are focused on incorporating **1** into an oligonucleotide to determine the fate of the phenoxyl radical within DNA.

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Supporting Information Available: Experimental procedures, NMR spectra of **5** and **6**, and Figures S1, S2 described in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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