Communications to the Editor

Reaction of *cis*- and *trans*-2-Butene-1,4-dial with 2′-Deoxycytidine to Form Stable Oxadiazabicyclooctaimine Adducts

Lakshmaiah Gingipalli and Peter C. Dedon*

Division of Bioengineering & Environmental Health
56-787, Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

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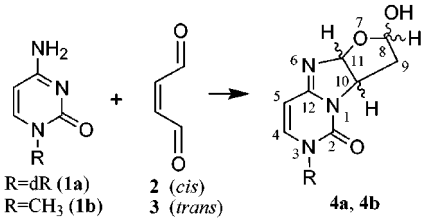
cis-2-Butene-1,4-dial (**2**) is a metabolite of furan,[1] an industrially important chemical found in food and cigarette smoke.[2] This electrophile is also produced by oxidation of the 5′-position of 2′-deoxyribose in DNA.[3] Like other unsaturated aldehydes, *cis*-2-butene-1,4-dial readily reacts with nucleophilic sites in proteins and glutathione,[4] and it has been shown to cause DNA damage.[5] With implications for the known carcinogenicity of furan,[2] recent studies have demonstrated that *cis*-2-butene-1,4-dial is mutagenic in the Ames test.[6] However, the DNA damage that is responsible for this mutagenicity, and possibly the carcinogenicity of furan, has eluded study. Structurally related compounds, such as the lipid peroxidation products malondialdehyde, 4-hydroxynonenal, and 2,4-decadienal, as well as 4-oxo-2-pentenal, arising from oxidation of α-acetoxy-*N*-nitrosopiperidine, react with DNA to form stable adducts,[7] many with demonstrated mutagenicity.[8] However, preliminary studies suggest that *cis*-2-butene-1,4-dial reacts with nucleosides to form unstable adducts.[6] We now report the structure of stable and novel adducts formed in a reaction of *cis*-2-butene-1,4-dial (**2**) with dC (Scheme 1) under biological conditions, adducts that may play a role in the mutagenic properties of this metabolite.

As judged by loss of parent 2′-deoxynucleoside, the reactivity of both *cis*- (**2**) and *trans*-2-butene-1,4-dial[9] (**3**) with 2′-deoxynucleosides occurred in the order dC ≫ dA > dG.[10] The extent of reaction with dC led us to focus on the sole products formed with *cis*- or *trans*-2-butene-1,4-dial, a pair of closely eluting HPLC peaks.[10] Three observations suggested that the two peaks contained adducts in equilibrium: (1) both peaks had identical UV spectra; (2) purification, concentration, and HPLC analysis of either peak produced a mixture of the two; and (3) the peaks were always present in the same proportion after several hours of incubation. Given these characteristics, all subsequent analyses were performed on the products contained in the pair of HPLC peaks.

Scheme 1



Analysis of the dC adducts by positive ion electrospray ionization (ESI) mass spectrometry revealed a single protonated molecular ion at *m*/*z* 312.[11] This result is consistent with the addition of a single molecule of 2-butene-1,4-dial to dC.

The ^{1}H NMR spectrum[12] revealed three D_2O-exchangeable and fourteen nonexchangeable protons. In DMSO, all signals were split except those assigned to 2′-deoxyribose. Expansion of the spectrum revealed splitting of the chemical shifts into four sets of signals. This is consistent with four diastereomeric adducts involving the cytosine base. Expansion of the ^{13}C NMR spectrum in DMSO also revealed four sets of signals for each carbon except the C5, C9, and C2′ positions (Scheme 1) for which the splitting was not completely resolved. This pattern of splitting for an adduct of dC with a single molecule of *cis*-2-butene-1,4-dial is again consistent with a mixture of four diastereomers. A DEPT experiment revealed three methylene carbons and eight methine carbons in each set of ^{13}C chemical shifts. The absence of signals for two carbons in the DEPT spectrum is consistent with two quaternary carbons.

To rule out reaction with 2′-deoxyribose, we treated 1-methylcytosine (**1b**) with *cis*- and *trans*-2-butene-1,4-dial.[13] Under conditions identical to those used with dC, HPLC analysis revealed a single major peak with a retention time of 14.2 min for both *cis*- and *trans*-2-butene-1,4-dial. Subsequent ^{1}H- and ^{13}C NMR analysis (vide infra) indicated that the peak contained two diastereomeric adducts that were identical for the two isomers of 2-butene-1,4-dial.[12] Given this fact and the ease of synthesis of *trans*-2-butene-1,4-dial, large-scale adduct synthesis was accomplished using the *trans* isomer.[14]

The 1-methylcytosine adducts exhibited a pH-sensitive UV spectrum with a single λ_{max} at 285 nm for pH 2.8 and 7.4, while two maxima were observed at 228 and 285 nm when the pH was

* To whom correspondence should be addressed. Telephone: 617-253-8017. Fax: 617-258-0225. E-mail: pcdedon@mit.edu.

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(9) *cis*- (**2**) and *trans*-2-butene-1,4-dial (**3**) were synthesized as described elsewhere.[1] Purity was verified by ^{1}H NMR.

(10) **1a** (2 mM) was reacted with **2** or **3** (0.2−8 mM) in 50 mM potassium phosphate pH 7.4 at 37 °C, and at various times, products were analyzed by C18 reversed phase HPLC (Hewlett-Packard Model 1100 with a model 1040A diode array detector) with a 0−10% acetonitrile gradient in 50 mM ammonium acetate. Both **2** and **3** reacted with **1a** to form products eluting at 15.2 and 15.5 min.

(11) Mass spectra were recorded on Hewlett-Packard 5989B electrospray ionization and Finnigan 8200 electron ionization mass spectrometers.

(12) Complete spectroscopic details will be published. Spectral data is available as Supporting Information. NMR spectra were recorded on 400 and 500 MHz Varian and Bruker spectrometers.

(13) **1b** was synthesized as described in Papaulis, A.; al-Abed, Y; Bucala, R. Biochemistry **1995**, *34*, 648−655; physical properties and spectral data were identical to reported values.

(14) **1b** (0.05M) was reacted with **3** (0.1M) in 50 mM potassium phosphate pH 7.4 for 12 h at 37 °C. Following extraction with CH_2Cl_2, the aqueous phase was concentrated under vacuum. The crude residue, dissolved in 1:1 CH_3OH and CH_2Cl_2 and passed through a silica gel plug to remove phosphate salts, was purified by silica gel chromatography (20% CH_3OH in CH_2Cl_2). Following analysis by silica gel TLC (12% CH_3OH in CH_2Cl_2), adduct-containing column fractions were combined and concentrated under vacuum. Pure adducts were obtained by recrystallization from ethanol to yield colorless crystals. Spectral data were identical for adducts produced by both **2** and **3**; see Supporting Information.

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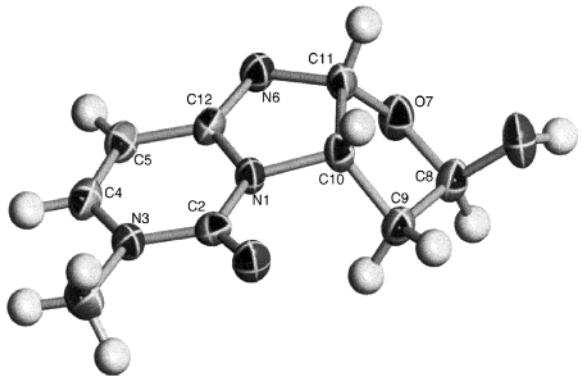

Figure 1. Crystal structure of the adduct of 2-butene-1,4-dial with 1-methylcytosine.

raised to 12. This pH sensitivity is characteristic of cytosine N^4-substitution.[15] The positive ion ESI mass spectrum of this compound exhibited a protonated molecular ion at *m*/*z* 210, while the EI mass spectrum revealed a molecular ion at *m*/*z* 209.[11] This again accounts for one molecule each of 1-methylcytosine and 2-butene-1,4-dial.

With the exception of the switch from deoxyribose to a methyl group, the ^{1}H NMR spectra of the 1-methylcytosine adducts in D_2O and DMSO were nearly identical to those for the dC adducts. A ^{1}H COSY experiment supported the assignment of signals, with connectivity established by H−H correlation. Unlike the dC adduct, however, there were two D_2O-exchangeable protons and only two sets of signals in DMSO,[12] results that are consistent with the presence of two diastereomers. This interpretation was supported by the ^{13}C NMR spectrum of the 1-methylcytosine adducts, which yielded two signals for each carbon. A DEPT experiment revealed one methylene carbon and one methyl carbon, with the remaining signals identified as methine carbons. Again, the absence of signals for two carbons was consistent with a pair of quaternary carbons.

Consideration of all of the data for the dC and 1-methylcytosine adducts led to several possible structures. For a more definitive structure, we subjected the 1-methylcytosine adduct to X-ray crystallographic analysis.[16] The structure shown in Figure 1 is consistent with the spectral data[12] and confirms the assignment of a novel oxadiazabicyclo(3.3.0)octaimine adduct of 1-methylcytosine (**4b** in Scheme 1). The crystal structure is also consistent with the spectral data for the dC adduct,[12] which leads us to conclude that the base adducts of dC and 1-methylcytosine are identical. With three chiral centers in the base adduct, there are eight possible disastereomers for the dC adduct and four diastereomers, each with an enantiomeric partner, for the 1-methylcytosine adduct. However, ring strain precludes formation of the four structures with a *trans* configuration about the C10 and C11 ring junction, which leaves four structures with a *cis* conformation (Figure 1). The NMR spectral data[12] is thus entirely consistent with the presence of four diastereomers for the dC adduct and two for the 1-methylcytosine adduct. Although the hemiacetal at C8 is relatively stable as a five-membered ring, equilibrium hydrolysis of the hemiacetal would explain the interconversion of the diastereomers.

The question of the stability of the dC and 1-methylcytosine adducts was addressed as a function of pH by HPLC.[10] At 37 °C, the adducts degraded with half-lives of 96 h at pH 2.8, 275 h at pH 7.4, and 13 h at pH 12.[17]

These results demonstrate that under mild, biological conditions, *cis*-2-butene-1,4-dial reacts efficiently[18] with cytosine to form a set of stable adducts. We also observed smaller quantities of *cis*-2-butene-1,4-dial adducts with dG and dA, and these adducts are currently under study. Although it is unusual for dC to be the preferential target for adduction, the facile formation of butenedialdehyde adducts of both dC and 1-methylcytosine was found to be highly reproducible for multiple preparations of both the *cis*- and *trans*-isomers of butenedialdehyde. Peterson et al. made a preliminary report of unstable nucleoside adducts with *cis*-2-butene-1,4-dial.[6] However, under conditions of neutral pH at 37 °C, the oxadiazabicyclo(3.3.0)octaimine adduct was found to be stable. The formation of stable adducts with *cis*-2-butene-1,4-dial is consistent with the reported mutagenicity of this compound in the Ames assay.[6] Given its reactivity to form base adducts and its mutagenicity, *cis*-2-butene-1,4-dial produced by 5′-oxidation of 2′-deoxyribose may also represent a source of endogenous DNA damage.

We conclude that *cis*- and *trans*-2-butene-1,4-dial are capable of modifying cytosine at the N^3- and N^4-positions to form stable adducts. This class of nucleoside adducts may have general importance in the mutagenesis and carcinogenesis associated with furan metabolism and oxidative DNA damage.

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Supporting Information Available: ^{1}H- and ^{13}C NMR chemical shifts and assignments and ^{1}H NMR spectra for 2′-deoxycytidine and 1-methylcytosine adducts **4a** and **4b** in $(CD_3)_2SO$ and D_2O (PDF). An X-ray crystallographic file for **4b** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Crystallographic analysis was performed on a Brucker SMART/CCD platform diffractometer. The colorless crystal had dimensions $a = 21.523$ (2) Å, $b = 23.449$ (3) Å, $c = 7.3211$ (8) Å, $\alpha = 90.00°$, $\beta = 90.00°$, $\gamma = 90.00°$, $Z = 16$. $R = 4.6\%$ and $R_w = 9.7\%$ for 1303 data. The structure was solved with SHELSX-97, SHELXL-97, and Bruker SHELXTL software; Sheldrick, G. M.; Schnieder, T. R. *Macromol. Cryst.* **1997**, *277*, 319−343; Sheldrick, G. M. *Acta Cryst.* **1990**, *46*, 467−473.

(17) **4a** (2 mM) was incubated in 50 mM potassium phosphate pH 7.4 at 37 °C, and at various times, aliquots were analyzed by HPLC.[10] Adduct degradation was quantified as the rate of loss of **4a**.

(18) Adducts were detectable by HPLC with UV detection in reactions of 2 mM **1a** with concentrations of **2** as low as 200 μM in 50 mM potassium phosphate buffer at pH 7.4 and 37 °C.