HHS Public Access

Author manuscript

J Am Chem Soc. Author manuscript; available in PMC 2017 December 07.

Published in final edited form as:

J Am Chem Soc. 2016 December 07; 138(48): 15559–15562. doi:10.1021/jacs.6b09657.

Mechanism of Nucleophilic Activation of (−)-Lomaiviticin A

Mengzhao Xue[†] and **Seth B. Herzon**[*,†,‡]

[†]Department of Chemistry, Yale University, New Haven, Connecticut 06520, United States

[‡]Department of Pharmacology, Yale School of Medicine, New Haven, Connecticut 06520, United States

Abstract

(−)-Lomaiviticin A (**1**) is a C_2-symmetric cytotoxin that contains two diazofluorene functional groups and which induces double-strand breaks (DSBs) in DNA. Evidence suggests DNA cleavage is initiated by hydrogen atom abstraction from the deoxyribose backbone. Here we demonstrate the formation of the vinyl radicals **1**· and **2**· from **1** by 1,7-addition of thiols to the diazofluorenes. These radicals can affect hydrogen atom abstraction from methanol and acetone. The first addition of thiol to **1** proceeds at a much greater rate than the second. The diazosulfide **5** formed en route to **1**· has been detected at −50 °C and undergoes decomposition to **1**· with a half-life of 110 min at −20 °C under air. These data, which constitute the first direct evidence for the generation of **1**· and **2**· from **1**, provide insights into the mechanism of DNA cleavage by **1**.

The C_2-symmetric bacterial metabolite (−)-lomaiviticin A (**1**, Scheme 1) induces double-strand breaks (DSBs) in DNA[1] and is undergoing preclinical evaluation as a combination[2] and monotherapy[1e] for the treatment of DNA DSB repair-deficient tumors.[3] **1** binds DNA by a mode of association involving penetration of both diazotetrahydrobenzo[*b*]fluorene (diazofluorene) residues into the duplex.[4] The related metabolite (−)-lomaiviticin C (2)[1b,c] contains only one diazo substituent and does not induce DNA DSBs.[1d] In vitro reactivity studies of synthetic diazofluorene analogs[5] led to the hypothesis that carbon-centered radical intermediates form from the diazofluorene. It was later proposed that **1** is transformed to the sp^2-radicals **1**· and **2**· in tissue culture and that these affect strand cleavage[6] by hydrogen atom abstraction from the deoxyribose backbone.[1d] A single hydrodediazotization of **1** generates **2**; 2-fold reaction of **1** forms the double hydrodediazotization product **3**.

Here we provide the first direct experimental evidence for the formation of **1**· and **2**· from **1** by 1,7-addition of thiol-based nucleophiles to the diazofluorene. Our studies lead to the unexpected observation that the rate of the first thiol addition (**1**→**2**) vastly exceeds that of

[*]**Corresponding Author** seth.herzon@yale.edu.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b09657.

Detailed experimental procedures and complete spectroscopic data for all new compounds (PDF)

Notes

The authors declare no competing financial interest.

ORCID

Seth B. Herzon: 0000-0001-5940-9853

the second (**2→3**). The radicals **1·** and **2·** affect hydrogen atom abstraction from methanol and acetone (BDE = 95 and 93.9 kcal/mol, respectively).[7]

We first studied the reactivity of **1** toward *N*-acetyl-L-cysteine methyl ester (NACME) in methanol-d_4 and acetone-d_6. Each experiment was conducted under air and argon. In the absence of base, mixtures of (−)-lomaiviticin A (**1**) bis(trifluoroacetate) (1.22 mM) and NACME (40 equiv) remained unchanged after at least 8 h at 25 °C.[8] When triethylamine (TEA, 40 equiv) was added to a solution of **1** (1.22 mM) and NACME (40 equiv) in methanol-d_4 at 25 °C under air, an instantaneous color change from vivid red to dark-brown red was observed. Immediate (<5 min) analysis by ^{1}H NMR spectroscopy revealed the formation of **2-*d*** (94%, entry 1, Table 1). Upon aging, the solution of **2-*d*** transformed to **3-*d*$_2$**, with a half-life of 49 min (83%). Under argon, the yield of **2-*d*** was 97%, and the rate of conversion to **3-*d*$_2$** was faster ($t_{1/2}$ = 5 h at 5 °C, entry 2). (−)-Lomaiviticin A (**1**) was also instantaneously converted to **2-*d*** in acetone-d_6 (84% and 96% yield under air and argon, entries 3, and 4, respectively) but **3-*d*$_2$** was not observed. Instead, **2-*d*** slowly transformed to unidentified decomposition products. This sequence was readily followed by ^{1}H NMR spectroscopy as transformation of the C_2-symmetric structure **1** to the C_1-symmetric structure **2-*d*** results in doubling of most signals (Figure 1). Loss of the remaining diazo substituent restores C_2-symmetry (as **3-*d*$_2$**), leading to simplification of the spectra. These experiments reveal that the rate of 1,7-addition to the first diazofluorene of **1** is faster than the remaining diazofluorene in **2**.

The source of deuterium at the vinylic position of **2-*d*** was elucidated by repeating the experiments separately in methanol and methanol-d_4, and analyzing the products by LC/HRMS. (−)-Lomaiviticin C (**2**) ionizes by ejection of the aminosugar residue proximal to the hydroxyfulvene, leading to a prominent daughter ion corresponding to **4** upon MS analysis (Figure 2A).[1b,9] LC/HRMS analysis of the first hydrodediazotization of **1** in methanol indicated formation of **4** ($[M]^+ = C_{60}H_{66}N_3O_{21}^+$: calculated, =1164.4183; observed = 1164.4169; error = 1.20 ppm), whereas the same experiment conducted in methanol-d_4 provided **4-*d*** ($[M]^+ = C_{60}DH_{65}N_3O_{21}^+$: calculated = 1165.4246; observed = 1165.4220; error = 2.23 ppm, Figure 2B). To identify the site of bond cleavage in methanol (e.g., C–H/D or O–H/D), and to remove any potential complications arising from O–H/D exchange, we conducted additional experiments in CH_3OD and CD_3OH. Mass spectral analysis of the hydrodediazotization of **1** in CH_3OD revealed generation of **4** (observed = 1164.4162; error = 1.80 ppm), and analysis of the same experiment in CD_3OH indicated generation of **4-*d*** (observed = 1165.4222; error = 2.06 ppm). Strictly analogous results were obtained when the 2-fold hydrodediazotization of **1** was conducted in CH_3OD or CD_3OH. Reaction in the former solvent formed the protiated product **3** whereas reaction in the latter solvent generated **3-*d*$_2$** (Figure S1). These results indicate that the newly formed C–H/D bonds in **2** and **3** derive from C–H/D bond cleavage in methanol and provide compelling evidence for the intermediacy of the sp^2 radicals **1·** and **2·**.

The requirement for base in the conversion of **1** to **2** and **3** is consistent with 1,7-addition of thiolate to generate a diazosulfide intermediate, followed by loss of dinitrogen and thiyl radical (Scheme 2). To probe this, we monitored the reactivity of **1** toward benzylthiol in the

presence of triethylamine at low temperature.[10] Addition of benzylthiol (10 equiv) and triethylamine (10 equiv) to a solution of **1** (1.22 mM) in acetone-d_6 at −50 °C under air instantaneously formed the diazosulfide **5** (81%, Scheme 3). The diazosulfide **5** was generated as a ~1:1 mixture of *E:Z* isomers that converted to a 3:1 mixture (presumably, *E:Z*) after standing for 1 h at −50 °C. The 3:1 mixture of diazosulfides **5** was stable for at least 12 h at −50 °C and was characterized by ^{1}H, HSQC, and HMBC NMR analysis. The protons α to sulfur in the major isomer of **5** appeared as two distinct doublets (J = 14.0 Hz) centered at 4.94 and 4.75 ppm. These were correlated to the same carbon atom (36.5 ppm; 28.4 ppm in free benzylthiol) in the HSQC spectrum and to the quaternary carbon of the phenyl ring in **5** (130.5 ppm; 128.8 in free benzylthiol) in the HMBC spectrum. Warming to −20 °C induced transformation of **5** to **2-*d***, with a half-life of 110 min (79% yield from **1** at 98% conversion of **5**). Under argon, the diazosulfide **5** was formed in quantitative yield and transformed to **2-*d*** with a half-life of 49 min (>99%). No intermediates were detected when the conversion of **2-*d*** to **3-d_2** was monitored carefully by NMR spectroscopy, suggesting decomposition of the putative diazosulfide derived from **2-*d*** is faster than its formation.

DFT calculations were employed to gain insight into the relative rates of addition to **1** and **2**.[1d] The optimized structure of **1** using the B3LYP 6-31G(d) level of theory and an aqueous solvent model is shown in Figure 3 and indicates that the distance from the diazo carbon to the opposing diazofluorene is 3.8 Å. We propose that the developing anionic charge in the transition state for addition to **1** is stabilized by a through-space interaction with the adjacent electron-deficient diazofluorene. The transformation of **1** to **2** converts a diazofluorene to an electron-rich hydroxyfulvene, and the transition state for the second addition may not benefit from the same stabilization. It is also possible that the hydroxyfulvene in **2** is strongly hydrogen-bound (or deprotonated) under these conditions, which would further decrease electrophilicity. The structure shown in Figure 3 parallels of **1** bound to DNA,[4] suggesting these transannular interactions are relevant in tissue culture.

These data provide several insights into the mechanism of DNA cleavage by **1**. First, these experiments show that the radicals **1**· and **2**· can be formed from **1** by nucleophilic addition, and that these are competent to cleave relatively strong C–H bonds, providing support for DSB induction by a hydrogen atom abstraction mechanism. Second, the faster rate of nucleophilic addition to **1** than **2** may provide an explanation for the greater proportion of single-strand breaks than DSBs produced by **1** (in an in vitro plasmid cleavage assay, this ratio was ~5:1[1d]): dissociation of **2** from the duplex may be competitive with the formation of **2**•. Finally, the facile conversion of **1** to **2** suggests that natural **2** derives from hydrodediazotization of **1** during bacterial growth. As **2** is several orders of magnitude less potent,[1b] this reactivity may constitute a fortuitous detoxification pathway for the producing strain. A question currently unresolved is the nature of the nucleophile in the presence of DNA. Given the short lifetimes of sp^2 radicals, we hypothesize that **1**· and **2**· are generated after binding, potentially by direct addition of a nucleotide[11] to the coordinated metabolite.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

Financial support from the National Institutes of Health (NIGMS R01-GM090000) and Yale University is gratefully acknowledged. We thank Dr. Eric Paulson for NMR assistance, Dr. Wei Shen for assistance with bacterial cultures, and Dr. Seewon Joung, Dr. Alan Healy, and Mr. Herman Nikolayevskiy for helpful comments on the paper.

REFERENCES

(1). (a) He H, Ding WD, Bernan VS, Richardson AD, Ireland CM, Greenstein M, Ellestad GA, Carter GT. J. Am. Chem. Soc. 2001; 123:5362. [PubMed: 11457405] (b) Woo CM, Beizer NE, Janso JE, Herzon SB. J. Am. Chem. Soc. 2012; 134:15285. [PubMed: 22963534] (c) Kersten RD, Lane AL, Nett M, Richter TKS, Duggan BM, Dorrestein PC, Moore BS. ChemBioChem. 2013; 14:955. [PubMed: 23649992] (d) Colis LC, Woo CM, Hegan DC, Li Z, Glazer PM, Herzon SB. Nat. Chem. 2014; 6:504. [PubMed: 24848236] (e) Colis LC, Hegan DC, Kaneko M, Glazer PM, Herzon SB. J. Am. Chem. Soc. 2015; 137:5741. For a review, see. [PubMed: 25849366] (f) Herzon SB, Woo CM. Nat. Prod. Rep. 2012; 29:87. [PubMed: 22037715]

(2). Colis LC, Herzon SB. Bioorg. Med. Chem. Lett. 2016; 26:3122. [PubMed: 27177826]

(3). (a) Rajski SR, Williams RM. Chem. Rev. 1998; 98:2723. For reviews of DNA-damaging natural products, see: [PubMed: 11848977] (b) Wolkenberg SE, Boger DL. Chem. Rev. 2002; 102:2477. [PubMed: 12105933] (c) Tse WC, Boger DL. Chem. Biol. 2004; 11:1607. [PubMed: 15610844]

(4). Woo CM, Li Z, Paulson EK, Herzon SB. Proc. Natl. Acad. Sci. U. S. A. 2016; 113:2851. [PubMed: 26929332]

(5). (a) Laufer RS, Dmitrienko GI. J. Am. Chem. Soc. 2002; 124:1854. For the first proposals for the generation of sp^2 radicals from the diazofluorene, see: [PubMed: 11866589] (b) Feldman KS, Eastman KJ. J. Am. Chem. Soc. 2006; 128:12562. [PubMed: 16984207]

(6). (a) Burrows CJ, Muller JG. Chem. Rev. 1998; 98:1109. For a review of DNA cleavage by hydrogen atom abstraction, see: For a review of natural products that affect hydrogen atom abstraction from DNA, see: [PubMed: 11848927] (b) Murphy JA, Griffiths J. Nat. Prod. Rep. 1993; 10:551. [PubMed: 8121647]

(7). (a) Bordwell FG, Cheng JP, Harrelson JA. J. Am. Chem. Soc. 1988; 110:1229.(b) Comprehensive Handbook of Chemical Bond Energies. CRC Press; Boca Raton: 2007. BDEs of C–H bonds; p. 19-145.

(8). The addition of TEA alone to solutions of **1** in methanol-d_4 or acetone-d_6 led to rapid precipitation of the metabolite, see Supporting Information

(9). Mulcahy SP, Woo CM, Ding WD, Ellestad GA, Herzon SB. Chem. Sci. 2012; 3:1070.

(10). Attempts to characterize a diazosulfide formed by the addition of NACME to **1** were complicated by line broadening

(11). (a) Chin A, Hung M-H, Stock LM. J. Org. Chem. 1981; 46:2203. For the addition of nucleotides to arenediazonium salts, see: (b) Hung MH, Stock LM. J. Org. Chem. 1982; 47:448.

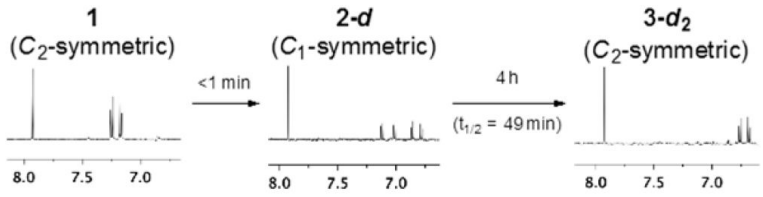


Figure 1.

Aryl region of the ^{1}H NMR spectra of **1**, **2-*d***, and **3-d_2**. Conditions: **1** (1.22 mM), TEA (40 equiv), NACME (40 equiv), methanol-d_4, air, 25 °C.

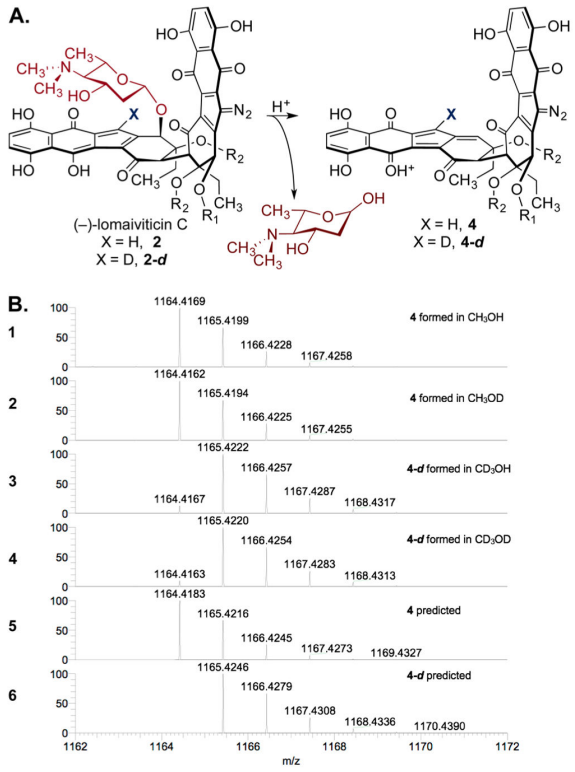


Figure 2.

(A) Ionization of **2** or **2-*d*** leads to ejection of the aminosugar residue and observation of the elimination products **4** or **4-*d*** by HRMS analysis.[1b,9] (B) 1, 2: Selected region of the HRMS spectrum of **4**, generated by hydrodediazotization of **1** in CH_3OH or CH_3OD, respectively. 3, 4: Selected region of the HRMS spectrum of **4-*d***, generated by hydrodediazotization of **1** in CD_3OH or CD_3OD, respectively. 5, 6: Predicted isotope distribution of **4** and **4-*d***, respectively.

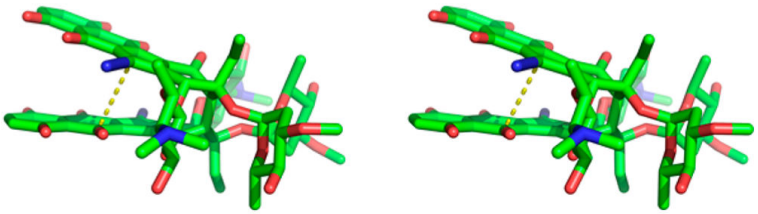
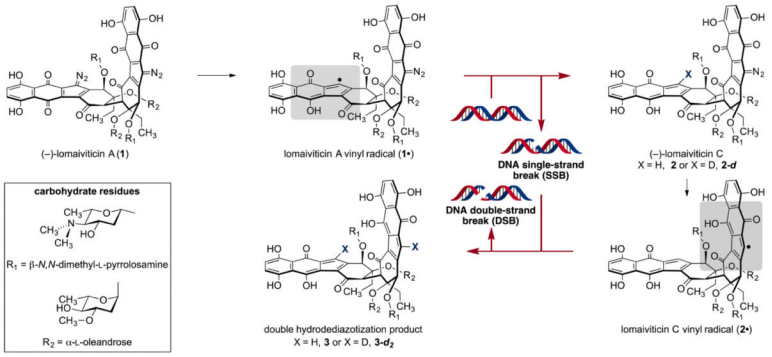
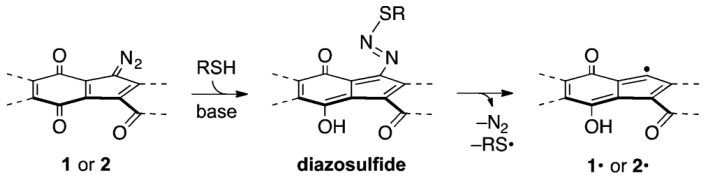


Figure 3.
Stereoview of DFT-minimized structure of **1** in water [B3LYP 6-31G(d)]. Hydrogen atoms are omitted for clarity.



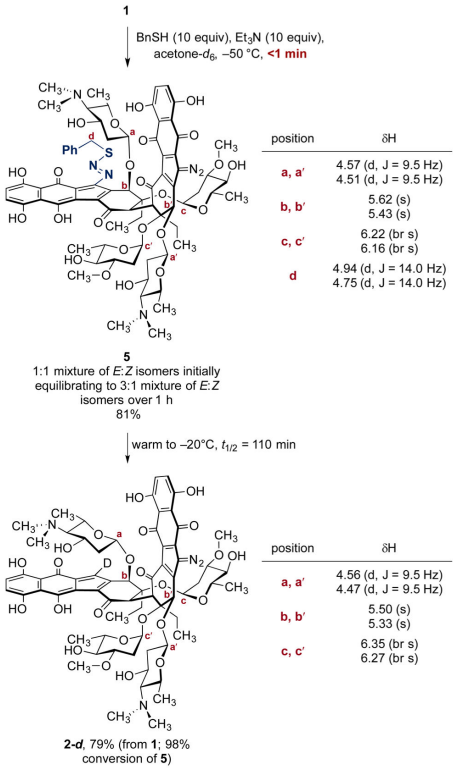
Scheme 1.

Proposed Pathway for the Conversion of (−)-Lomaiviticin A (1) to (−)-Lomaiviticin C (2) and the Double Hydrodediazotization Product 3 via the Vinyl Radical Intermediates 1· and 2·



Scheme 2.

Postulated Pathway for the Formation of **1**· and **2**· via Nucleophilic Addition of Thiol



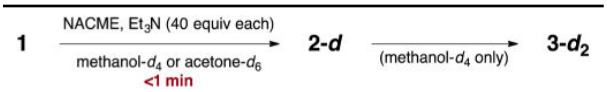
Scheme 3.

Generation and Decomposition of the Diazosulfide 5.[a]

[a]Spectroscopic data shown corresponds to the major diazosulfide isomer. Reaction was run under air. ^{1}H NMR data were acquired at −20 °C.

Table 1

Hydrodediazotization Studies of 1



entry	atmos.	solvent	yield 2-d_2[a]	yield 3-d_2[a] ($t_{1/2}$, T)
1	air	CD_3OD	94%	83% (49 min, 25 °C)
2	argon	CD_3OD	97%	87% (5 h, 5 °C)
3	air	acetone-d_6	84%	n/d[b]
4	argon	acetone-d_6	96%	n/d[b]

[a] Yields were determined by ^{1}H NMR spectroscopy using 1,4-dicyanobenzene as an internal standard and are based on **1**.

[b] n/d = not detected.