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Mechanisms for the Formation of Major Oxidation Products of Adenine upon 365-nm Irradiation with 2-Methyl-1,4-naphthoquinone as a Sensitizer

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Received September 7, 2002

Recently we reported the isolation and characterization of N^6-formyl- and N^6-acetyladenine from 365-nm irradiation of dinucleoside monophosphates d(ApA), d(ApC), and d(CpA) in the presence of 2-methyl-1,4-naphthoquinone (menadione) (Wang et al. *Biochem. Biophys. Res. Commun.* **2002**, *291*, 1252−7). In this article we investigated the mechanisms for the formation of the two major products by carrying out photoirradiation with isotopically labeled menadione and 2,3-dimethyl-1,4-naphthoquinone. HPLC and electrospray ionization (ESI)-mass spectrometry (MS) and tandem MS studies of the products unambiguously established that the carbonyl group in the products arises from the photosensitizer: The N^6-formyl group comes from oxidation of the methyl group and the N^6-acetyl group stems from the methyl group and the adjacent ring carbon in menadione. From above results, we proposed mechanisms for the formation of the two products.

Introduction

Nucleic acid damage induced by endogenous or exogenous reactive oxygen species (ROS) is an important pathway that contributes to a number of pathological conditions including cancer, neurological diseases, and aging.[1−6] Solar irradiation is a common exogenous ROS source, among which UV irradiation is the most harmful and mutagenic component.[7,8] DNA bases can directly absorb incident UVB light (280−320 nm); on the other hand, UVA light (320−400 nm) can be absorbed by endogenous photosensitizers which can damage DNA through photooxidation. The latter process can occur through a type I and/or type II mechanism.[9] The type I mechanism involves either initial electron or hydrogen abstraction by an excited-state photosensitizer. The type II mechanism, however, occurs through the reaction of guanine with singlet oxygen ($^1O_2$).[9]

Some quinone species can damage DNA by the above photooxidation mechanisms. Among them 2-methyl-1,4-naphthoquinone (MQ), or menadione, belongs to the vitamin K family (vitamin K_3). The triplet state of MQ has been shown to be an effective electron acceptor.[10−14] MQ-induced damage of nucleobases, nucleosides, and short oligonucleotides has been investigated.[15−20] Although, among the four DNA bases, guanine was shown to be the easiest to be oxidized by MQ photosensitization in a mixture of four mononucleosides and in isolated DNA,[21] oxidation of other DNA bases is also important. In this respect Melvin and co-workers[17] showed that photoirradiation in the presence of MQ can give rise to the molecular weight increase of single-stranded poly(dA). The origin of the molecular weight increase, however, has not been determined.

Recently we reported the isolation and characterization of two major oxidation products of adenine, N^6-formyl- and N^6-acetyladenine (Scheme 1), in dinucleoside monophosphates d(ApA), d(ApC), and d(CpA).[22] This is a

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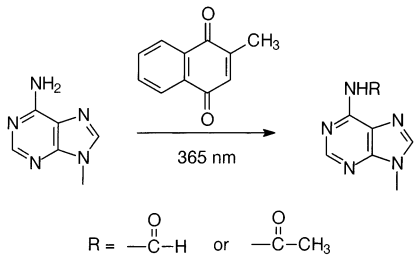
10.1021/jo0264170 CCC: $22.00
Published on Web 11/05/2002

SCHEME 1



continuous study with aims at determining the origins of the formyl and acetyl groups and rationalizing the mechanisms for the formation of the two products. To this end, we synthesized menadione and 2,3-dimethyl-1,4-naphthoquinone whose methyl group is labeled with a stable isotope, ^{13}C or D. We will demonstrate that the formyl group arises from the oxidation of the methyl group and the acetyl group originates from the methyl group and the adjacent ring carbon in menadione. We will also propose tentative mechanisms for the formation of the two major products.

Results and Discussion

Effect of Oxygen on the Formation of the Acetylated and Formylated Products. As we reported previously,[22] the acetylation and formylation were found to be major reactions of adenine while dinucleoside monophosphates d(ApA), d(CpA), and d(ApC) were irradiated with 365-nm light in the presence of 2-methyl-1,4-naphthoquinone. We did the MQ-sensitized photoirradiation of d(ApC) for a time period varying from 1 min to 2 h, and HPLC analysis showed that the yield of the acetylated product increases with time and remains constant when the irradiation time is longer than 30 min. This result may suggest that the N^6-acetyladenine product in d(ApC) can further react with the sensitizer. However, we did not identify any new product even from the 2-h irradiation mixture. Moreover, the acetylated product was the major product formed independent of the irradiation time (data not shown).

The formation of those two products appears unusual, and it is important to investigate the mechanisms for their formation, for which we need to determine the importance of oxygen in the reaction and to investigate the origins of the acetyl and formyl groups. To this end, we carried out photoirradiations under three otherwise identical conditions except that the amounts of oxygen present are different (details shown in Experimental Section). Indeed HPLC and ESI-MS studies show that the amount of oxygen in solution plays a significant role in the formation of the two products (HPLC traces shown in Figure 1). Under deoxygenated condition, neither the acetylated nor the formylated product was produced (Figure 1a). Furthermore, the yields for the products are higher for the reaction with air-bubbling ("oxygenated condition") than the control experiment where the solution was prepared and irradiated without air or Ar bubbling. The ratio of the peak area of the acetylated product to that of the starting material d(ApC) increased from 11% in the control experiment to 27% in the experiment with air-bubbling (Figure 1b,c). The results clearly show that oxygen is necessary for the formation

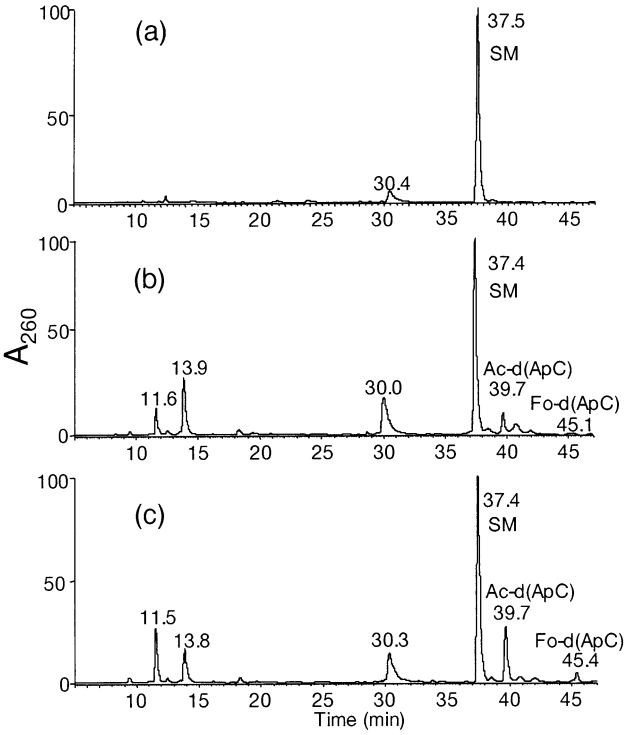
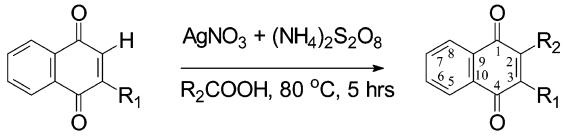


FIGURE 1. HPLC traces for the separation of menadione-sensitized irradiation of d(ApC) under deoxygenated (a), control (b), and oxygenated solutions (c). SM is the starting material d(ApC). Ac-d(ApC) and Fo-d(ApC) are the acetylated and formylated products of d(ApC), respectively.

SCHEME 2



1 - 5

1 R_1 = H, R_2 = CD_3
2 R_1 = H, R_2 = $^{13}CH_3$
3 R_1 = CH_3, R_2 = CH_3
4 R_1 = CH_3, R_2 = CD_3
5 R_1 = CH_3, R_2 = $^{13}CH_3$

of the two major products. Similarly, Wagner and coworkers[13] found that the presence of oxygen is important for the menadione-sensitized photooxidation of thymidine. The authors attributed the effect to that the molecular oxygen is an electron quencher and it prevents back electron transfer from the radical anion of menadione to the radical cation of thymidine.[13]

Photoreactions of d(ApC) with Different Isotope-Labeled MQ. To determine the origin of the carbonyl group in the products, we synthesized 2-methyl-d_3-1,4-naphthoquinone (**1**) and 2-methyl-^{13}C-1,4-naphthoquinone (**2**) (structures shown in Scheme 2) following the same procedure as reported in the literature.[23] In our hands, however, we were unable to obtain the pure labeled menadione via recrystallization. Instead we used reverse-phase HPLC separation to obtain the pure product. Using

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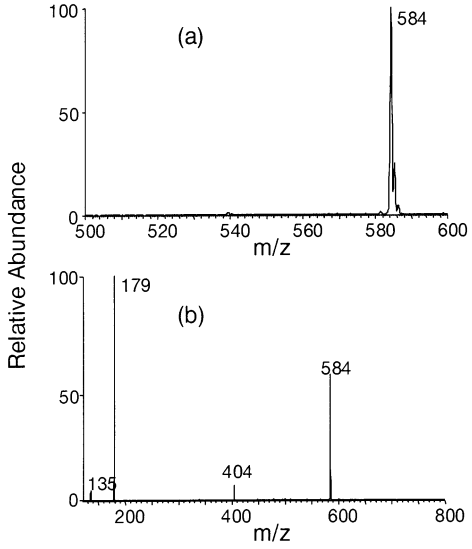

FIGURE 2. ESI-MS (a) and product-ion spectrum for the $[M - H]^-$ ion (b) of the acetylated product of d(ApC) when 2-methyl-d_3-1,4-naphthoquinone (**1**) was used as photosensitizer.

a 40:60 (by volume) mixture of acetonitrile and water as the mobile phase, we were able to separate the three components, 1,4-naphthoquinone, menadione, and 2,3-dimethyl-1,4-naphthoquinone, from each other (chromatograms shown in the Supporting Information). From the HPLC peak areas with absorbance at 350 nm and by assuming that the three components have similar extinction coefficients at 350 nm, we estimated that the ratio for the unreacted 1,4-naphthoquinone, 2-methyl-d_3-1,4-naphthoquinone (**1**), and 2,3-dimethyl-*2*-d_3-1,4-naphthoquinone (**4**) is approximately 20:60:20. Similarly, we separated the three components from the reaction mixture of 1,4-naphthoquinone and $^{13}CH_3COOH$.

The irradiation products from use of **1** and **2** as sensitizers were again separated by HPLC and characterized by ESI-MS and MS/MS and the identities of the products established the origins of carbonyl groups. Negative-ion ESI-MS (Figure 2a) for the acetylated product from the photoirradiation with **1** as sensitizer shows an ion of *m*/*z* 584, which is 3 mass units higher than the corresponding acetylated product isolated from photoirradiation with the unlabeled photosensitizer. The result indicates that the acetylated product carries the D_3-label. In addition, ions of *m*/*z* 179 and 135 were observed in the MS/MS of *m*/*z* 584 ion (Figure 2b), those two product ions are the anions of N^6-acetyladenine and adenine, respectively. It is very likely that a deuterium transfers from the methyl group to adenine upon the loss of ketene (CD_2CO) moiety from the N^6-acetyladenine, which results in an anion of *m*/*z* 135 for adenine. The above results show clearly that the methyl group in N^6-acetyladenine arises from the methyl group in menadione. Interestingly, no formylated product was detected in the photoreaction of d(ApC) with **1**.

To pinpoint the origin of the carbonyl group in N^6-formyladenine and to gain further evidence for the origin of carbonyl groups in the N^6-acetyladenine, we used 2-methyl-^{13}C-1,4-naphthoquinone (**2**) as a sensitizer for the photoreaction. Our results show that the formylated product formed and the negative-ion ESI-MS showed an

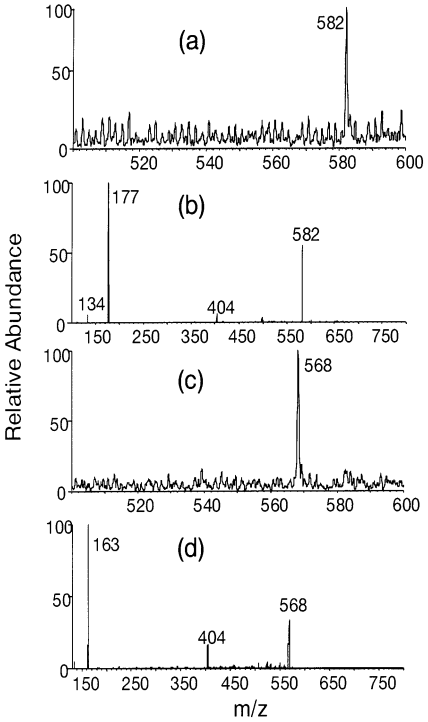

FIGURE 3. ESI-MS and MS/MS of the $[M - H]^-$ ions of the acetylated and formylated products of d(ApC) when 2-methyl-^{13}C-1,4-naphthoquinone (**2**) was used as photosensitizer: (a) ESI-MS of the acetylated product, (b) MS/MS of the $[M - H]^-$ ion of the acetylated product, (c) ESI-MS of the formylated product, and (d) MS/MS of the $[M - H]^-$ ion of the formylated product.

ion of *m*/*z* 568 (Figure 3c). Upon collisional activation, a fragment ion of *m*/*z* 163, which is the anion of N^6-formyladenine, was observed in the product-ion spectrum (Figure 3d). The formylated product and the N^6-formyladenine fragment are one mass unit higher than the corresponding ions observed in the control experiment where unlabeled menadione is used as sensitizer. We, therefore, establish unambiguously that the carbonyl group arises from the oxidation of the methyl group in menadione because the formyl group carries the ^{13}C label.

Similarly, an ion of *m*/*z* 582 was observed in negative-ion ESI-MS for the acetylated product (Figure 3a). In addition, an ion of *m*/*z* 177, which is the anion of N^6-acetyladenine, was observed in the MS/MS of the *m*/*z* 582 ion (Figure 3b), indicating that the acetylated product contains the ^{13}C label. Furthermore, ^1H and ^{13}C NMR spectra of the acetylated product demonstrate that the methyl carbon in the acetyl group carries the ^{13}C label. ^1H NMR shows that the spectrum is very similar to that of the unlabeled acetylated product except that the methyl proton resonance at δ 2.4 is split into two peaks, which is due to a one-bond coupling between ^{13}C and ^1H (J = 129 Hz, shown in the Supporting Information). In addition, ^{13}C NMR shows a single resonance at δ 24, which is also in line with the presence of ^{13}C in the methyl group of the acetyl functionality.

To summarize, we have demonstrated that both the formyl group in N^6-formyladenine and the methyl group in N^6-acetyladenine originate from the methyl group in menadione. The results also indicate that the carbonyl

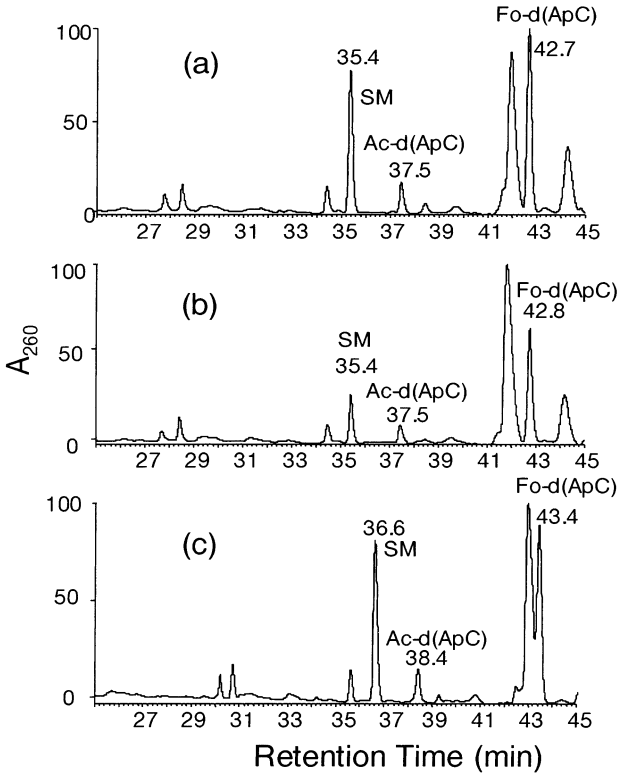

FIGURE 4. HPLC traces for the separation of 365-nm irradiation of d(ApC) photosensitized with 2,3-dimethyl-1,4-naphthoquinone (a), 2,3-dimethyl-*2*-d_3-1,4-naphthoquinone (b), and 2,3-dimethyl-*2*-^{13}C-1,4-naphthoquinone (c).

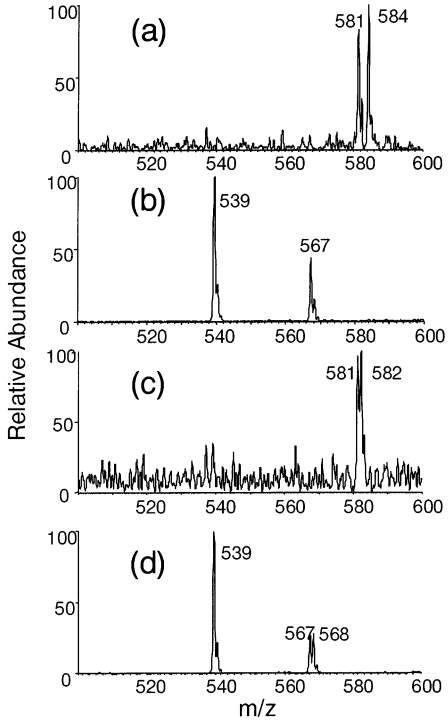

FIGURE 5. Negative-ion ESI-MS of the acetylated and formylated products of adenine in d(ApC) while 2,3-dimethyl-*2*-d_3-1,4-naphthoquinone (**4**) and 2,3-dimethyl-*2*-^{13}C-1,4-naphthoquinone (**5**) were used as photosensitizers. (a, b) MS for the acetylated and formylated product while **4** was used and (c, d) MS for the acetylated and formylated product while **5** was used.

in N^6-acetyladenine is probably from the oxidation of C2 in menadione.

Photoreactions of d(ApC) with 2,3-Dimethyl-1,4-naphthoquinone (3), 2,3-Dimethyl-*2*-d_3-1,4-naphthoquinone (4), and 2,3-Dimethyl-*2*-^{13}C-1,4-naphthoquinone (5). The fact that 2,3-dimethyl-1,4-naphthoquinone is present in the product mixture from the reaction of 1,4-naphthoquinone with acetic acid indicates that methylation of menadione is also an efficient process under the reaction condition. This result motivates us to prepare the 2,3-dimethyl-1,4-naphthoquinone with ^{13}C or D or without isotope label in one of the methyl groups (**3**−**5**, Scheme 2). It turns out that we can readily obtain pure 2,3-dimethyl-1,4-naphthoquinones (HPLC traces for the separation of the reaction mixtures are shown in the Supporting Information).

To obtain additional evidence regarding the origins of the formyl and acetyl groups, we carried out experiments with those 2,3-dimethyl-1,4-naphthoquinones (**3**−**5**) as photosensitizers. Interestingly, the presence of an additional methyl group drastically changes the product distribution. Although the yield for the formation of N^6-acetyladenine is not significantly different from the experiment when menadione was used as sensitizer, that of N^6-formyladenine is much higher when 2,3-dimethyl-1,4-naphthoquinones were used as sensitizers (Figure 4 shows the HPLC traces for the separation of the photoirradiation mixtures). For the photosensitized reaction with **4**, both D_3-labeled (*m*/*z* 584) and unlabeled (*m*/*z* 581) acetylated products were obtained, whereas only the unlabeled (*m*/*z* 567) formylated product was observed (Figure 5a,b). The result is consistent with that obtained from the photoreaction with 2-methyl-d_3-1,4-naphthoquinone (**1**), indicating the isotope effect for the formation of the formylated product. When **5** was used as the photosensitizer, acetylated and formylated products were obtained in both unlabeled and ^{13}C-labeled forms (Figure 5c,d). The HPLC peak immediately before the formylated product in each panel of Figure 4 is also present in the control photoirradiation of **3** by itself but absent in the control HPLC separation of **3** without photoirradiation (data shown in the Supporting Information). Therefore, it is a photoinduced decomposition product of 2,3-dimethyl-1,4-naphthoquinone whose structure, however, remains unknown.

Proposed Mechanism for the Product Formation. From the results of the O_2-dependent photoirradiation and the studies with different stable-isotope incorporated menadiones (**1** and **2**) and 2,3-dimethyl-1,4-naphthoquinones (**3**−**5**) (results were summarized in Table 1), we proposed mechanisms for the formation of the N^6-acetyl- and N^6-formyladenine (Schemes 3 and 4). In both mechanisms, the formation of the N^6 radical of adenine is an important step. One-electron photooxidation of adenine gives rise to its cation radical,[17,24] which is known to be very acidic (pK_a < 1) and deprotonates readily to form the N^6-centered adenine radical.[24]

For the formation of N^6-formyladenine, menadione tautomerizes to form a quinone methide,[25] which can react with the N^6-centered adenine radical (Scheme 3

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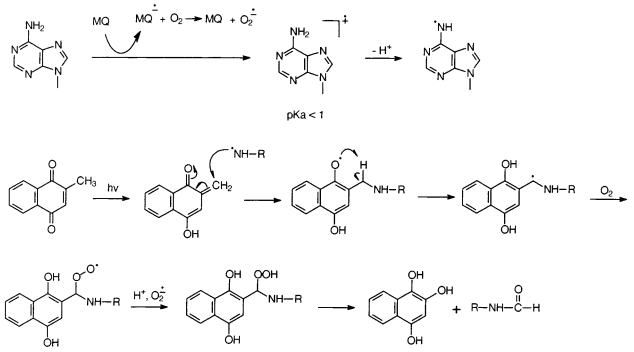
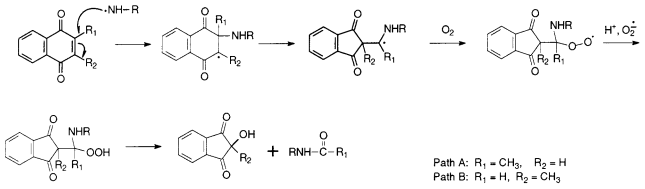
TABLE 1. Summary for the Photosensitization Reactions with Different Isotope-Labeled Photosensitizers

Sensitizers	MQ	1	2	3	4	5
Acetylation	—C(=O)—CH_3	—C(=O)—CD_3	—C(=O)—$^{13}CH_3$	—C(=O)—CH_3	—C(=O)—CH_3 + —C(=O)—CD_3 (~ 1:1)	—C(=O)—CH_3 + —C(=O)—$^{13}CH_3$ (~ 1:1)
Formylation	—C(=O)—H	None	$^{-13}$C(=O)—H	—C(=O)—H	—C(=O)—H	—C(=O)—H + $^{-13}$C(=O)—H (~ 1:1)

SCHEME 3



SCHEME 4



shows the reaction with unlabeled menadione as an example). After hydrogen rearrangement, the resulting carbon-centered radical can combine with molecular oxygen to give a peroxyl radical. The peroxyl radical is then reduced to a hydroperoxide, from which the formylated product can form via α-cleavage. The α-cleavage has been observed for alkyl hydroperoxy ketones,[26−28] and it has also been found in the decomposition of 5-hydroperoxy-6-hydroxy-5,6-dihydrothymidine.[16]

Regarding the proposed mechanism, *o*-quinone methide has been observed in the photolysis of vitamin K1, which has a similar structure as menadione.[29] Likewise, photolysis (254, 266, or 308 nm) of pyridoxine (vitamin B6) gives *o*-quinone methide.[30] The formation of a peroxyl radical through the combination of the 6-hydroxy-5-yl radical of thymidine with molecular oxygen was also proposed for the degradation of thymidine under similar irradiation conditions.[13] From the proposed mechanism, we expect that the formation of *o*-quinone methide and the following hydrogen transfer steps have the primary isotope effect, which accounts for the absence of the formylated product from the oxidation of the CD_3 group when **1** and **3** are used as photosensitizers (Table 1).

For the formation of N^6-acetyladenine, we propose that the N^6-centered radical attacks C2 to give a C3-centered radical, which can rearrange to form an exocyclic carbon-centered radical. Similarly, the latter radical can combine with molecular oxygen to form a peroxyl radical, which can again be reduced to form a hydroperoxide. The resultant hydroperoxide can follow a familiar α-cleavage to give the acetylated product (Scheme 4).

From the proposed mechanism in Scheme 4, it appears that both path A and path B, which lead to the formation of the acetylated and formylated products, are feasible. A closer look, however, reveals that path A should be a more facile process than path B. While the ring radical is rearranged to form the exocyclic radical, a secondary radical is changed to a tertiary one in path A, which is energetically favorable. However, it is the other way around in path B.

The argument is also consistent with the relative yields for the N^6-acetyladenine and N^6-formyladenine when menadione and 2,3-dimethyl-1,4-naphthoquinone are photosensitizers. While the former is used as a sensitizer, the yield for the acetylated product is much higher than that for the formylated product (Figure 1), whereas the relative yields are reversed while the latter is used as a sensitizer (Figure 4). We expect that acetylation and formylation compete against each other. For the formation of the acetylated product, a tertiary radical is changed to another tertiary one while 2,3-dimethyl-1,4-naphthoquinone is the sensitizer. However, as stated previously, a secondary radical is changed to a tertiary one while 2-methyl-1,4-naphthoquinone is the sensitizer. Therefore, we anticipate that the formation of the acetylated product is a more facile process for photosensitization with menadione than that with 2,3-dimethyl-1,4-naphthoquinone. Collectively, our proposed mechanisms are consistent with all the experimental results that we have obtained.

Conclusions

Upon 365-nm irradiation with menadione or 2,3-dimethyl-1,4-naphthoquinone as a photosensitizer, adenine in dinucleoside monophosphate d(ApC) undergoes reaction to form N^6-acetyl- and N^6-formyladenines. Pho-

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toirradiation in the presence of different amounts of oxygen shows that oxygen is required for the formation of those two products.

Photoirradiations with 2-methyl-^{13}C-1,4-naphthoquinone (**1**) and 2-methyl-d_3-1,4-naphthoquinone (**2**) as sensitizers show that the formyl group in the product arises from the methyl group in the sensitizer and the perdeuterated methyl group in the sensitizer does not give rise to the formylated product. The results also show that the methyl in N^6-acetyladenine originates from the methyl group in menadione, which suggests that the carbonyl in the acetyl group is very likely from the oxidation of C2 in menadione. Photoirradiation with dimethylated naphthoquinones (**3**−**5**) gave similar results. On the basis of the above observations and the known acidity of adenine cation radical,[24] we proposed reaction mechanisms for the formation of those two types of products.

Experimental Section

Spin multiplicities for proton NMR spectra are given as s (singlet), d (doublet), dd (doublet of doublet), dq (doublet of quartet), or m (multiplet). Coupling constants are given in Hz.

2-Methyl-d_3-1,4-naphthoquinone (Scheme 2, 1). We followed the procedure reported by Fauler and co-workers.[23] Glacial acetic-d_3 acid-*d* (99.9% D, 1 mL), 1,4-naphthoquinone (2 g), and silver nitrate ($AgNO_3$, 1.28 g) were dissolved in 120 mL of a solvent mixture of acetonitrile and water at a volume ratio of 2:1. Ammonium persulfate (3.8 g), dissolved in 50 mL of water, was added gradually to the stirred solution in 15 min. After the mixture was incubated at 80 °C for 5 h and at room temperature overnight, 200 mL of water was added and the solution was extracted twice with 50 mL of dichloromethane. The organic layer was washed twice with 100 mL of water and dried with sodium sulfate. After filtration, the solvent was distilled off under reduced pressure. The oily product was purified by reverse-phase HPLC as detailed in the HPLC section. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.85 (s, 1H), 7.74 (m, 2H), 8.08 (m, 2H). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 126.4, 126.8, 132.4, 132.5, 133.9, 134.9, 136.0, 185.3, 185.9. EI-MS (positive-ion): *m*/*z* 175, 147, 118, 104, 76, 50.

2-Methyl-^{13}C-1,4-naphthoquinone (2). The procedure was the same as above except that acetic-2-^{13}C acid (99% ^{13}C, 0.25 g) was used instead of acetic-d_3 acid-*d* (1 g), and the quantities of other reagents were adjusted accordingly. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.20 (dd, 3H, $J_{C-H}^1 = 129.2$, $J_{H-H}^4 = 1.6$, $^{13}CH_3$), 6.84 (dq, 1H, $J_{H-H}^4 = 1.6$, $J_{C-H}^3 = 5.9$, H_3), 7.74 (m, 2H), 8.09 (m, 2H). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.2. EI-MS (positive-ion): *m*/*z* 173, 145, 117, 116, 104, 76, 50.

The procedure for the synthesis of 2,3-dimethyl-1,4-naphthoquinones was the same as that for the synthesis of 2-methyl-d_3-1,4-naphthoquinone except that the starting material was 2-methyl-1,4-naphthoquinone instead of 1,4-naphthoquinone, and acetic acid, acetic-d_3 acid-*d*, and acetic-2-^{13}C acid were used for the synthesis of 2,3-dimethyl-1,4-naphthoquinone (**3**), 2,3-dimethyl-2-d_3-1,4-naphthoquinone (**4**), and 2,3-dimethyl-2-^{13}C-1,4-naphthoquinone (**5**), respectively.

2,3-Dimethyl-1,4-naphthoquinone (3). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.20 (s, 6H), 7.70 (m, 2H), 8.10 (m, 2H). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.1, 126.3, 132.2, 133.3, 143.4, 184.9. EI-MS (positive-ion): *m*/*z* 186, 158, 157, 129, 115, 104, 76, 50.

2,3-Dimethyl-2-d_3-1,4-naphthoquinone (4). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.19 (s, 3H), 7.70 (m, 2H), 8.08 (m, 2H). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.1, 126.4, 132.3, 133.5, 143.6, 185.1. EI-MS (positive-ion): *m*/*z* 189, 161, 132, 121, 104, 76, 50.

2,3-Dimethyl-2-^{13}C-1,4-naphthoquinone (5). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.19 (s, 3H), 2.19 (d, 3H, $J_{C-H}^1 = 129.2$, $^{13}CH_3$), 7.70 (m, 2H), 8.09 (m, 2H). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 12.9. EI-MS (positive-ion): *m*/*z* 187, 159, 158, 130, 116, 104, 76, 50.

Photosensitized Reactions. Dinucleoside monophosphate d(ApC) (300 nmol) was dissolved in a 10-mL aqueous solution that was saturated with the photosensitizer (approximately 0.3 mM as determined by UV absorbance at 350 nm[21]). The solution was then transferred to a 10.2-cm i.d. Petri dish, irradiated on ice for 1 h under air with two 15-W Spectroline light tubes emitting at 365 nm (Spectronics Corporation, Westbury, NY), and dried by using a speed-vac. The dried residue was redissolved in water and subjected to HPLC analysis.

Photoirradiation experiments with various amounts of oxygen were carried out under three different conditions. Under the deoxygenated condition, the solution was degassed by bubbling the solution with Ar for 1 h, transferred to a Petri dish, inserted into an Ar-filled zip-lock bag, and sealed. In the control experiment, the Petri dish with the sample solution was inserted into a similar zip-lock bag that was open to air. Under oxygenated condition, the sample solution was again dispersed in a Petri dish and inserted into a zip-lock bag, but the solution was continuously bubbled with air during irradiation. The photoirradiation was otherwise under the same condition as stated above.

HPLC and Mass Spectrometry. The HPLC separation was performed on a system with a photodiode array detector, and a 4.6 × 250 mm reverse-phase C18 column (5 μm in particle size, and 300 Å in pore size) was used. The flow rate was 1.0 mL/min. An isocratic elution with 40:60 acetonitrile/water (by volume) and a gradient of 35 min 6−12% acetonitrile in 50 mM triethylammonium acetate (pH 6.8) were employed for the separation of the synthetic mixtures of isotopically labeled menadiones and the photoirradiation products, respectively.

Electrospray ionization (ESI)-mass spectrometry (MS) and tandem MS (MS/MS) experiments were carried out on an LCQ Deca XP ion-trap mass spectrometer (ThermoFinnigan, San Jose, CA). An equal-volume solvent mixture of acetonitrile and water was used as the carrier and electrospray solvent, and a 1-μL aliquot of a 5 μM sample solution was injected in each run. The spray voltage was 4.0 kV, and the capillary temperature was maintained at 200 °C. MS/MS was done by selecting the $[M - H]^-$ ions for collisional activation. The mass width for precursor selection was 1 *m*/*z* unit and the collision gas was helium. Each spectrum was obtained by averaging approximately 50 scans, and the time for each scan was 0.1 s.

Acknowledgment. The authors acknowledge the NIH (grant no. CA 96909) and the University of California at Riverside for supporting this research. Y.W. also acknowledges Professors William H. Okamura, Thomas H. Morton, and John-Stephen A. Taylor for very helpful discussions.

Supporting Information Available: HPLC traces for the separation of product mixtures of the preparation of **1**−**5**; ^{1}H NMR and ^{13}C NMR spectra of ^{13}C-labeled N^6-acetyladenine in d(ApC); ^{1}H NMR spectra of compounds **1**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0264170