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Novel oxidation products from guanine nucleosides reacted with dimethyldioxirane

R. Jeremy H. Davies,*[a] Clarke Stevenson,[a] Shiv Kumar,[a] Jason Lyle,[a] Lisa Cosby,[a] John F. Malone,[b] Derek R. Boyd,[b] Narain D. Sharma,[b] Ann P. Hunter[c] and Bridget K. Stein[c]

[a] *School of Biology and Biochemistry, Queen's University, Belfast, UK BT7 1NN*

[b] *School of Chemistry, Queen's University, Belfast, UK BT7 1NN*

[c] *EPSRC National Mass Spectrometry Service Centre, University of Wales Swansea, Swansea, UK SA2 8PP. E-mail: j.davies@qub.ac.uk; Fax: +44-28-9023-6505; Tel: +44-28-9027-2102*

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Treatment of guanosine or 2′-deoxyguanosine with dimethyldioxirane, followed by heating in aqueous solution, generates respectively 4-amidinocarbamoyl-5-hydroxyimidazole (1) or its 2-(2,3,4-trihydroxybutyl) derivative (2).

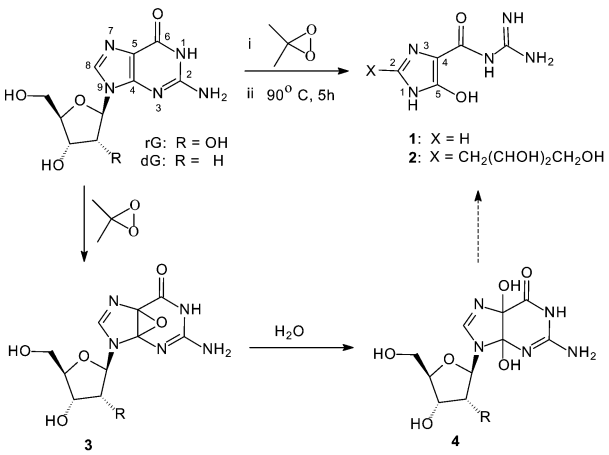
Reflecting its relatively low redox potential, guanine is more susceptible to oxidative modification than the other nucleobases in DNA.[1] It is thus the main target for genotoxic damage caused by type I photosensitizers,[2–4] singlet oxygen,[5] and a variety of one- and two-electron oxidants.[6,7] In each of these cases, the cognate reaction mechanisms have been extensively investigated by using oligonucleotides and nucleosides as model compounds. It has emerged that the guanine base moiety can be transformed into a remarkable range of intermediate species and final oxidation products that include *inter alia* 8-oxo-7,8-dihydroguanine,[1] 5,8-dihydroxy-7,8-dihydroguanine,[7] 2,5-diaminoimidazol-4-one,[8] 2,2,4-triamino-oxazolone,[8] spiroiminodihydantoin[9] and oxaluric acid.[7]

Here, we present evidence for a new pathway of guanine nucleoside oxidation that is initiated by treatment with dimethyldioxirane (DMD). Our interest in the action of this powerful oxidant stems from our earlier demonstration[10] that DMD is a useful chemical sequencing reagent which selectively introduces piperidine-labile lesions at the sites of guanine bases in single- and double-stranded DNA. DMD was prepared according to Adam *et al.*[11] as a 0.1 M solution in acetone. To carry out the oxidation of guanosine (rG) or 2′-deoxyguanosine (dG), a 2.5-fold molar excess of DMD was added to an aqueous solution containing 50 mM of the nucleoside at room temperature. The addition of DMD was accompanied by transient development of a red–purple coloration that quickly faded to leave a solution that had completely lost the characteristic UV absorption of the guanine chromophore. It was discovered, however, that when each reaction mixture was subsequently heated at 70–90 °C to drive off acetone this led to the gradual formation of a UV-absorbing product with λ_{max} 302 nm. The maximal yield (estimated spectroscopically as ~25% for rG, and ~35% for dG) was obtained by heating at 90 °C for 5 h.

The UV-absorbing species derived from rG and dG, denoted **1** and **2** respectively (Scheme 1), were isolated by the same procedure. First the concentrated reaction mixture was loaded onto a column of Sephadex® G-10 which was washed with 3 volumes of water to remove unwanted material. Crude product was then eluted from the column with 0.05% trifluoroacetic acid (TFA). The eluant was concentrated by rotary evaporation and then fractionated by semi-preparative reversed-phase HPLC on a phenomenex® Jupiter 10μ C18 column. When a linear gradient of 0–30% methanol in 0.05% TFA was applied, **1** and **2** were eluted as single peaks (detected at 254 nm) which were collected and evaporated to obtain homogeneous samples. Their elemental compositions, as determined by HRMS-ES, were $C_5H_7N_5O_2$ in the case of **1** (Found: *m*/*z* 170.0679; [*M* + H^+] requires 170.0678) and $C_9H_{15}N_5O_5$ in the case of **2** (Found: *m*/*z* 274.1152; [*M* + H^+] requires 274.1151). When heated with 1 M NaOH at 65 °C for 8 h, both compounds liberated guanidine which was detected by adduct formation with 1,2-naphthoquinone-4-sulfonic acid.[8]

The identity of product **1** as 4-amidinocarbamoyl-5-hydroxyimidazole was assigned on the following basis. Comparison of ^{1}H NMR spectra recorded in DMSO-d_6 and D_2O showed it to contain six D_2O-exchangeable protons, accounted for by broad resonances in the range δ 7.6–13.5, and one very slowly exchanging proton represented by a sharp singlet at δ 8.15. The latter characteristics are typical of a proton attached to C-2 of an imidazole ring and this interpretation was supported by ^{13}C NMR and ^{13}C-^{1}H heteronuclear correlation measurements. The proton at δ 8.15 was shown by HMQC to be directly bonded to a carbon atom with δ_C = 131.0 ppm, while HMBC revealed multibond couplings to carbons at 100.6 and 159.3 ppm respectively. These values are very similar to the chemical shifts reported for the corresponding imidazole ring carbons in 4-carbamoyl-5-hydroxyimidazole,[12] *viz.* 126.3, 100.3 and 156.3 ppm respectively. The two remaining carbons from the amidinocarbamoyl side chain of **1** give rise to ^{13}C signals at 155.5 and 160.0 ppm. In keeping with the structure proposed for **1**, MS/MS analysis of its molecular ion at *m*/*z* 170 showed that it decomposes to three fragment ions that can be attributed as follows: *m*/*z* 86 [$NH_2C(NH)NHCO^+$], *m*/*z* 85 [protonated 5-hydroxyimidazole] and *m*/*z* 60 [protonated guanidine]. Confirmation of the molecular structure was provided by X-ray crystallographic analysis[13] of the perchlorate salt of **1** (Fig. 1). Within the crystal, the molecules of **1** exist as imidazolium-5-olate zwitterions[12] and are protonated on the exocyclic guanidino group. Notably, the UV absorption of **1** is considerably red-shifted (by ~25 nm) relative to compounds bearing a simple 4-carbamoyl substituent but, like them, it shows little change in λ_{max} over a wide range of pH.[12]

The dG oxidation product **2** exhibits the same UV absorption profile as **1** but is optically active with $[\alpha]_D$ = −17 (*c* = 0.5, H_2O), showing it to be a non-racemic chiral compound. Its structure was established by NMR spectroscopy. Remarkably,



Scheme 1

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the ^{1}H spectrum of **2** in D_2O was not only devoid of signals downfield of δ 4.2, thus indicating substitution at C-2 of the imidazole ring, but it was practically superimposable on the spectrum[14] of 8-(2,3,4-trihydroxybutyl)guanine. The presence of a trihydroxylated *n*-butyl group was confirmed by 2D COSY measurements which led to the same peak assignments as in reference 14. The ^{13}C NMR spectrum of **2** showed four signals (all assigned) in the range δ_C 30–75 for the sp^3 carbons of the side chain, and five more for the residual sp^2 carbons. Four of the latter were within 0.5 ppm of the chemical shifts recorded for **1** but a peak at δ_C 142.8 replaced that at δ_C 131.0 in the spectrum of **1**. Assignment of this signal to C-2 was confirmed by HMBC analysis. As in the case of **1**, MS/MS measurements revealed that the molecular ion of **2** fragments predominantly to a [M + H − 85] species with m/z 189 that corresponds to the protonated 2-substituted hydroxyimidazole moiety. Subsequent fragmentation sequences are consistent with the successive elimination of three water molecules from the trihydroxybutyl side-chain, generating major ions at m/z 171, 153 and 135.

It is evident that **1** and **2** are formed by multi-step reaction mechanisms that remain to be clarified. We postulate, however, that (as indicated by chromophore loss) the reaction is initiated by oxygen insertion across the central 4,5-double bond of the guanine nucleus to yield an epoxide **3** (Scheme 1) which then undergoes hydrolysis to a 4,5-dihydrodiol **4**. Such epoxidation within the fused pyrimidine ring of guanine, and subsequent *vic*-diol formation, finds precedent in the reaction of DMD with methylated uracils.[15]

Ostensibly, DMD differs fundamentally from other types of oxidants in its action towards guanine nucleosides. However, a parallel for the introduction of a trihydroxybutyl group into the imidazole ring is afforded by the conversion of dG into 8-(2,3,4-trihydroxybutyl)guanine when it is subjected to photosensitization with acetone under anaerobic conditions.[14] The mechanism of this process has not yet been elucidated. Finally, it is important to ascertain whether the reactivity described here for dG extends to the polynucleotide level and thereby underpins the function of DMD as a guanine-specific DNA sequencing agent.[10] The oxidative lesions produced in DNA by DMD may offer interesting new substrates for studies of molecular mutagenesis and DNA repair.

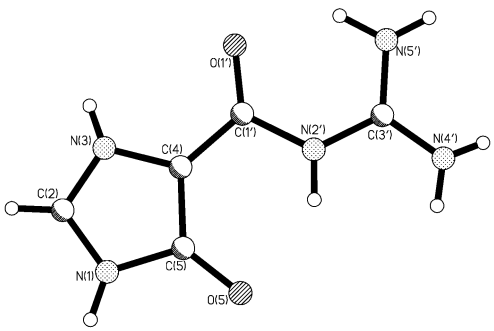

Fig. 1 X-Ray structure of the perchlorate salt of **1** (anion omitted).

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13 Crystal data for **1**. $HClO_4$: $C_5H_8ClN_5O_6$, M = 269.6, thin colourless plate, 0.30 × 0.12 × 0.015 mm, triclinic, space group $P1$, a = 4.825(3), b = 9.812(5), c = 10.052(5) Å, α = 100.44(1), β = 94.62(1), γ = 91.42(1)°, V = 466.1(4) Å^3, Z = 2, D_c = 1.921 g cm^{-3}, T = 150(2) K, $F(000)$ = 276, Bruker SMART CCD diffractometer, 2123 reflections, 1359 observed ($>2\sigma(I)$), 186 parameters, Final R_1 = 0.049, wR_2 = 0.125 (all data), GoF = 0.98. CCDC reference number 184182. See http://www.rsc.org/suppdata/cc/b2/b201418c/ for crystallographic files in .cif format.
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