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Oxidative hydrolysis of a cyclic 1,N^2-propano-2′-deoxyguanosine, an adduct of 2′-deoxyguanosine with acetaldehyde or crotonaldehyde☆

Magoichi Sako,* Shinsuke Inagaki, Yukihiro Esaka and Yoshihiro Deyashiki

Gifu Pharmaceutical University, 5-6-1, Mitahora-higashi, Gifu 502-8585, Japan

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Abstract—The $SO_4^{\bullet-}$-oxidation of cyclic 1,N^2-propano-2′-deoxyguanosine, chemo- and regioselectively produced in the reaction of 2′-deoxyguanosine with excessive acetaldehyde or crotonaldehyde, resulted in the smooth formation of (4-hydroxy-5-hydroxymethyltetrahydrofuran-2-ylimino)-(4-hydroxy-6-methyltetrahydropyrimidin-2-ylideneamino)acetic acid, 3-(4-hydroxy-5-hydroxymethyltetrahydrofuran-2-yl)-6-methyl-3*H*-1,3,4,5,8a-pentaazacyclopenta[*b*]naphthalen-9-one, and 2′-deoxyguanosine even under neutral conditions. The formation of the guanine-ring opened product during the reaction is very interesting and appears to closely relate to the mechanisms for the point-mutations of DNA by these mutagenic and carcinogenic aldehydes.

Current attention has been paid to the characteristic chemical reactivity of cyclic 1,N^2-propanoguanine adducts (cf. **1**) (Fig. 1), chemo- and regioselectively produced in the reactions of DNA and 2′-deoxyguanosine with excessive acetaldehyde and crotonaldehyde, in relation to genotoxic, mutagenic, and carcinogenic effects of these aldehydes and their precursors.[1,2] These aldehydes have been shown to mainly induce G–A transitions and G–T transversions in the HPRT reporter gene in human peripheral T cells[3,4] and in the supF gene of the shuttle vector plasmid pMY189 replicated in human fibroblast cells.[5] Recently, the cyclic adducts have been documented to form reversible interchain cross-links by an intermolecular condensation of the aldehyde group in its ring-opening equivalent (cf. **1′**) with the exocyclic amino group of another juxtaposed guanine moiety in the double-helical DNA[6] or with the N-terminal amines of peptides.[7] The point-

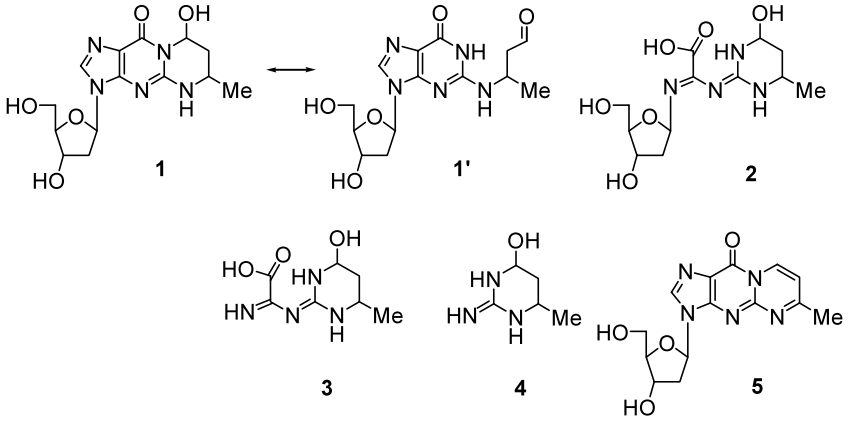

Figure 1.

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* Corresponding author. Tel.: +81-(58)237-3931; fax: +81-(58)237-5979; e-mail: sako@gifu-pu.ac.jp

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mutations by acetaldehyde and crotonaldehyde, however, cannot be explained by considering the interchain cross-link formation since they induce, in principle, absolute blocking of DNA replication. During the course of our investigations on the formation and chemical reactivity of cyclic 1,N^2-propano-2′-deoxyguanosine (**1**),[8,9] we found that the adduct **1** is very sensitive during a single-electron oxidation and easily causes an oxidative degradation of the guanine ring even under neutral conditions.

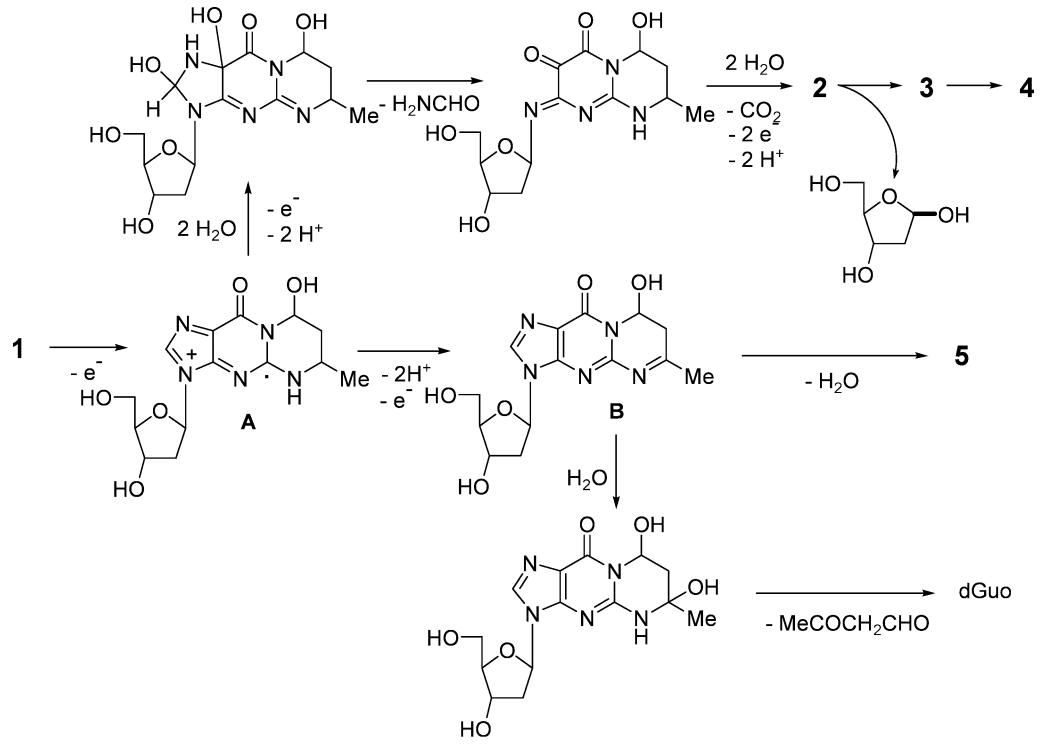
The cyclic adduct **1** is stable in pH 5.5–8.5 phosphate buffers even under reflux for 8 h.[10] In sharp contrast to the high stability under thermal conditions, the adduct **1** [E^{ox}_p = +1.19 V versus SCE (in DMF)] was extremely labile in the presence of a single-electron acceptor, e.g. sulfate radical anion ($SO_4^{\bullet-}$) which can be generated almost quantitatively by the simple treatment of ammonium persulfate at 80°C,[11] resulting in a smooth consumption even under neutral conditions (e.g. in pH 7.0 phosphate buffer under argon atmosphere for 1 h) to give complex mixtures. On the other hand, N^2-unsubstituted 2′-deoxyguanosine [E^{ox}_p = +1.33 V versus SCE (in DMF)] and N^2-ethyl-2′-deoxyguanosine [E^{ox}_p = +1.30 V versus SCE (in DMF)][12] were very stable under the employed oxidation conditions in which the starting materials were recovered even after a prolonged reaction time (e.g. 3 h). The ODS column chromatographic separation of the reaction mixture that was obtained in the $SO_4^{\bullet-}$-oxidation of **1** allowed the isolation of (4-hydroxy-5-hydroxymethyltetrahydrofuran-2-ylimino)(4-hydroxy-6-methyltetrahydropyrimidin-2-ylideneamino)acetic acid (**2**) (25%), (4-hydroxy-6-methyltetrahydropyrimidin-2-ylideneamino)iminoacetic acid (**3**) (8%), 2-imino-6-methylhexahydropyrimidin-4-ol (**4**) (26%), 3-(4-hydroxy-5-hydroxymethyltetrahydrofuran-2-yl)-6-methyl-3*H*-1,3,4,5,8a-pentaazacyclopenta[*b*]-naphthalen-9-one (**5**) (3%), ribose (trace), and 2′-deoxyguanosine (4%), together with the recovered starting adduct **1** (16%) and small amounts of undetermined products. An analogous product distribution was observed for the $SO_4^{\bullet-}$-oxidation of the cyclic adduct **1** at 55°C, though most of the starting **1** was recovered. The structures of the products **2**, **3**, **4**, and **5** were assigned on the basis of their spectral data and/or independent syntheses. The remarkable spectral changes in the key product **2** from the starting adduct **1** are as follows: (a) disappearance of one proton signal for the imidazole-ring from the aromatic region [δ_H 7.72 (δ_C 137.9) ppm for **1**] and of a carbonyl group [ν 1686 (δ_C 157.8 ppm) cm^{-1} for **1**], and the appearance of a new carbonyl moiety [ν 1730 cm^{-1} for **2**] in the IR and ^{1}H NMR spectra were observed, (b) a significant shift in the two proton signals assignable to the C_8- (for **1**: C_4- for **2**) and $C_{1'}$-protons to the high fields [δ_H 6.05 (m) and 5.97 (t, J = 6 Hz) ppm for **1**; δ_H 5.07 (m) and 5.66 (t, J = 6 Hz) ppm for **2**] in the ^{1}H NMR spectrum was observed, implying a drastic structural change in the guanine ring during the reaction.

To determine the oxygen-atom sources of the carboxyl group in the ring-opened product **2**, the $SO_4^{\bullet-}$-oxidation of **1** was carried out in 0.1 M phosphate buffer (pH 7.0) containing ^{18}O-labeled water under similar conditions. The FAB-MS and IR spectra of the product obtained in this reaction showed the regioselective insertion of the ^{18}O-labeled oxygen atoms into the carboxyl and C_4-hydroxyl moieties in **2** {a molecular ion peak (m/z 323; calcd for $C_{12}H_{21}N_4{}^{16}O_3{}^{18}O_3$) for the labeled **2**, a loss of $C^{18}O_2$ from the labeled molecular ion [m/z 275 (MH^+–48)], and a fragment ion peak (m/z 132) for the ^{18}O-labeled hexahydropyrimidinol $C_5H_{12}N_3{}^{18}O$ (cf. **4**) under ionizing radiation were observed in the mass spectrum; the stretching vibration band of the carboxyl group [ν 1730 cm^{-1} for **2**] was shifted to a lower field [ν 1710 (sh) cm^{-1} for ^{18}O-labeled **2**]}. These facts strongly suggest that the oxygen atoms at the carboxyl group and C_4-hydroxyl group in **2** originated from the water molecule in the media. Based on these observations, we present a possible reaction sequence for the formation of **2** in the present reaction involving a single-electron oxidation[13] of the electron-rich guanine ring by $SO_4^{\bullet-}$ followed by hydrolytic degradation of the guanine ring as shown in Scheme 1.

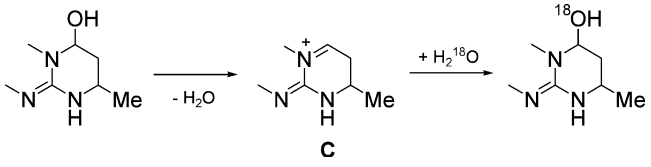
During this reaction, the exchange of the hydroxyl group in the hexahydropyrimidine ring by water also proceeds via an intermediary formation of the corresponding immonium salt (**C**) (Scheme 2). The mode of this exchange reaction at the C_4-position in the hexahydropyrimidine ring is analogous to that proposed for the interchain cross-linking.[6,7] Under the employed conditions, the product **2** appears to undergo further hydrolysis to give the deglycosidated products **3** and **4**.

The formation of the minor products, a tricyclic compound **5** and 2′-deoxyguanosine, in this reaction can be reasonably explained taking the chemical reactivity of the radical cation intermediate (**A**) into consideration; the radical cation **A** should undergo dehydrogenation proceeding via further single-electron oxidation by another $SO_4^{\bullet-}$ to give a transient intermediate (**B**) which could undergo the dehydration to give the ultimate product **5** and the concurrent hydrolysis leading to 2′-deoxyguanosine with a loss of butane-1,3-dione. Therefore, the isolation of these minor products provide productive evidence for the initial formation of the radical cation **A** in the $SO_4^{\bullet-}$-oxidation of **1**.

Characteristics of the cyclic adduct **1** on the chemical reactivity are 1) the adduct itself is very stable under thermal conditions in the pH 5.5–8.5 buffer solutions and more easily undergoes a single-electron oxidation compared with the case of the unmodified guanines which are most sensitive to oxidations among the nucleobases,[14] and 2) the degradation of the guanine moiety proceeds via the hydrolysis of the generated radical cations not via the homolytic coupling with a molecular oxygen.[15–19] The G–A transitions and G–T transversions mainly induced by acetaldehyde and crotonaldehyde in human cells can be reasonably explained by considering the effects of base-pairings suitably fitted with a tautomeric form of the ring-opened product (cf.



Scheme 1. A reaction sequence for the $SO_4^{\bullet-}$-oxidation of **1**.



Scheme 2. A plausible mode for the OH-exchange at the C_4-position.

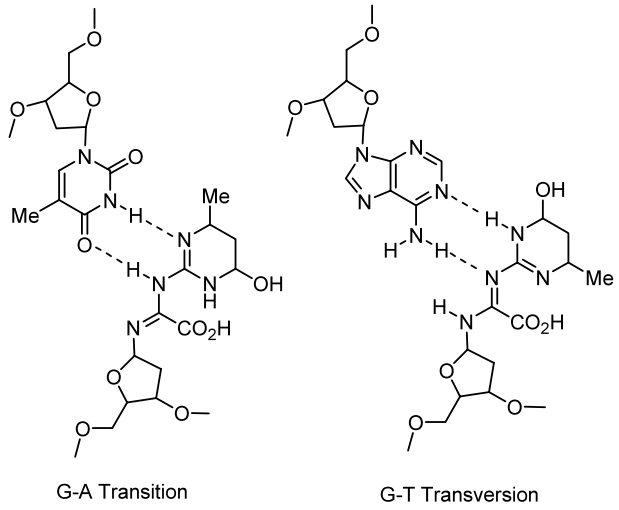


Figure 2. Possible base-pairings of the ring-opened product **2**.

2) (see Fig. 2). Thus, the formation of the guanine-ring opened product **2** during the oxidative hydrolysis of the cyclic 1,N^2-propanoguanine adduct **1** is very interesting and appears to closely relate to the mechanisms for the point-mutations of DNA by these mutagenic and carcinogenic aldehydes.

Experimental

*Oxidation of the cyclic adduct **1** with ammonium persulfate*: To a solution of the adduct **1** (163.6 mg, 0.5 mmol) in 0.1 M phosphate buffer (pH 7.0) (4.0 mL) was added $(NH_4)_2S_2O_8$ (228.2 mg, 1.0 mmol) and then the mixture was stirred at 80°C under argon atmosphere for 1 h. The resulting mixture was subjected to Sep-Pak column eluting with 0%, 10%, and 20% methanol-containing water (each 50 mL) to isolate (4-hydroxy-5-hydroxymethyltetrahydrofuran-2-yl-imino)-(4 - hydroxy - 6 - methyltetrahydropyrimidin - 2 - ylideneamino)acetic acid (**2**) (39.5 mg, 25%), (4-hydroxy-6-methyltetrahydropyrimidin-2-ylideneamino)iminoacetic acid (**3**) (8.0 mg, 8%), 2-imino-6-methylhexahydropyrimidin-4-ol (**4**) (16.7 mg, 26%), 3-(4-hydroxy-5-hydroxymethyltetrahydrofuran-2-yl)-6-methyl-3*H*-1,3,4,5,8a-pentaazacyclopenta[*b*]naphthalen-9-one (**5**) (4.8 mg, 3%), and 2′-deoxyguanosine (5.7 mg, 4%), together with the recovered starting adduct **1** (26.1 mg, 16%), ribose (trace; identified with ^{1}H NMR spectral and TLC analyses), and undetermined product (ESI MS *m*/*z* 185, 168).

For **2**: mp 190°C (decomp., from MeOH); IR (KBr) 3400, 3300 (sh), 1730, 1650, 1620, 1525, 1450 cm^{-1}; UV (H_2O) (ε) λ_{max} 225 (sh, 1.1×10^4) nm; ^{1}H NMR (D_2O) δ 1.19 (3H, d, $J=6$ Hz), 1.53 (1H, br t, $J=12$ Hz), 1.94 (1H, br d, $J=12$ Hz), 2.16 (2H, br t), 3.55 (2H, m), 3.69 (1H, m), 3.86 (1H, br s), 4.30 (1H, br s), 5.07 (1H, br s),

5.66 (1H, t, $J=6$ Hz); ^{13}C NMR (CD_3OD) δ 163.2, 162.9, 157.8, 89.0, 84.4, 73.7, 73.4, 64.2, 43.5, 41.9, 36.6, 20.8; FAB MS m/z 317 $[MH]^+$, 273, 201, 130; HR-FAB MS m/z $[MH]^+$ 317.1470 (calcd for $C_{12}H_{21}N_4O_6$ $[MH]^+$ 317.1461).

For **3**: mp 220–225°C (decomp., from MeOH–Et_2O); IR (KBr) 3308, 3224, 1726, 1658, 1626 cm^{-1}; UV (MeOH) λ_{max} 231 (sh), 213 nm; ^{1}H NMR (DMSO-d_6) δ 1.34 (3H, d, $J=6$ Hz), 1.63 (1H, br t, $J=12$ Hz), 2.00 (1H, br d, $J=12$ Hz), 3.82 (1H, m), 5.19 (1H, br s), 6.59 (1H, br d, $J=4$ Hz), 9.19 (1H, br s), 9.68 (1H, br s), 11.5 (1H, br); ESI MS m/z 202 $[MH]^+$, 156, 130.

For **4**: mp 188–190°C (from MeOH–Et_2O); IR (KBr) 3356, 3224, 3091, 1678, 1620 cm^{-1}; UV (H_2O) (ε) λ_{max} 227 (sh, 4.0×10^3) nm; ^{1}H NMR (DMSO-d_6) δ 1.23 (3H, d, $J=6$ Hz), 1.45 (1H, br t, $J=12$ Hz), 1.89 (1H, br d, $J=12$ Hz), 3.63 (1H, m), 4.95 (1H, br s), 6.32 (1H, br), 7.70 (1H, br), 9.10 (1H, br); ^{13}C NMR (DMSO-d_6) δ 155.9, 72.9, 42.9, 37.4, 21.6. HR-FAB MS m/z $[MH]^+$ 130.0988 (calcd for $C_5H_{12}N_3O$ $[MH]^+$ 130.0980). The structure of this compound was confirmed by its independent synthesis involving the thermal condensation of guanidine with crotonaldehyde.

For **5**: mp 256–258°C (decomp., from MeOH); IR (KBr) 3376, 3253, 3112, 1731, 1699, 1644 cm^{-1}; UV (H_2O) (ε) λ_{max} 335 (sh, 3.3×10^3), 317.5 (4.4×10^3), 249 (1.65×10^4), 214 (2.18×10^4) nm; Fl (H_2O) λ_{max} 495 (Ex 350) nm; ^{1}H NMR (CD_3OD) δ 2.49 (1H, m), 2.70 (3H, s), 2.79 (1H, m), 3.81 (2H, dd, $J=12$ and 3 Hz), 4.05 (1H, m), 4.59 (1H, m), 6.53 (1H, t, $J=6$ Hz), 7.20 (1H, d, $J=7$ Hz), 8.45 (1H, s), 9.29 (1H, d, $J=7$ Hz); FAB MS m/z 318 $[MH]^+$, 202; HR-FAB MS m/z $[MH]^+$ 318.1208 (calcd for $C_{14}H_{16}N_5O_4$ $[MH]^+$ 318.1202).

The structures of isolated 2′-deoxyguanosine and ribose were identified in every respects with those of the authentic compounds.

The sources of oxygen atoms in the ring-opened product ***2***: To a solution of the adduct **1** (33.8 mg, 0.1 mmol) in the ^{18}O-labeled buffer [prepared by dissolving the residue, which was obtained after evaporation of the non-labeled 0.1 M phosphate buffer (pH 7.0) (1.0 mL) to dryness, in ^{18}O-labeled water (EURISO-TOP, 99.8% atom ^{18}O) (1.0 mL)] was added $(NH_4)_2S_2O_8$ (45.6 mg, 0.2 mmol) and then the mixture was stirred at 80°C under argon atmosphere for 1 h. The resulting mixture was subjected to Sep-Pak column eluting with 0%, 10%, and 20% methanol-containing water (each 50 mL). The UV-positive fractions eluted with 10% methanol-containing water were collected, evaporated to dryness, and triturated with diethyl ether to obtain the desired product (1.8 mg) as a 3: 2 mixture of triply ^{18}O-labeled **2** and doubly ^{18}O-labeled **2**. The product ratio was estimated by the intensity of the fragment peaks (the intensity of m/z 323: 321, m/z 275: 273, and m/z 132: 130 were each 3: 2) in its mass spectrum.

^{18}O-labeled **2**: IR (KBr) 1710, 1650, 1620, 1525, 1450 cm^{-1}; HR-FAB MS m/z $[MH]^+$ 323.1597 (calcd for triply ^{18}O-labeled **2**: $C_{12}H_{21}N_4{}^{18}O_3{}^{16}O_3$ $[MH]^+$ 323.1588), m/z $[MH]^+$ 321.1550 (calcd for doubly ^{18}O-labeled **2**: $C_{12}H_{21}N_4{}^{18}O_2{}^{16}O_4$ $[MH]^+$ 321.1546), m/z 275.1607 (calcd for $C_{11}H_{21}N_4{}^{18}O^{16}O_3$ $[MH-C^{18}O_2]^+$ 275.1605), m/z 273, m/z 132 ($C_5H_{11}N_3{}^{18}O$), m/z 130 ($C_5H_{11}N_3{}^{16}O$).

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