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ESR studies on DNA cleavage induced by enediyne C-1027 chromophore

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Dedicated to Professor Koji Nakanishi in recognition of his outstanding contributions to bioorganic and natural products chemistry.

Abstract—C-1027 belongs to the family of chromoprotein antitumor antibiotics, which contain a carrier apoprotein and a highly unstable enediyne chromophore. The enediyne spontaneously aromatizes to generate *p*-benzyne biradical, and subsequently abstracts hydrogens from the DNA sugar backbone, resulting in cleavage of the double strand. Using spin-trapping methods, we obtained direct proof of radical intermediates during an DNA cleavage, and found intriguing difference in behavior between the trapping agents 2-methyl-2-nitrosopropane (MNP) and 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO): MNP added to the sugar radicals of the DNA, whereas DMPO directly trapped a phenyl radical or *p*-benzyne biradical derived from the C-1027 chromophore.

1. Introduction

In 1988, Otani and co-workers isolated the antitumor antibiotic C-1027 from the culture broth of *Streptomyces globisporus* C-1027.[1] C-1027 possessed potent antitumor activity against cultured human cancer cells and some transplantable tumors in mice.[2] Elucidation of the structure of C-1027 revealed that this compound belongs to the family of chromoproteins[3] containing a highly unstable chromophore (**1**, Fig. 1)[4] and a carrier apoprotein (**6**, Fig. 2).[5] Although enediyne **1** is reasonably stable when bound to apoprotein **6**, compound **1**, in its free form, quickly aromatizes via a Masamune–Bergman rearrangement, even at room temperature (**1** → **5**, $t_{1/2}$ = 50 min in EtOH).[4d] Previous studies reported that the nine-membered enediyne **1** equilibrates with *p*-benzyne **2** in apoprotein **6**, and is kinetically stabilized.[6–8] In addition, electron spin resonance (ESR) and mass spectroscopic (MS) analysis of the C-1027 powder revealed that apoprotein **6** is cleaved gradually through hydrogen abstraction from Gly96 by **2**.[6a,7] Conversely, chromophore **1** released from the apoprotein interacts with the minor groove of the double-stranded DNA, causing oxidative cleavage after O_2 addition with remarkable sequence selectivity (Fig. 2).[9–11] Goldberg and co-workers demonstrated that the most preferred sequence for **1** was 5′-GTTAT/5′-ATAAC (restriction sites underlined).[10] Although the outcome of C-1027-mediated DNA damage has been studied in considerable detail, no direct observation of radical intermediates has been reported.[12] As part of our continuing interest in the chemical behavior of C-1027, we report in this article the results of ESR and spin-trapping studies on the DNA-cleavage reaction induced by C-1027.[13,14]

2. Results and discussion

2.1. Direct observation of ESR signals of C-1027

Although the C-1027 powder exhibits a steady ESR signal at room temperature,[6a] no signal due to C-1027 was observed in a diluted (~1 mM) and highly dielectric aqueous solution. Interestingly, upon cooling the C-1027 aqueous solution to −233 °C, a small signal arose, presumably from carbon radical(s) produced by C-1027

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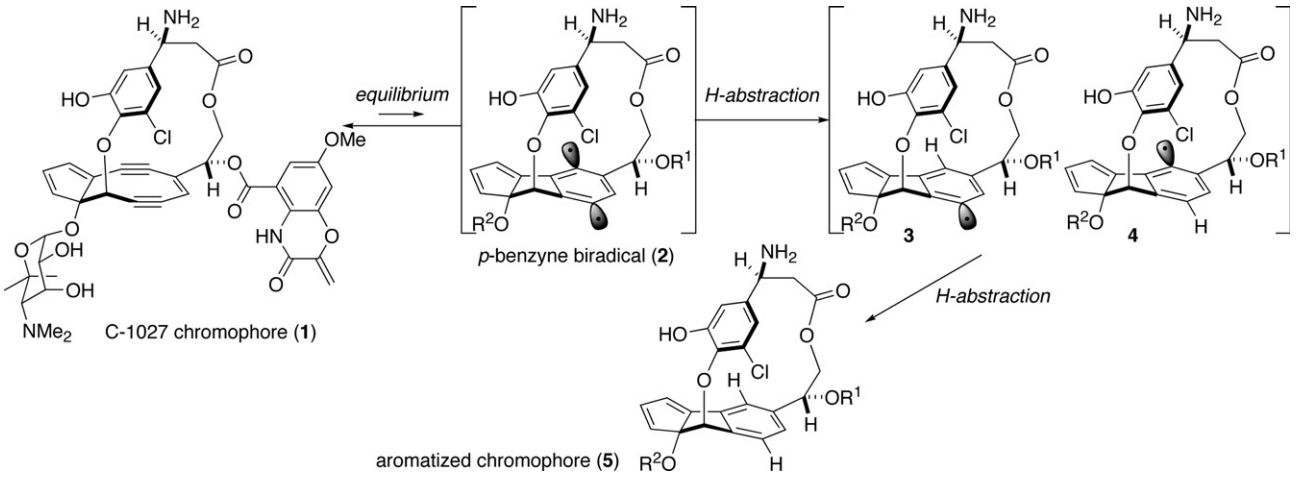

Figure 1. Structures of C-1027 chromophore **1** and the aromatized product **5**.

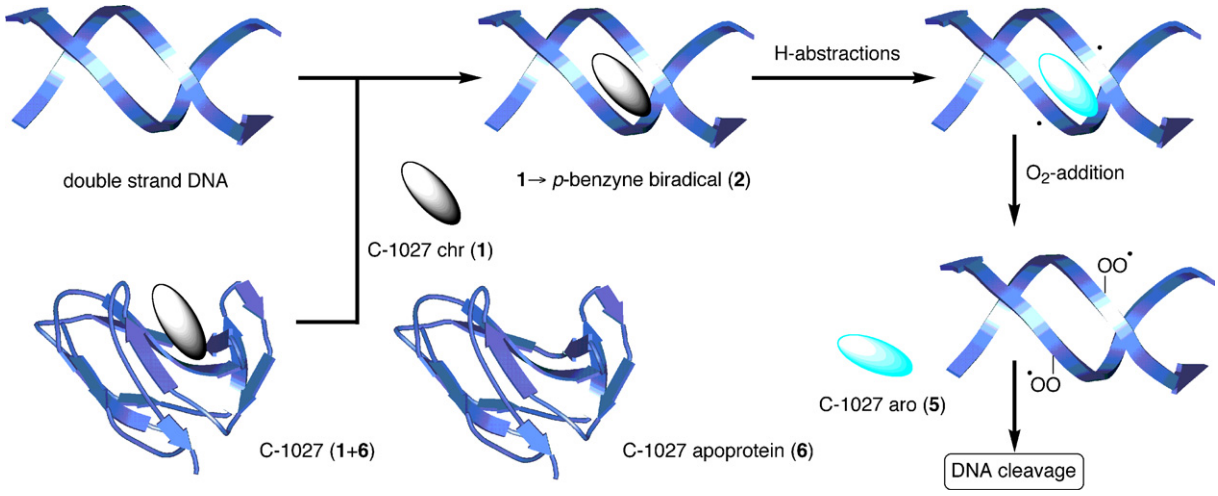

Figure 2. Overview of the C-1027-mediated DNA-cleavage reaction.

(Fig. 3A, $g = 2.003$). Furthermore, when C-1027 and double-stranded DNA (5′-TGCCATC/5′-GATGGCA: restriction sites underlined)[9b] were mixed in a aqueous buffer and then cooled to −233 °C, the intensity of the ESR signal was enhanced (Fig. 3B), indicating an increase in concentration of the carbon radical(s). However, this preliminary study revealed the difficulty in assignment of the radical species, because of a low S/N ratio and the potential generation of carbon radicals of similar *g*-values through C-1027-induced DNA cleavage. Thus, we abandoned the direct ESR measurement approach to observing the behavior of C-1027.

2.2. Spin-trapping study of C-1027

In response to the negative data in the previous section, we applied a spin-trapping method using (2-methyl-2-nitrosopropane, MNP; Fig. 4) and 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO).[15–17] Spin-trapping allows the capture of a reactive radical, which is converted to a more stable radical adduct detectable by ESR at room temperature. Importantly, hyperfine coupling (hfc) parameters of the adducts permit identification of the initially trapped radical.

The dodecamer 5′-GCC**GTTA**$_1$**T**GCCG/5′-CGGC**A** **TA**$_2$**A**$_3$**C**GGC (**7**) was chosen as the DNA substrate, because Goldberg showed, from an extensive gel electrophoresis study, that **1** abstracts hydrogen atoms from the C4′, C1′, and C5′ positions of the A_1, A_2, and A_3 nucleotide sugars of **7**, respectively, through sequence selective binding, as indicated in bold face.[10]

First, the nitroso spin-trapping reagent MNP was applied to our system. A mixture of C-1027 and MNP in aqueous buffer did not produce a signal, indicating that MNP did not trap the radicals **2–4**. No spin adduct was observed, when single-stranded DNA (5′-GCCGTTAT GCCG, **8**) and C-1027 were added to MNP. In contrast, a combination of double-stranded DNA **7**, MNP, and C-1027 in buffer provided the highly anisotropic spectrum shown in Figure 5A, which contains at least two signals—the relatively sharp triplet ($A_N = 1.5$ mT) and a broad overlapping signal. These results indicate that **1** was bound to **7** and subsequently abstracted hydrogen from **7** to generate the DNA radicals trapped by MNP. Since the intensity of the signals increased in a time-dependent manner (Fig. 6), the trapped radicals accumulated at room temperature as expected. Impor-

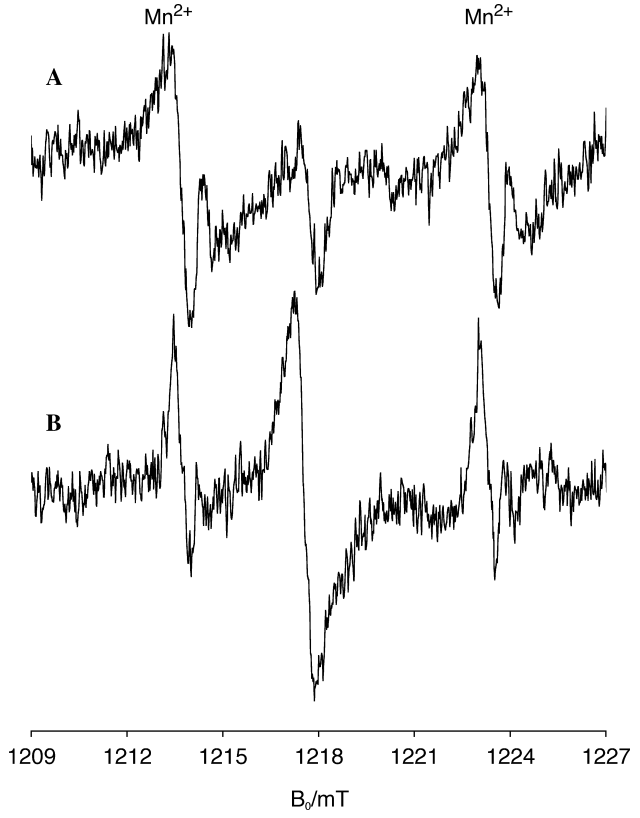

Figure 3. Q-band ESR spectra of C-1027. (A) C-1027 in NTE buffer (100 mM NaCl, 20 mM Tris–HCl, 1 mM EDTA, pH 7.5) at −233 °C; (B) C-1027 with double-stranded DNA [d(TGCCATC)/d(GATGGCA)] at −233 °C. The Mn^{2+} signals were derived from the sample.

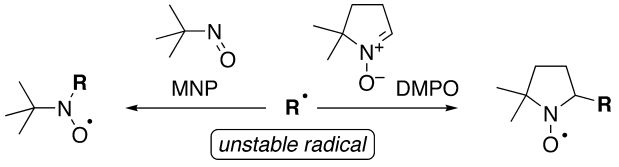

Figure 4. Spin-trapping reagents used in this study.

tantly, an identical ESR spectrum was obtained under anaerobic conditions (Fig. 5B),[18] excluding the possibility that the radicals produced after oxidative cleavage of the DNA were trapped. Thus, these data strongly suggest that the DNA radicals that formed initially reacted directly with MNP.

Considering Goldberg's results,[10] the sharp triplet (A_N = 1.5 mT) and overlapping broadened signal in the present spectrum could be attributed to the A_1–C4′ adduct (**10**), and A_2–C1′ and/or A_3–C5′ adducts (**9**, **11**), respectively (Fig. 7).[19] These assignments are compatible with the hfc constants reported for MNP adducts of thymidine monophosphate: C4′ (A_N = 1.60 mT), C1′ (A_N = 1.44 mT, $A_{N\beta}$ = 0.32 mT), and C5′ (A_N = 1.50 mT, $A_{H\beta}$ = 0.56 mT). Therefore, we conclude that MNP did capture radical intermediates of the DNA cleavage and acted as a substitute for O_2 (Fig. 2).

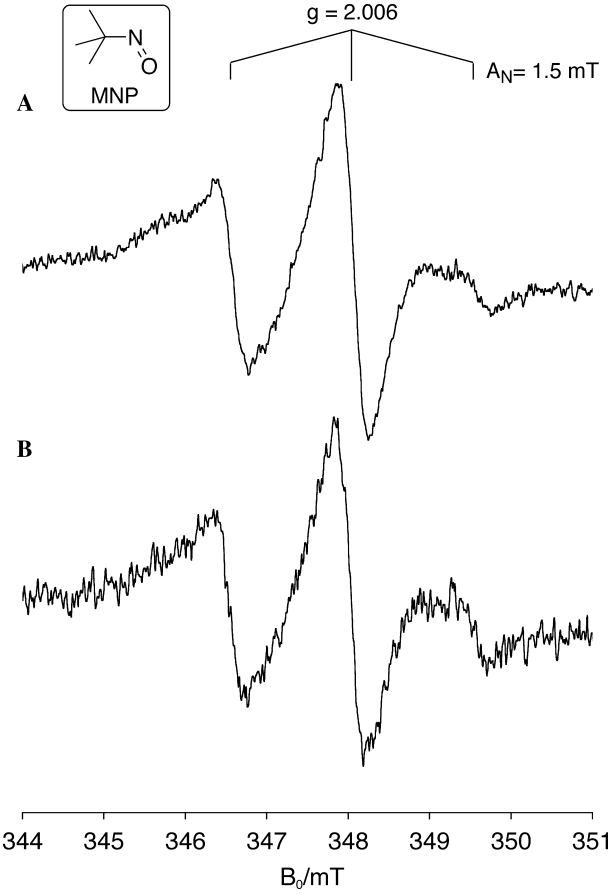

Figure 5. X-band ESR spectra of MNP spin adducts. (A) C-1027 with double-stranded DNA **7** and MNP in NTE buffer (100 mM NaCl, 20 mM Tris–HCl, 1 mM EDTA, pH 7.5) under aerobic conditions; (B) the same mixture under anaerobic conditions.

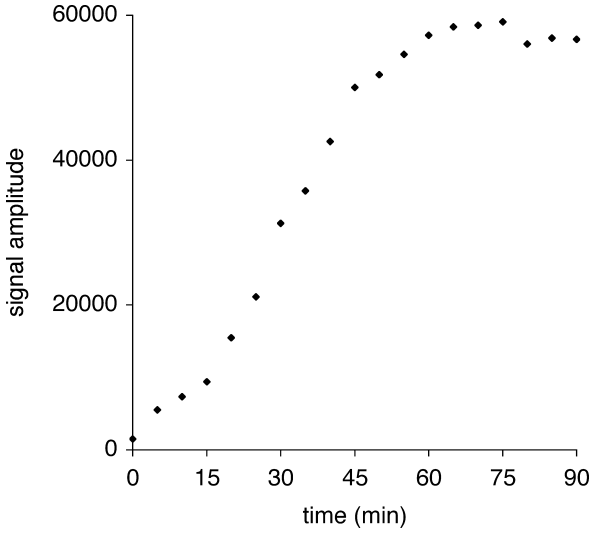

Figure 6. Time-course of amplitude of the ESR signal (g = 2.006). C-1027 with double-stranded DNA **7** and MNP in NTE buffer (100 mM NaCl, 20 mM Tris–HCl, 1 mM EDTA, pH 7.5) under aerobic conditions.

Next, we performed ESR experiments using DMPO, which possesses a nitrone function. Surprisingly, when only C-1027 was added to DMPO in buffer solution,

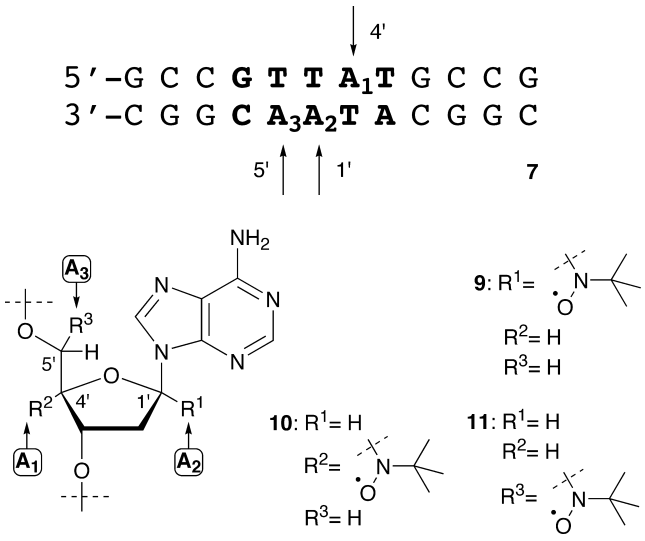

Figure 7. Possible MNP adducts of double-stranded DNA **7**.

an ESR signal emerged (Fig. 8A, $A_N = 1.51$ mT, $A_H = 2.25$ mT). DMPO clearly possessed a different reactivity compared to MNP. The same hfc values were observed even in the presence of double-stranded DNA **7**, while the spectrum broadened somewhat, presumably due to increased viscosity caused by the addition of **7** (Fig. 8B). When the isolated chromophore **1**[7,20] was treated with DMPO in MeCN–H_2O, an ESR spectrum (Fig. 8C) similar to that shown in Figures 8A and B was obtained, eliminating the possibility of trapped radicals derived from apoprotein **6**.[6a] The observed hfc values agreed well with those reported for phenyl radicals[21] and no doubly trapped spin adduct of biradical **2** was detected. Consequently, spin adducts were assigned as **13** and/or **14** (Fig. 9), which resulted from single trapping of the *p*-benzyne biradical **2** or from the trapping of phenyl radical **3** or **4** (Fig. 1). In addition, the presence of **13** and/or **14** in the reaction mixture was supported by time-of-flight mass spectra (MALDI-TOF MS) spectroscopy [Calcd for $C_{49}H_{56}O_{14}N_4Cl$ 959.348 (M^-+2H^+), found 959.350].

We determined concentrations of the MNP and DMPO adducts to be between 10^{-6} and 10^{-7} M using 2,2,6,6-tetramethyl-1-pyperidinyloxy (TEMPO, free radical) as a standard. Thus, the yields of the radical-trapping reactions were ~0.1% for both the cases. This low yield is reasonable in view of the extremely low estimated equilibrium constant of the process supplying *p*-benzyne **2**[6,22] and the competing decomposition reaction of the initially formed unstable radical species.

In our experiments, MNP reacted with DNA sugar radicals, while DMPO added to the chromophore radical **2**, **3**, or **4**. Generally, MNP is strongly electrophilic in water because of the increased polarity of the NO bond (*t*-Bu-$N^{\delta+}$=$O^{\delta-}$) in highly dielectronic media.[23] Therefore, MNP tends to add to the highly electron-rich DNA α-oxo-radicals even though these radicals are sterically shielded in the minor groove. Since the trapping efficiency of MNP is insensitive to O_2 (Figs. 5A

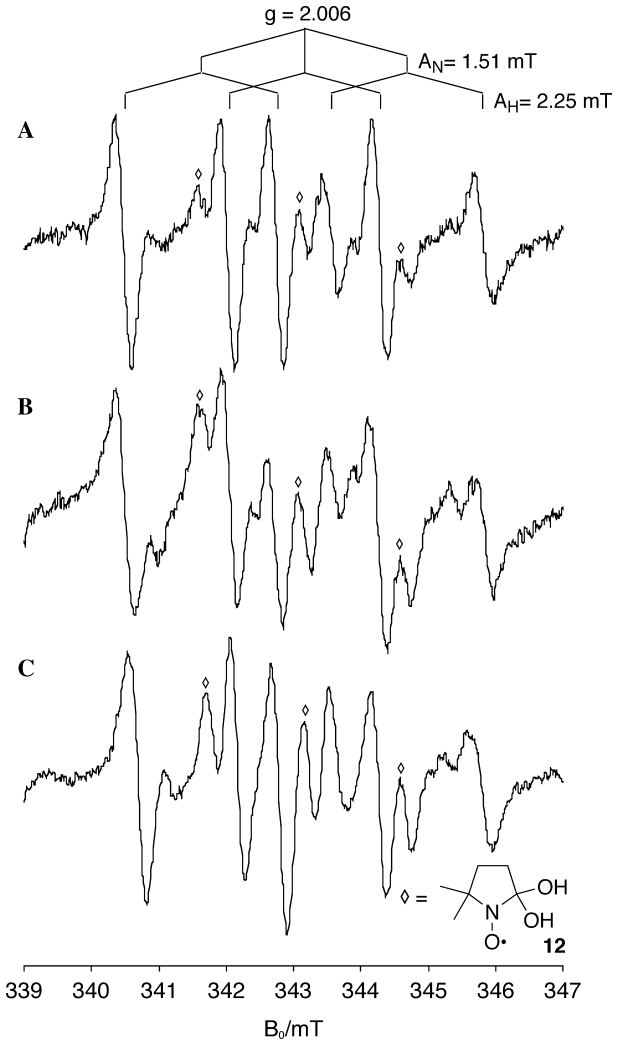

Figure 8. X-band ESR spectra of DMPO adducts. (A) C-1027 and DMPO in NTE buffer (100 mM NaCl, 20 mM Tris–HCl, 1 mM EDTA, pH 7.5); (B) C-1027 with double-stranded DNA **7** and DMPO in NTE buffer; (C) C-1027 chromophore **1** and DMPO in MeCN (pH 6.8) phosphate buffer (1:1). The hfc constants are $A_N = 1.48$ mT and $A_H = 2.09$ mT. The triplet signal [◊, $A_N = 1.53$ mT (A,B), $A_N = 1.44$ mT (C)] was assigned as the oxidized product of DMPO (**12**, $A_N = 1.53$ mT in water; Makino, K.; Hagi, A.; Ide, H.; Murakami, A. *Can. J. Chem.* **1992**, *70*, 2818).

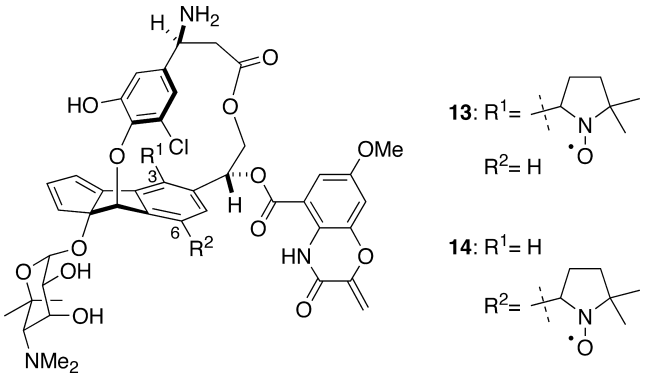

Figure 9. Possible DMPO-adducts of C-1027 chromophore **1**.

and B), MNP appeared to compete with O_2 in attacking DNA radicals. In addition, trapping of phenyl radicals by MNP is reportedly comparable to self-coupling of phenyl radicals,[24,25] and thus MNP is unlikely to represent an efficient agent for the capture of phenyl radicals.[26] MNP would therefore trap nucleophilic DNA radicals in preference to phenyl radicals.

Conversely, DMPO appears more reactive than MNP toward phenyl radicals,[21,27] and the nitrone function should not exhibit the strong electrophilic character of MNP. Phenyl radicals would thus be trapped more readily than would hindered sugar radicals by sterically demanding DMPO. Furthermore, O_2 adds more quickly to alkyl radicals than to phenyl radicals.[28] Therefore, it is conceivable that DMPO competes with O_2 in trapping phenyl radicals, but not the DNA alkyl radicals that degrade before DMPO reacts; whereas the strongly electrophilic MNP can rival O_2, leading to the DNA spin adducts observed.

3. Conclusion

The radical intermediates formed during C-1027-induced DNA cleavage have been observed using spin-trapping methods under physiologically relevant conditions. Although the different reactivities of nitroso and nitrone spin-trapping reagents have been recognized for a long time, this study is the first application of the distinct preferences of MNP and DMPO toward radicals, which enables the selective trapping of DNA or phenyl radicals. MNP was found to be particularly suitable for trapping DNA radicals because of its electrophilicity and insensitivity to steric hindrance. In addition, our results support the mechanism of C-1027-mediated DNA cleavage mediated through benzyne radicals and subsequently formed DNA sugar radicals. The present method will be useful for analyzing other radical-mediated biological processes. Further studies of the chemistry and biology of C-1027 are currently underway in this laboratory.

4. Experimental

4.1. General method

Q-band (34 GHz) and X-band (9.6 GHz) ESR spectra were recorded on a Bruker ESP 300 and a Bruker ESP 380E, respectively, with reference to a standard sample (Mn^{2+}/MgO). The Q-band ESR settings are as follows: scan field, 1217.77 ± 10.0 mT; modulation frequency, 100 kHz; modulation amplitude, 0.5 mT; receiver gain, 1.6×10^6; time constant, 163.84 ms; sweep time, 167.77 s; scan, 7; temperature, −233 °C. The X-band ESR settings are as follows: scan field, 343.11 ± 6.0 mT; modulation frequency, 100 kHz; modulation amplitude, 0.25 mT; receiver gain, 1.6×10^6; time constant, 163.84 ms; sweep time, 163.77 s; scan, 15 for Figure 5, 1 for Figure 6, 25 for Figure 8; temperature, room temperature. High performance liquid chromatography (HPLC) was conducted on a HITACHI D-7200 HPLC system. MALDI-TOF MS were recorded on a Applied Biosystem Voyager DE STR SI-3 instrument using α-cyano-4-hydroxy cinnamic acid as a matrix. C-1027 (lot number 941221) was kindly provided by Dr. Otani (Taiho Pharmaceutical). Synthetic DNA samples were purchased from Fasmac (Kanagawa, Japan). All the experiments were conducted at room temperature under aerobic conditions unless otherwise stated. All the reagents were used as supplied.

4.2. Q-band ESR spectra

(A) Powder C-1027 (0.5 mg, 44 nmol) was dissolved in water (15 μL). The solution was introduced into a Φ 1 mm quartz tube, which was inserted into the cavity of the ESR spectrometer at −233 °C.

(B) C-1027 (0.5 mg, 44 nmol) in water (5 μL) and the double-stranded DNA [d(TGCCATC)/d(GATGGAC)] (30 nmol) in NTE buffer (10 μL, 100 mM NaCl, 20 mM Tris–HCl, 1 mM EDTA, pH 7.5) were mixed at room temperature, and centrifuged for 1 min. The solution was introduced into a Φ 1 mm quartz tube, which was inserted to the cavity of the ESR spectrometer at −233 °C.

4.3. Spin-trapping experiments

4.3.1. MNP spin-trapping experiments. MNP (10 mg) in NTE buffer (1 mL), in a vial, was stirred at room temperature overnight in the dark, before being used in experiments (ca. 12 mM solution). The MNP solution (50 μL, ca. 0.6 μmol) was added to a solution of C-1027 (1.0 mg, 88 nmol) and **7** (30 nmol) in NTE buffer (30 μL), and the resultant solution was incubated for 1 min at room temperature before being introduced into a quartz flat cell (8 × 48 mm). Experiments without DNA or with **8** (30 nmol) were performed in a similar manner. For anaerobic experiments, NTE buffer was degassed using the freeze–evacuate–thaw procedure, prior to adding substrates (C-1027, MNP). Double-stranded DNA **7** in NTE buffer was similarly degassed and annealed under standard conditions before use. These reagents were mixed under a nitrogen atmosphere.

4.3.2. DMPO spin-trapping experiments. C-1027 (1.0 mg, 88 nmol) in NTE buffer (30 μL) and DMPO (2.5 mg, 22 μmol) in NTE buffer (50 μL) were mixed, and the resultant solution was incubated for 1 min at room temperature before being introduced into a quartz flat cell (8 × 48 mm). For experiments using C-1027 (1.0 mg, 88 nmol) and double-stranded DNA **7** (30 nmol) in NTE buffer (30 μL) were incubated for 1 min before the addition of DMPO (2.5 mg, 22 μmol) in NTE buffer (50 μL).

4.3.3. DMPO spin-trapping experiments using C-1027 chromophore. C-1027 (10 mg, 880 nmol) in phosphate buffer (PB, pH 6.8, 100 μL) was injected into a reverse-phase ODS column [Cosmosil 5C18-MS (10 × 250 mm, Nacalai Tesque, Kanagawa, Japan); sol-

vent, MeCN/20 mM PB (pH 6.8) 50/50; flow rate, 2.0 mL/min; detection, 350 nm; temperature, 40 °C]. In this solvent system, **1** was partially liberated from the holoprotein. C-1027 chromophore (t_R = 19 min) was isolated into a vial in the presence of DMPO (80 mg, 706 μmol), and the resultant solution was incubated for 1 min at room temperature before being introduced into a quartz flat cell (8 × 48 mm).

Acknowledgments

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References and notes

1. Otani, T.; Minami, Y.; Marunaka, T.; Zhang, R.; Xie, M.-Y. *J. Antibiot.* **1988**, *41*, 1580.
2. Zhen, Y.; Ming, S.; Bin, Y.; Otani, T.; Saito, H.; Yamada, Y. *J. Antibiot.* **1989**, *42*, 1294.
3. For recent reviews on chromoprotein antibiotics and other enediyne natural products, see: (a) Neocarzinostatin Maeda, H., Edo, K., Ishida, N., Eds.; Springer: Tokyo, 1997; (b) Xi, Z.; Goldberg, I. H. In *Comprehensive Natural Products Chemistry*; Barton, D. H. R., Nakanishi, K., Eds.; Elsevier, 1999; Vol. 7, p 553; (c) Galm, U.; Hager, M. H.; Van Lanen, S. G.; Ju, J.; Thorson, J. S.; Shen, B. *Chem. Rev.* **2005**, *105*, 739.
4. (a) Minami, Y.; Yoshida, K.; Azuma, R.; Saeki, M.; Otani, T. *Tetrahedron Lett.* **1993**, *34*, 2633; (b) Yoshida, K.; Minami, Y.; Azuma, R.; Saeki, M.; Otani, T. *Tetrahedron Lett.* **1993**, *34*, 2637; (c) Iida, K.; Ishii, T.; Hirama, M.; Otani, T.; Minami, Y.; Yoshida, K. *Tetrahedron Lett.* **1993**, *34*, 4079; (d) Yoshida, K.; Minami, Y.; Otani, T.; Tada, Y.; Hirama, M. *Tetrahedron Lett.* **1994**, *35*, 5253; (e) Iida, K.; Fukuda, S.; Tanaka, T.; Hirama, M.; Imajo, S.; Ishiguro, M.; Yoshida, K.; Otani, T. *Tetrahedron Lett.* **1996**, *38*, 4997.
5. Tanaka, T.; Fukuda-Ishisaka, S.; Hirama, M.; Otani, T. *J. Mol. Biol.* **2001**, *309*, 267.
6. (a) Hirama, M.; Akiyama, K.; Tanaka, T.; Noda, T.; Iida, K.; Sato, I.; Hanaishi, R.; Fukuda-Ishisaka, S.; Otani, T.; Leet, J. E *J. Am . Chem. Soc.* **2000**, *122*, 720; (b) Iida, K.; Hirama, M. *J. Am. Chem. Soc.* **1995**, *117*, 8875.
7. Usuki, T.; Inoue, M.; Hirama, M.; Tanaka, T. *J. Am. Chem. Soc.* **2004**, *126*, 3022.
8. For a review of benzynes, see Wenk, H. H; Winkler, M.; Sander, W. *Angew. Chem. Int. Ed.* **2003**, *42*, 502.
9. (a) Sugiura, Y.; Matsumoto, T. *Biochemistry* **1993**, *32*, 5548; (b) Okuno, Y.; Iwashita, T.; Sugiura, Y. *J. Am. Chem. Soc.* **2000**, *122*, 6848.
10. (a) Xu, Y.; Zhen, Y.; Goldberg, I. H. *Biochemistry* **1994**, *33*, 5947; (b) Xu, Y.; Zhen, X.; Zhen, Y.; Goldberg, I. H. *Biochemistry* **1995**, *34*, 12451.
11. For a review on the oxidative cleavage of DNA, see Pogozelski, W. K.; Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089–1107.
12. Myers et al. studied the reactive intermediates of neocarzinostatin chromophore in detail, see Myers, A. G.; Cohen, S. B.; Kwon, B.-M. *J. Am. Chem. Soc.* **1994**, *116*, 1670.
13. For a preliminary account of this work, see Usuki, T.; Inoue, M.; Akiyama, K.; Hirama, M. *Chem. Lett.* **2002**, 1148.
14. (a) Some ESR studies were reported on neocarzinostatin and auromomycin in the absence of DNA without defining the structures. Neocarzinostatin Edo, K.; Iseki, S.; Ishida, N.; Horie, T.; Kusano, G.; Nozoe, S. *J. Antibiot.* **1980**, *33*, 1586; (b) Sheridan, R. P.; Gupta, R. K. *Biochem. Biophys. Res. Commun.* **1981**, *99*, 213; (c) Edo, K.; Koide, Y. In *Neocarzinostatin*; Maeda, H., Edo, K., Ishida, N., Eds.; Springer: Tokyo, 1997, p 23; Auromomycin: (d) Suzuki, H.; Kirino, Y.; Tanaka, N. *J. Antibiot.* **1983**, *36*, 583.
15. Spin trapping study of the *p*-benzyne generated by Masamune–Bergman cyclization of the bicyclic nine-membered model enediyne was reported Usuki, T.; Mita, T.; Lear, M. J.; Das, P.; Yoshimura, F.; Inoue, M.; Hirama, M.; Akiyama, K.; Tero-Kubota, S. *Angew. Chem. Int. Ed.* **2004**, *43*, 5249.
16. For a review on the spin-trapping method, see Janzen, E. G. *Acc. Chem. Res.* **1971**, *4*, 31.
17. Spin-trapping study of DNA degradation has been extensively studied by Kuwabara et al. as well as by Gilbert and Davies; see, for examples (a) Hiraoka, W.; Kuwabara, M.; Sato, F.; Matsuda, A.; Ueda, T. *Nucleic Acids Res.* **1990**, *18*, 1217; (b) Kuwabara, M.; Ohshima, H.; Sato, F.; Ono, A.; Matsuda, A. *Biochemistry* **1993**, *32*, 10599, and references therein; (c) Hazlewood, C.; Davies, M.; Gilbert, B. C.; Packer, J. E. *J. Chem. Soc., Perkin Trans.* **1995**, *2*, 2167; (d) Ho, W. F.; Gilbert, B. C.; Davies, M. J. *J. Chem. Soc., Perkin Trans.* **1997**, *2*, 2525; (e) Gilbert, B. C.; Silvester, S.; Walton, P. H.; Whitwood, A. C. *J. Chem. Soc., Perkin Trans.* **1999**, *2*, 1891, and references therein.
18. Goldberg et al. reported a electrophoresis study of C-1027-mediated DNA cleavage under anaerobic conditions, see (a) Xu, Y.; Zhen, Y.; Goldberg, I. H. *J. Am. Chem. Soc.* **1997**, *119*, 1133; (b) Xu, Y.; Xi, Z.; Zhen, Y.; Goldberg, I. H. *Biochemistry* **1997**, *36*, 14975.
19. Kuwabara, M.; Hiraoka, W.; Sato, F. *Biochemistry* **1989**, *28*, 9625.
20. Otani, T.; Minami, Y.; Sakawa, K.; Yoshida, K. *J. Antibiot.* **1991**, *44*, 564.
21. The hfc parameters of DMPO adducts of various phenyl radicals in water are A_N = 1.54–1.64 mT, and A_H = 2.35–2.50 mT, see (a) Kirino, Y.; Ohkuma, T.; Kwan, T. *Chem. Pharm. Bull.* **1981**, *29*, 29; (b) Reszka, K. J.; Chignell, C. F. *Chem. Biol. Interact.* **1995**, *96*, 223, in benzene: A_N = 1.39–1.42 mT, A_H = 1.91–2.05 mT; (c) Augusto, O.; Kunze, K. L.; Ortiz de Montellano, P. R. *J. Biol. Chem.* **1982**, *257*, 6231.
22. (a) Logan, C. F.; Chen, P. *J. Am. Chem. Soc.* **1996**, *118*, 2113; (b) Schottelius, M. J.; Chen, P. *J. Am. Chem. Soc.* **1996**, *118*, 4896.
23. Taniguchi, H.; Madden, K. P. *J. Am. Chem. Soc.* **1999**, *121*, 11875.
24. Madden, K. P.; Taniguchi, H. *J. Am. Chem. Soc.* **1991**, *113*, 5541, The rate constant of self-coupling of phenyl radicals was determined to be 7.6×10^{6} M^{-1} s^{-1}; Madhavan, V.; Scholer, R. H.; Fessenden, R. W. *J. Am. Chem. Soc.* **1978**, *100*, 888.
25. We are aware that these discussion should include the reactivity of *p*-benzyne biradical **2** toward the spin-trapping reagents. Since there are no available data for this type of reaction for the biradicals, we have hypothesized that the biradical would react with the trapping reagents at a rate similar to that of the phenyl radical. For discussions about the hydrogen abstraction rate of *p*-benzyne biradicals, see Refs. 6,22.
26. The phenyl radical–MNP adducts were formed when the simple enediyne model was used in CD_2Cl_2 (Ref. 15). Lack of MNP-C-1027 adducts in the present experiments is attributable to the greater steric hindrance of the radical-

forming carbon of the C-1027 chromophore and/or the decreased reactivity of MNP toward the phenyl radicals in water.
27. The rate constant of a reaction between a phenyl radical and DMPO in water is 7×10^7 M^{-1} s^{-1} Kalyanaraman, B. In *Reviews in biochemical toxicology*; Hodgson, E., Bend, J. R., Philpot, R. M., Eds.; Elsevier Biomedical: New York, 1982; Vol. 4, p 73.
28. Preidel, M.; Zellner, R. *Ber. Bunsen-Ges. Phys. Chem.* **1989**, *93*, 1417.