Table I. Rate Constants and Kinetic Isotope Effects in the Solvolysis of Isopropyl β-Naphthalenesulfonate[a]

	EtOH	20E–80T[b]	TFE
$10^5(^Hk)$, s^{-1}	7.05 ± 0.08	6.34 ± 0.03	6.08 ± 0.07
$^Hk/^Dk$ at α[c]	1.07 ± 0.01	1.11 ± 0.01	1.15 ± 0.01
$^Hk/^Dk$ at β (D_6)[c]	1.22 ± 0.01	1.49 ± 0.01	1.77 ± 0.03
$^{12}k/^{14}k$ at α[d]	1.095 ± 0.004	1.089 ± 0.003	1.055 ± 0.002
$^{12}k/^{14}k$ at β[d]	1.009 ± 0.002	1.015 ± 0.002	1.019 ± 0.001

[a] 0.036 M in substance at 65 °C with added 1.1 equiv of 2,6-lutidine. [b] 20% EtOH-80% TFE (v/v). [c] Average of two runs. [d] For errors of $^{12}k/^{14}k$ see ref 9.

EtOH–TFE is a nearly isodielectric solvent system, in which nucleophilicity and electrophilicity change distinctly in opposite directions.[7,8,11] Almost identical rate constants observed in the three solvents indicate that these two factors are energetically counterbalanced with each other.

Results of deuterium isotope effects support the expected variation of the mechanisms. α-Deuterium effects in EtOH ($^Hk/^Dk$ = 1.07) and TFE (1.15) at 65 °C are almost the same as those reported for the solvolysis of the brosylate in 90% EtOH (1.083) and 97% TFE (1.16) at 25 °C, respectively,[12,13] when the difference in temperature is taken into consideration. These values reflect the steric congestion of the transition states: tight nucleophilic attachment of a solvent and a leaving group in EtOH and loose attachment in TFE. β-Deuterium effects indicate a strong hyperconjugative electron demand in TFE and a much reduced demand in EtOH. The observed difference between these two extremes is much larger than that between the values reported for 97% TFE (1.46 per D_6) and 90% EtOH (1.28 per D_6).[12]

The primary ^{14}C effect for the α carbon varied considerably with the solvent used. The effect in EtOH is large and close to the largest value ($^{12}k/^{14}k$ = 1.105) reported for the nucleophilic substitution of a secondary substrate,[14] while the effect is much smaller in TFE. Thus, it is confirmed experimentally with the simplest member of secondary alkyl derivatives that the carbon isotope effect at the reaction center is sensitive to the mechanism of the solvolysis and is large in S_N2 and small in S_N1.

It is surprising that significant isotope effects, $^{12}k/^{14}k$ = 1.01–1.02, were observed for carbon-14 at the β carbon, adjacent to the reaction center. These are the first reported examples of secondary carbon-14 isotope effects of significant magnitude in solvolysis without neighboring group participation.[9,15] A larger effect in TFE and a smaller one in EtOH indicate that the effects originate from the C_β–H bond weakening incident to hyperconjugation. It is noteworthy that these values are much larger than the secondary carbon-13 equilibrium isotope effect observed in 1,2-dimethylcyclopentyl caton, where one methyl group was labeled with carbon-13.[6]

In 20E–80T, all the effects other than that of α carbon-14 are just the averages of the corresponding values for EtOH and TFE. The characteristics of the transition state, e.g., steric congestion and hyperconjugative electron demand, should be in the middle between the two extremes. The different behavior of the α-carbon-14 effect reflects the fact that the symmetry of the transition state is the chief determining factor of the effect.[16] In this mixed solvent, the transition state seems fairly symmetrical, though it is looser than that in EtOH.

The present investigation verifies that α- and even β- carbon-14 kinetic isotope effects are sensitive to mechanistic changes in the borderline solvolysis. Model calculations of kinetic isotope effects using the observed data for all the carbons and deuteriums will enable us to describe the variable transition-state structures of the solvolysis of simple secondary substrates,[9,17] which is still a subject of much debate in recent years.[18] A study along this line is now in progress.

Acknowledgment. The present work was supported in part by Grants-in-Aid for Science Research (Nos. 347018 and 574197) from the Ministry of Education, Science and Culture, Japan.

Registry No. Isopropyl β-naphthalenesulfonate, 67199-42-2; carbon-14, 14762-75-5; deuterium, 7782-39-0.

(10) The product analysis by ^1H NMR showed that no olefin was produced in TFE (in the absence of 2,6-lutidine) or in EtOH-d_6. Although the addition of 2,6-lutidine into TFE resulted in the formation of 6% propene, almost no change was observed in the deuterium isotope effects: $^Hk/^Dk$ = 1.16 ± 0.01 for α-D and $^Hk/^Dk$ = 1.82 ± 0.02 for β-D_6 in the absence of 2,6-lutidine. Thus, the occurrence of the elimination does not affect the following discussion. In 20E–80T, 56% 2,2,2-trifluoroethyl ether and 36% ethyl ether were obtained (by ^{13}C NMR) along with 8% propene (by ^1H NMR).

(11) (a) Rappoport, Z.; Ben-Yacov, H.; Kaspi, J. *J. Org. Chem.* **1978**, *43*, 3678–3684. (b) Ando, T.; Tsukamoto, S.-I. *Tetrahedron Lett.* **1977**, 2775–2778.

(12) Shiner, V. J., Jr. In "Isotope Effects in Chemical Reactions"; Collins, C. J., Bowman, N. S., Eds.; Van Nostrand-Reinhold: New York, 1970; Chapter 2.

(13) Shiner, V. J., Jr.; Fisher, R. D.; Dowd, W. *J. Am. Chem. Soc.* **1969**, *91*, 7748–7749.

(14) Raaen, V. F.; Juhlke, T.; Brown, F. J.; Collins, C. J. *J. Am. Chem. Soc.* **1974**, *96*, 5928–5930.

(15) Ando, T.; Yamataka, H.; Morisaki, H.; Yamawaki, J.; Kuramochi, J.; Yukawa, Y. *J. Am. Chem. Soc.* **1981**, *103*, 430–436.

(16) Yamataka, H.; Ando, T. *J. Phys. Chem.* **1981**, 85, 2281–2286 and references cited therein.

(17) McLennan, D. J. *J. Chem. Soc., Perkin Trans. 2* **1981**, 1316–1324.

(18) For recent reviews of the topic see: (a) Harris, J. M. *Prog. Phys. Org. Chem.* **1974**, *11*, 89–173. (b) Bentley, T. W.; Schleyer, P. v. R. *Adv. Phys. Org. Chem.* **1977**, *14*, 1–67.

Identification of Degradation Products of d(C-G) by a 1,10-Phenanthroline–Copper Ion Complex

Seiichi Uesugi, Toshio Shida, Morio Ikehara,*
Yuji Kobayashi, and Yoshimasa Kyogoku

Faculty of Pharmaceutical Sciences and Institute for Protein Research
Osaka University, Suita, Osaka 565, Japan

Received May 24, 1982

The 1,10-phenanthroline–cuprous complex $(OP)_2Cu^+$ has been reported to cleave double-stranded DNA in an oxygen-dependent reaction.[1-4] It is assumed that hydrogen peroxide is formed as an essential reactant[3,4] and that hydroxyl radical is the reactive species in DNA degradation.[3] It is also known that an antitumor drug, bleomycin, cleaves DNA in the presence of Fe^{2+} and oxygen by a similar mechanism involving hydroxyl radicals.[5] Recently some DNA degradation products by the drug have been identified.[6] However, in the case of phenanthroline, the mode of action on DNA is not well understood.[7] Investigation of the DNA cleavage products will provide information for a general understanding of DNA cleavage reactions catalyzed by free radicals as observed for many antitumor drugs such as neocarzinostatin,[8] daunomycin,[9] and mitomycin[10] and in radiolysis.[11] In this communication, we report identification of degradation products (**1**, **2**, cytosine, guanine, and deoxyguanosine 5′-phosphate) from a self-complementary dinucleoside monophosphate, d(C-G),[12] by phenan-

(1) Sigman, D. S.; Graham, D. R.; D'Aurora, V.; Stern, A. M. *J. Biol. Chem.* **1979**, *254*, 12269–12272.

(2) Downey, K. M.; Que, B. G.; So, A. G. *Biochem. Biophys. Res. Commun.* **1980**, *93*, 264–270.

(3) Que, B. G.; Downey, K. M.; So, A. G. *Biochemistry* **1980**, *19*, 5987–5991.

(4) Marshall, L. E.; Graham, D. R.; Reich, K. A.; Sigman, D. S. *Biochemistry* **1981**, *20*, 244–250.

(5) Lown, J. W.; Sim, S.-K. *Biochem. Biophys. Res. Commun.* **1977**, *77*, 1150–1157.

(6) Giloni, L.; Takeshita, M.; Johnson, F.; Iden, C.; Grollman, A. P. *J. Biol. Chem.* **1981**, *256*, 8608–8615.

(7) Marshall, L. E.; Reich, K. A.; Graham, D. R.; Sigman, D. S. *Fed. Proc.* **1981**, *40*, 1730.

(8) Sim, S.-K.; Lown, J. W. *Biochem. Biophys. Res. Commun.* **1978**, *81*, 99–105.

(9) Lown, J. W.; Sim, S.-K.; Majumdar, K. C. Chang, R.-Y. *Biochem. Biophys. Res. Commun.* **1977**, *76*, 705–710.

(10) Lown, J. W.; Begleiter, A.; Johnson, D.; Morgan, A. R. *Can. J. Biochem.* **1976**, *54*, 110–119.

(11) Kapp, D. S.; Smith, K. C. *Radiat. Res.* **1970**, *42*, 34–49.

0002-7863/82/1504-5494$01.25/0

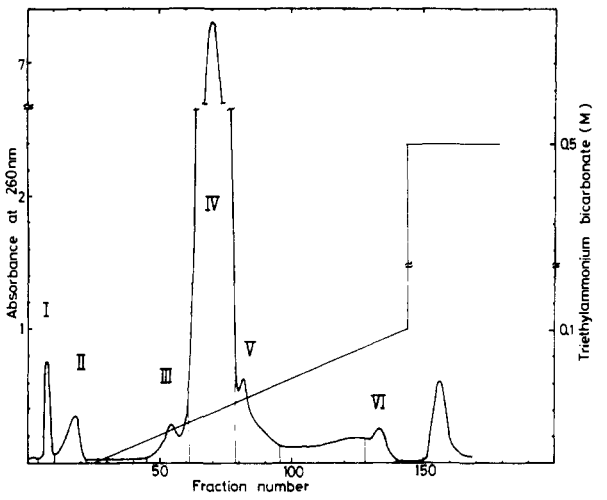

Figure 1. Separation of degradation products of d(C-G) by the phenanthroline–Cu^+ complex on a column (1.7 × 37.5 cm) of DEAE-cellulose (HCO_3^- form). Elution was carried out with a linear gradient of triethylammonium bicarbonate (0–0.1 M, total 2 L).

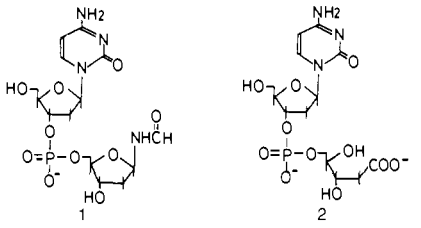

Figure 2. Structures of degradation products.

throline–Cu^+ complex and oxygen.

The complete reaction mixture contained 10 mM d(C-G), 500 μM 1,10-phenanthroline, 50 μM $CuSO_4$, 100 mM 3-mercaptopropionic acid, and 100 mM sodium phosphate buffer (pH 7.5). Oxygen was introduced to the reaction mixture by aeration at 0 °C for 24 h. The reaction mixture was passed through a column of activated charcoal.[13] The desalted mixture was chromatographed on a DEAE–cellulose column. The elution profile is shown in Figure 1. d(C-G) was eluted in the major peak, IV. The compounds in the minor peaks I, II, and VI were identified by UV and ^1H NMR spectra and paper chromatography or paper electrophoresis.[14] They were shown to be cytosine, guanine, and deoxyguanosine 5′-phosphate (5′-dGMP), respectively. Two compounds in peak III were further separated by chromatography on a column of Dowex 1 resin (HCO_3^- form).[15] Compounds **1** and **2** from the first and second peaks, respectively, were proved to have the structures shown in Figure 2. **1** shows UV spectra similar to those of deoxycytidine at three pHs and migrates as fast as d(C-G) on paper electrophoresis at pH 7.5. The ^1H NMR (360 MHz) and ^{13}C NMR (50 MHz) spectra show the presence of a dCp- residue and a pair of isomeric sugar derivatives. **1** was hydrolyzed by spleen phosphodiesterase to yield dCp and a pair of sugar derivatives (cis–trans isomers about the amide linkage). The latter derivatives show ^1H NMR spectra identical with that of *N*-(2′-deoxy-β-D-erythropentofuranosyl)formamide (**3**),[16] which was synthesized from deoxythymidine (Figure 3). **2** also shows UV spectra similar to those of deoxycytidine and comigrates with

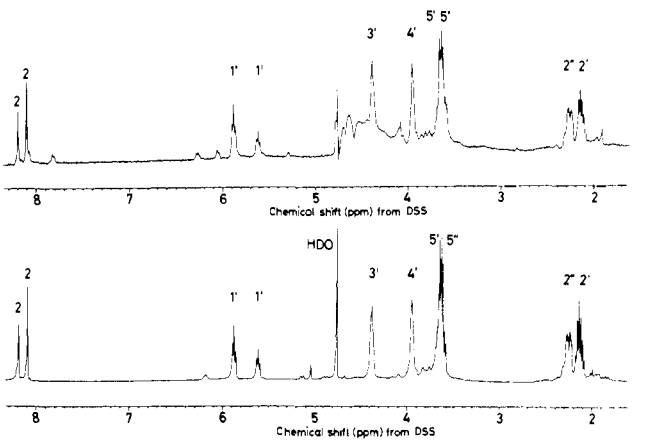

Figure 3. ^1H NMR (360 MHz) spectra of *N*-(2′-deoxy-β-D-erythropentofuranosyl)formamide (**3**) obtained from **1** by enzymic digestion (upper spectrum) and synthesized from deoxythymidine (lower spectrum); measured in D_2O.

dCp on paper electrophoresis at pH 7.5. The ^1H and ^{13}C NMR spectra show the presence of a dCp- residue and a sugar residue containing a carboxyl group (^{13}C chemical shift in D_2O, pD 7.5, 182.11 ppm from DSS reference) and are identical with those of a compound that was synthesized from d(C-G) by depurination with 30% formic acid and oxidation with silver oxide.

The relative yields of the degradation products were as follows: cytosine, 2.0%; guanine, 2.1%, 5′-dGMP, 2.1%; **1**, 1.6%; **2**, 2.4%. Two control reactions, where either phenanthroline or $CuSO_4$ was omitted, were examined. DEAE–cellulose chromatography gives no degradation products in either case. It is assumed that the phenanthroline–Cu^+ complex is intercalated between the base pairs of the d(C-G) duplex and oxygen radicals generated by the complex and oxygen attack nearby carbon atoms. **2** may be formed by hydroxylation at C1′ of a dG residue, elimination of the guanine base, and hydrolysis of the deoxyribonolactone residue.[17] Cytosine and 5′-dGMP may be formed by similar oxidation at C1′ of a dC residue, subsequent base release, and elimination of 5′-dGMP residue from the 3′-O-esterified deoxyribonolactone derivative. The lability of this type of compound was confirmed by the following reactions. d(G-C), a sequence isomer of d(C-G), was depurinated with 30% formic acid. Oxidation of the product with silver oxide gave 5′-dCMP but no compound corresponding to **2**. The first step for production of **1** may be oxidation at the C4–C5 double bond[18] of the guanine residue. Similar oxidation occurs at the C5–C6 double bond of deoxythymidine under γ irradiation in aqueous solution, and **3** is obtained as one of many products.[19,20] Deoxyribonolactone is also formed in the radiolysis.

From the present results on d(C-G), it is deduced that DNA cleavage by the $(OP)_2Cu^+$ complex is initiated by oxidation of C1′ with oxygen free radicals followed by release of the attached base and successive cleavage of C3′–OP and C5′–OP bonds, presumably by β-elimination reactions. The same mechanism resulting in the direct release of bases and concomitant cleavage of phospho diester bonds also could be involved in the case of bleomycin.[6] The present work revealed that even a dimer can be cleaved by the phenanthroline–Cu^+ complex. However, further studies on longer oligonucleotides may be necessary to prove that the dimer case can be extrapolated to the case of large DNA.

Acknowledgment. We thank Dr. Alexander F. Markham for reading the manuscript.

Registry No. 1, 82871-74-7; **2**, 82871-75-8; **3**, 57609-73-1; $(OP)_2Cu^+$, 17378-82-4; d(C-G), 15178-66-2; cytosine, 71-30-7; guanine, 73-40-5; deoxyguanosine-5′-phosphate, 902-04-5.

(12) Uesugi, S.; Shida, T.; Ikehara, M. *Chem. Pharm. Bull.* **1981**, *29*, 3573–3585.
(13) Elution was carried out with 50% aqueous ethanol containing 0.5% concentrated ammonium hydroxide.
(14) The solvent for paper chromatography was 7:3 ethanol–1 M ammonium acetate buffer (pH 7.5). The buffer for electrophoresis was 0.05 M triethylammonium bicarbonate (pH 7.5).
(15) The column size was 1.3 × 11.5 cm. Elution was carried out with a linear gradient of triethylammonium bicarbonate buffer (pH 7.5, 0–0.2 M, total 3 L).
(16) Cadet, J.; Nadrian, R.; Voituriez, L.; Remin, M.; Hruska, F. E. *Can. J. Chem.* **1981**, *59*, 3313–3318.
(17) This hydrolysis may occur during the workup.
(18) Holian, J.; Garrison, W. M. *Chem. Commun.* **1967**, 676–677.
(19) Cadet, J.; Teoule, R. *Tetrahedron Lett.* **1972**, 3225–3228.
(20) Cadet, J.; Teoule, R. *Bull. Soc. Chim. Fr.* **1975**, 891–895.