Bioorganic & Medicinal Chemistry Letters 19 (2009) 2070–2074

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Photoirradiation products of flavin derivatives, and the effects of photooxidation on guanine

Katsuhito Kino *, Teruhiko Kobayashi, Eiji Arima, Rie Komori, Takanobu Kobayashi, Hiroshi Miyazawa

Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, 1314-1 Shido, Sanuki, Kagawa 769-2193, Japan

ARTICLE INFO

Article history:
Received 8 October 2008
Revised 6 January 2009
Accepted 21 January 2009
Available online 14 February 2009

Keywords:
Guanine oxidation
Flavin
UV-A radiation
VIS radiation

ABSTRACT

Photoirradiation in the presence of riboflavin led to guanine oxidation and the formation of imidazolone. Meanwhile, riboflavin itself was degraded by ultraviolet light A (UV-A) and visible light (VIS) radiation, and the end product was lumichrome. VIS radiation in the presence of riboflavin oxidized guanine similarly to UV-A radiation. Although UV-A radiation with lumichrome oxidized guanine, VIS radiation with lumichrome did not. Thus, UV-A radiation with riboflavin can oxidize guanine even if riboflavin is degraded to lumichrome. In contrast, following VIS radiation degradation of riboflavin to lumichrome, VIS radiation with riboflavin is hardly capable of oxidizing guanine. The consequences of riboflavin degradation and guanine photooxidation can be extended to flavin mononucleotide and flavin adenine dinucleotide. In addition, we report advanced synthesis; carboxymethylflavin was obtained by oxidation of formylmethylflavin with chlorite and hydrogen peroxide; lumichrome was obtained by heating of formylmethylflavin in 50% AcOH; lumiflavin was obtained by incubation of formylmethylflavin in 2 M NaOH, followed by isolation by step-by-step concentration.

Guanine is highly susceptible to oxidative stress in the DNA, as it has the lowest redox potential among the four DNA bases. Riboflavin (vitamin B2) (**RF**) (Fig. 1A) is a photosensitizer that causes electron transfer reactions[1,2] and generates singlet oxygen.[3] Ultraviolet light A (UV-A) radiation with **RF** oxidizes guanine, and imidazolone (Iz) is formed as a guanine oxidative product (Fig. 1B),[1,4] in addition to spirohydantoin and other products.[5] Iz may be responsible for DNA mutations, which might cause G:C to C:G transversions.[4,6–8] Thus, **RF** contained in our cells, especially basal cells and dermis, may be involved in the photooxidation of DNA, despite the fact that **RF** is degraded by light. Therefore, it is quite important to understand the photochemical effects of the degradation products of **RF**. Previously, **RF** degradation to lumichrome (**LC**), lumiflavin (**LF**), formylmethylflavin (**FMF**) and carboxymethylflavin (**CMF**) (Fig. 1A) by light was reviewed.[9] In addition, **FMF** and hydroxyethylflavin (**HEF**) (Fig. 1A) degradation to **LC** and **LF** by light were reported,[10,11] and the mechanism of photo-degradation from **RF** was described.[12,13] However, little is known about the quantitative analysis of photo-degradation products of the flavin derivatives under identical conditions. In addition, it has not yet been reported whether these degradation products from photoirradiated **RF** can oxidize guanine and whether blue visible light (VIS) instead of UV-A can oxidize guanine with flavin derivatives. In this study, UV-A or VIS radiation were found to generate **LC** from **RF**, **FMF**, **CMF**, **HEF**, flavin adenine dinucleotide (**FAD**) and flavin mononucleotide (**FMN**) under near-physiological conditions, such as pH 8. In addition, we found that the photochemical effects of **LC** on guanine oxidation are different from those of **RF**, **FAD** and **FMN**.

We first prepared **FMF**, **HEF**, **CMF**, **LC**, and **LF** from **RF**. The reaction of **RF** with periodate ion led to **FMF**, and **HEF** was obtained by reduction with sodium borohydride.[14,15] We applied the oxidation method[16] to the preparation of **CMF**; **FMF** was oxidized by chlorite and hydrogen peroxide (Scheme 1).[17] The procedure reported here is a simple method relative to the previous synthesis of **CMF**.[18,19]

LC was obtained by incubation of **FMF** in a hot acidic solution (Scheme 1).[20] This procedure is an easier and simpler method for large-scale synthesis of **LC** than previous methods: construction of isoalloxazine,[21] chromatographic separation of **RF** photolysis,[22] and hydrolysis of **FMF** by alkali.[23,24]

Although it was previously reported that treatment with 2 M sodium hydroxide resulted in the conversion of **FMF** to **LF**,[25] our HPLC analysis of the reaction showed that the obtained **LF** contained a small amount of **LC**.[26] By trial and error, pure **LF** was obtained by preferential removal of **LC** in a concentrated acidic solution (Scheme 1).[27]

Figure 2 shows HPLC analysis of the degradation of flavin derivatives by UV-A or VIS radiation. UV-A radiation at 366 nm degraded **RF** to **LC** as a major product, and **FMF** was formed as a minor product at 5 min in the reaction, and then degraded (Fig. 2A, open symbols). Moreover, the photolysis of **FMF** lead to

* Corresponding author. Tel.: +81 87 894 5111; fax: +81 87 894 0181.
E-mail address: kkino@kph.bunri-u.ac.jp (K. Kino).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.01.112

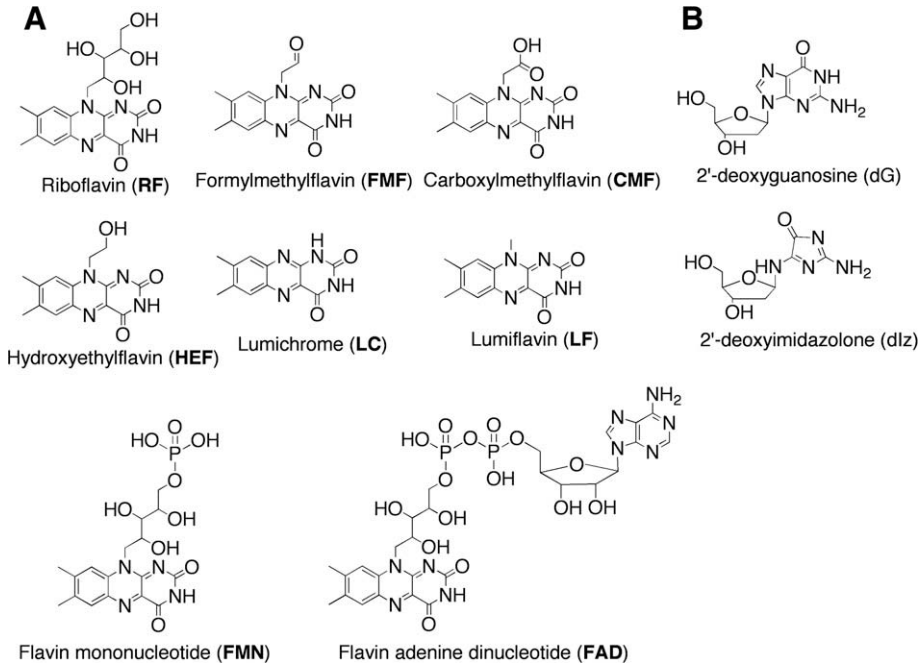


Figure 1. The structures of (**A**) flavin derivatives and (**B**) deoxynucleoside.

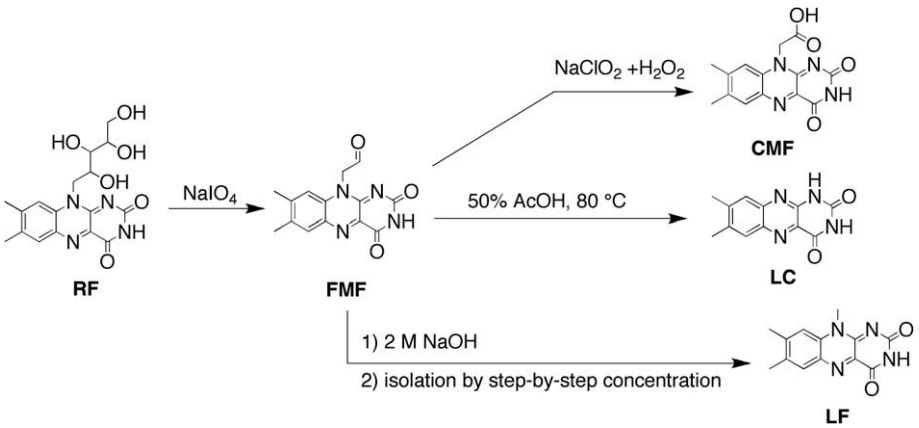
LC, and **FMF** was more rapidly degraded than **RF** (Fig. 2B, open symbols). Hence, it was confirmed that **FMF** is an intermediate product in the photolysis of **RF** to **LC**.[10,25] In addition, **CMF** was rapidly degraded, but **HEF** was slowly degraded (Fig. 2C and D, open symbols). **RF**, **FMN**, **FAD** and **HEF** have 2'-hydroxyl group, and **reduced FMF** arises from the flavins (step 2 in Scheme 2), followed by air-oxidation (step 3 in Scheme 2).[9] In the pathway leading to **reduced FMF**, the hydrogen abstraction reaction (step 1 in Scheme 2) is likely to be slow and rate-limiting. In contrast, McBride et al. suggested that **reduced FMF** could readily undergo cyclization (step 4 in Scheme 2).[10,11] Additionally, this cyclic reduced form can lead to the formation of **LC** (step 5 in Scheme 2). Therefore, the degradation rates of **FMF** and **CMF**, which have 2'-carbonyl group, were faster than those of the flavins which have 2'-hydroxyl group. These results are consistent with the previously reported mechanism.[12,13]

Since **RF** has two absorption maxima at 360–380 nm and 440–450 nm,[13,28,29] VIS radiation at 440 nm, as well as UV-A radiation at 366 nm, degraded **RF** to **LC** (Fig. 2A, closed symbols). This result confirmed that **RF** excited at 366 nm or 440 nm falls to the lowest excited state. **FMF**, **CMF** and **HEF** were also degraded to **LC** by VIS radiation (Fig. 2B–D, closed symbols). Like UV-A radiation, VIS radiation rapidly degraded **FMF** and **CMF**, and slowly degraded **RF** and **HEF** (Fig. 2A–D, closed symbols). In addition, **LF** was not further degraded by UV-A or VIS radiation.

Moreover, **FMN** and **FAD** were also degraded to **LC** by UV-A or VIS radiation (Fig. 2E and F). In addition, the degradation rate of **FMN** was similar to that of **RF**, whereas **FAD** was slowly degraded. The difference between the **FMN** and **FAD** degradation rate is likely dependent on whether adenine nucleotide exists.

Under all conditions used in Figure 2, the quantity of detected **LF** was less than 2%, and **CMF** and **HEF** intermediates were not detected at all. Therefore, UV-A or VIS radiation was concluded to convert from **RF**, **FAD,** and **FMN** to **LC** at least via **FMF**.

Figure 3 shows HPLC analysis of deoxyguanosine irradiated by UV-A or VIS radiation with **RF**, **LC**, **LF**, **FMN** or **FAD**. UV-A radiation at 366 nm with **RF** oxidized guanine and generated Iz[30] (Fig. 3A), and this result is compatible with the previous finding.[4] VIS radiation, as well as UV-A radiation, with **RF** can oxidize guanine (Fig. 3A). UV-A radiation with **LC** can oxidize guanine, but VIS radi-



Scheme 1. Synthesis of **CMF**, **LC** and **LF**.

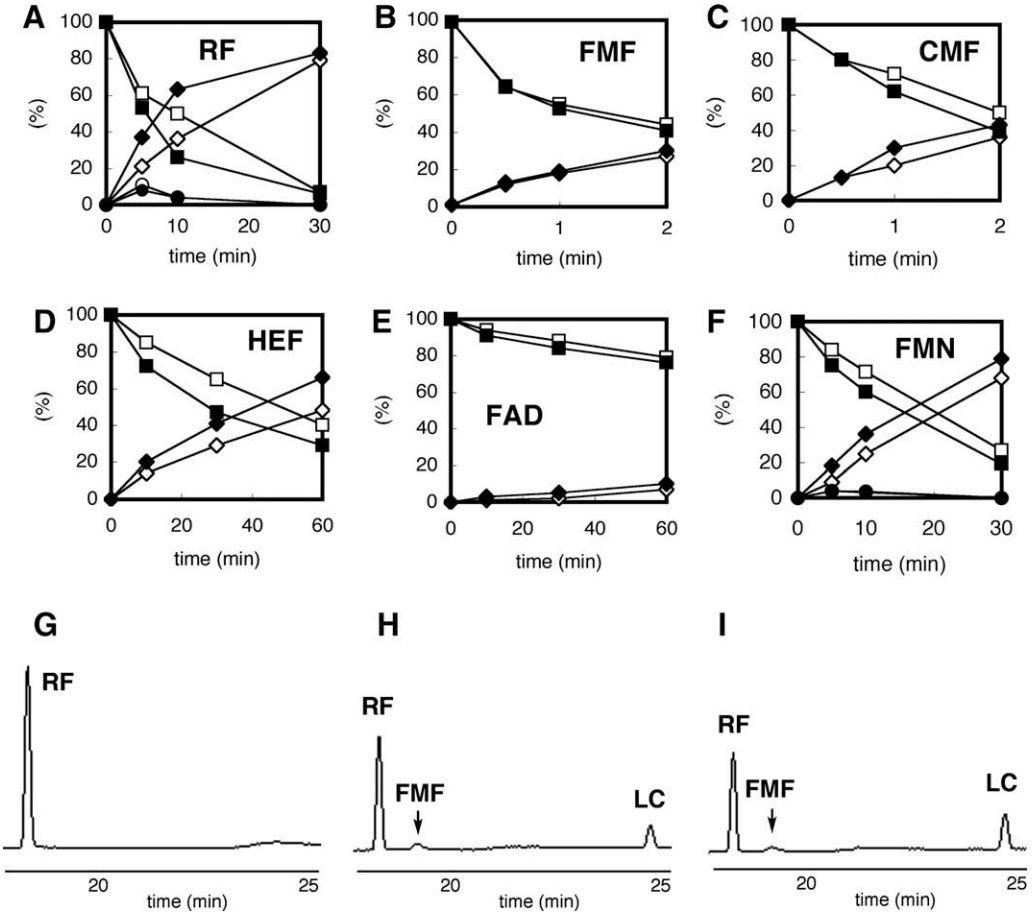
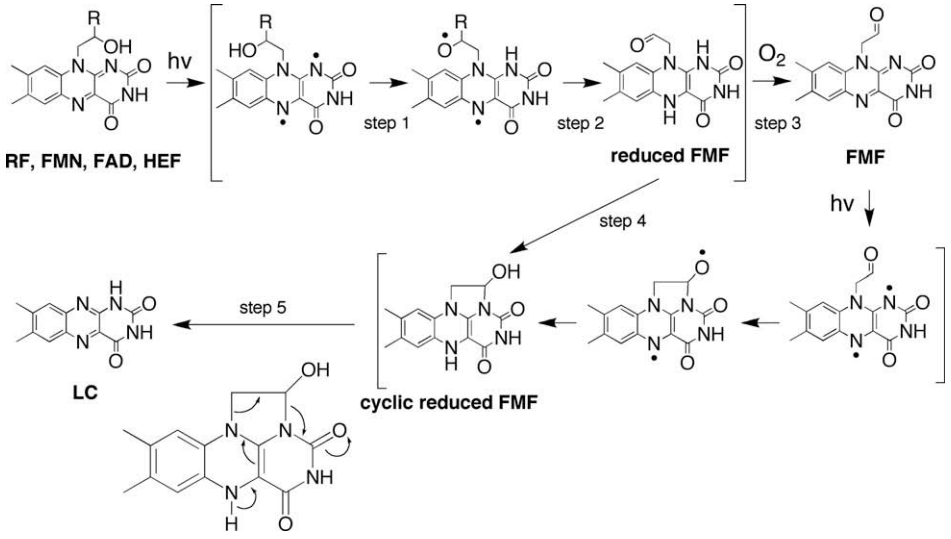


Figure 2. Photodegradation of flavin derivatives by UV-A or VIS radiation. Each 23 μM of (**A**) **RF**, (**B**) **FMF**, (**C**) **CMF**, (**D**) **HEF**, (**E**) **FAD** or (**F**) **FMN** in 9 mM sodium phosphate buffer (pH 8.0) was irradiated by UV-A light at 366 nm (open symbols) or VIS light at 440 nm (closed symbols). The samples were analyzed by HPLC using a 5C4-MS column (Nakalai Tesque, 5 μm, 150 × 4.6 mm, elution with a solvent mixture of water, 0–30% CH_3CN/30 min at a flow rate of 1.0 ml/min) in panel **A**–**D**. In panel **E** and **F**, the samples were analyzed by HPLC using a 5C18-MS column (Nakalai Tesque, 5 μm, 150 × 4.6 mm, elution with a solvent mixture of 10 mM TEAA, 0–30% CH_3CN/30 min at a flow rate of 1.0 ml/min). The amount of flavin derivatives was monitored at 366 nm absorbance. In the UV-A radiation experiments, squares indicate the amount of the starting materials, and diamonds indicate that of **LC**. In panel **A** and **F**, circles indicate the amount of intermediate **FMF**. (**G**–**I**) HPLC analysis of reaction mixtures. **RF** (**G**) was irradiated for 5 min by UV-A light at 366 nm (**H**) or VIS light at 440 nm (**I**).



Scheme 2. Suggested photodegradation mechanism of **RF**, **FMN**, **FAD**, **HEF**, and **FMF**.

ation at 440 nm with **LC** was hardly capable of oxidizing guanine (Fig. 3B). In contrast to **RF**, **LC** can hardly absorb light at 440 nm.[13,28,31] **LF** has the same flavin chromophore as **RF**, and the absorption of **LF** is almost the same as that of **RF**.[13,29] Hence,

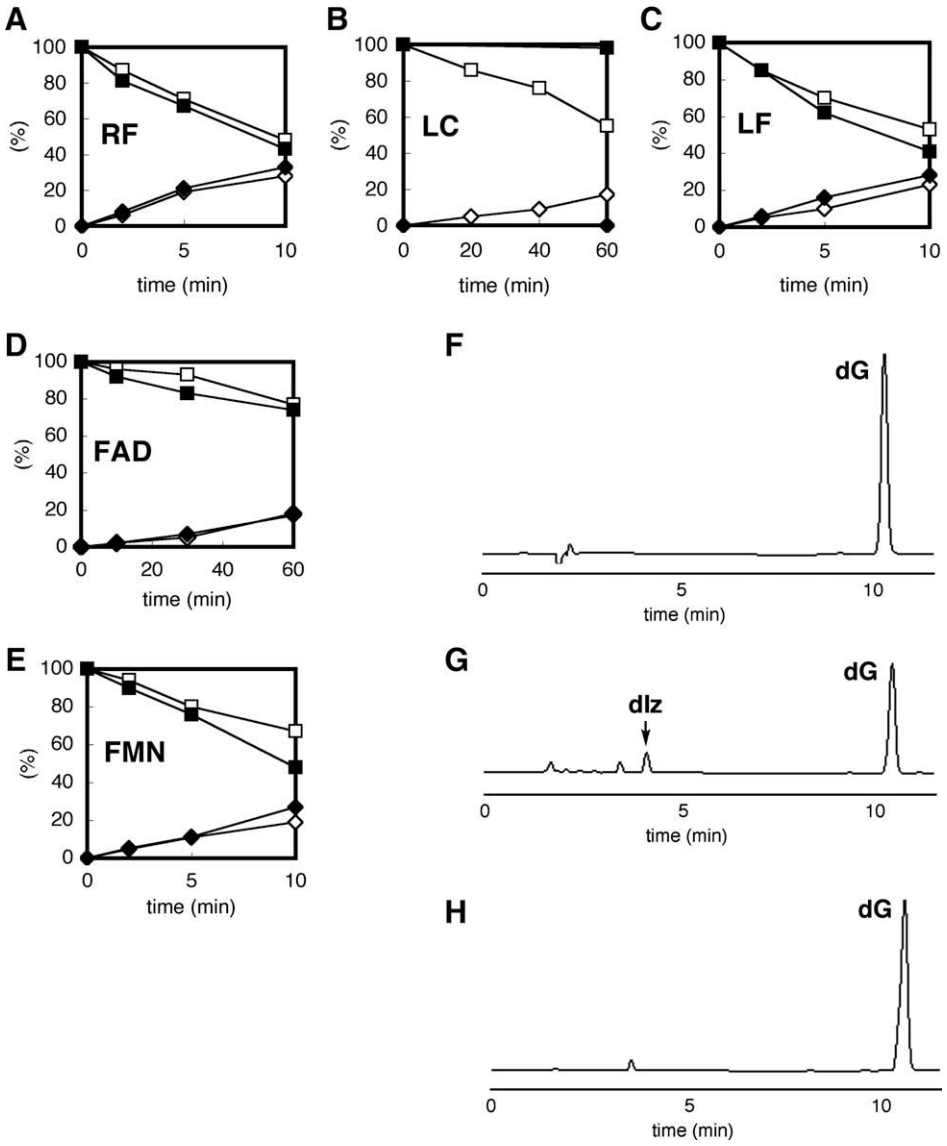


Figure 3. Photooxidation of deoxyguanosine under UV-A or VIS radiation. Deoxyguanosine (100 μM) with each 20 μM of (**A**) **RF**, (**B**) **LC**, (**C**) **LF**, (**D**) **FAD** or (**E**) **FMN** in 20 mM sodium phosphate buffer (pH 8.0) was irradiated by UV-A light at 366 nm (open symbols) or VIS light at 440 nm (closed symbols). The samples were analyzed by HPLC using a 5C4-MS column (Nakalai Tesque, 5 μm, 150 × 4.6 mm, elution with a solvent mixture of water, 0–7% CH_3CN/20 min at a flow rate of 1.0 ml/min) and the amount of dG (square) and dIz (diamond) was monitored at 254 nm absorbance. (**F**–**H**) HPLC analysis of reaction mixtures. Deoxyguanosine with **LC** (**F**) was irradiated for 60 min by UV-A light at 366 nm (**G**) or VIS light at 440 nm (**H**).

the rate of guanine oxidation by **LF** was similar to that by **RF** (Fig. 3C). The fluorescence lifetime of **LC** is shorter than that of **RF** and almost the same as that of **LF**.[13] The lifetime of the triplet-excited **LC** is shorter than that of **LF**.[13] Thus the oxidation rate of guanine greatly depends not only on the molar absorption coefficients but also on the lifetimes of the singlet-excited and triplet-excited states; VIS radiation with **LC** was not capable of oxidizing guanine, and UV-A radiation with **LC** oxidized guanine more slowly than that with **RF** or **LF**.

Moreover, UV-A or VIS radiation in the presence of **FAD** and **FMN** can oxidize guanine (Fig. 3D and E). Notably, the photoreactivity of guanine in the presence of **FMN** was almost the same as **RF**, while the rate of guanine photooxidation with **FAD** was slower than that with **RF**. These different results are likely to depend on whether adenine nucleotide exists, similar to the results in Figure 2.

In summary, even though **RF**, **FAD** and **FMN** were degraded to **LC** by UV-A radiation, UV-A radiation can oxidize guanine and generate Iz. Conversely, once **RF**, **FAD** and **FMN** are degraded completely to **LC** by VIS radiation, guanine is hardly oxidized. **RF** is a vitamin, and **FMN** and **FAD** are natural constituents of living organisms. Since UV-A and VIS are easy to transmit to basal cells and dermis, the effects of UV-A and VIS radiation in the presence of flavin derivatives are not negligible. We speculate that guanine oxidation by UV-A radiation in the presence of flavin derivatives is sustained, but that by VIS radiation is not sustained. Guanine oxidation causes point mutation and telomere shortening, and relates to cancer and senescence.[32] Thus, the different photoreactivity of UV-A and VIS is expected to affect the ease and persistence of such phenomena as the cytotoxicity of **RF** activation by UV or VIS radiation.[33,34] In addition, **RF** photosensitized inactivation of phage.[35] The selection of light wavelength for photoirradiation can decide the duration of oxidation and cytotoxicity of tumour cells, and our results seems to be available to improve laser therapies.

Acknowledgment

This work was supported by grants from Tokushima Bunri University.

References and notes

1. Cadet, J.; Berger, M.; Buchko, G. W.; Joshi, P. C.; Raoul, S.; Ravanat, J.-L. *J. Am. Chem. Soc.* **1994**, *116*, 7403–7404.
2. Cadet, J.; Decarroz, C.; Wang, S. Y.; Midden, W. R. *Isr. J. Chem.* **1983**, *23*, 420–429.
3. García, N. A.; Criado, S. N.; Massad, W. A. In *Flavins: Photochemistry and Photobilogy*; Silva, E., Edwards, A. M., Eds.; RSC Publishing: Cambridge, 2006; pp 61–82.
4. Kino, K.; Saito, I.; Sugiyama, H. *J. Am. Chem. Soc.* **1998**, *120*, 7373–7374.
5. Luo, W.; Muller, J. G.; Burrows, C. J. *Org. Lett.* **2001**, *3*, 2801–2804.
6. Kino, K.; Sugiyama, H. *Chem. Biol.* **2001**, *8*, 369–378.
7. Kino, K.; Sugiyama, H. *Mutat. Res.* **2005**, *571*, 33–42.
8. Neeley, W. L.; Delaney, J. C.; Henderson, P. T.; Essigmann, J. M. *J. Biol. Chem.* **2004**, *279*, 43568–43573.
9. Cairns, W. L.; Metzler, D. E. *J. Am. Chem. Soc.* **1971**, *93*, 2772–2777.
10. Mcbride, M. M.; Metzler, D. E. *Photochem. Photobiol.* **1967**, *6*, 113–123.
11. Mcbride, M. M.; Moore, W. M. *Photochem. Photobiol.* **1967**, *6*, 103–111.
12. Moore, W. M.; Spence, J. T.; Raymond, F. A.; Colson, S. D. *J. Am. Chem. Soc.* **1963**, *85*, 3367–3372.
13. Ahmad, I.; Vaid, F. H. M. In *Flavins: Photochemistry and Photobilogy*; Silva, E., Edwards, A. M., Eds.; RSC Publishing: Cambridge, 2006; pp 13–40.
14. Kino, K.; Miyazawa, H.; Sugiyama, H. *Genes Environment.* **2007**, *29*, 23–27.
15. Fall, H. H.; Petering, H. G. *J. Am. Chem. Soc.* **1956**, *78*, 377–380.
16. Dalcanale, E.; Montanari, F. *J. Org. Chem.* **1986**, *51*, 567–569.
17. The synthesis of **CMF**: As well as the procedure in Ref. 14, **FMF** was generated from **RF** (3.0 g, 8.0 mmol). Without isolation of **FMF**, $NaClO_2$ (6.0 g) was added to the suspension containing **FMF**, and then 6.9% H_2O_2 aqueous solution (13 ml) was added dropwise. The suspension was vigorously stirred at room temperature for 1.5 h in the dark. The reaction was monitored by TLC (CH_2Cl_2/MeOH, 4:1), which showed the absence of **FMF**. Conc. HCl (1 ml) was added, and the solid was isolated from the suspension by filtration, washed with cold water, and dried. The product **CMF** was prepared in 96% total yield (2.3 g, 7.7 mmol) as an orange solid.
18. Föry, W.; Mackenzie, R. E.; Mccormick, D. B. *J. Heterocycl. Chem.* **1968**, *5*, 625–630.
19. Yagi, K.; Matsuoka, Y. *J. Biochem.* **1960**, *48*, 93–100.
20. The synthesis of **LC**: Prepurified **FMF** (2.16 g, 7.59 mmol) was dissolved in 50% acetic acid aqueous solution (220 ml), and then was heated at 80 °C for 3 h. During heating, the solution had a color varying from initially yellow to finally black. The black solution was cooled to room temperature on standing, and a green precipitate was isolated by filtration, washed with water and methanol, and dried. The product **LC** was prepared in 61% yield (1.12 g, 4.64 mmol).
21. Seng, F.; Ley, K. *Angew. Chem., Int. Ed.* **1972**, *11*, 1010–1011.
22. Suzuki, A. T.; Ohishi, N.; Yagi, K. *J. Chromatogr.* **1979**, *169*, 459–461.
23. Song, P.-S.; Smith, E. C.; Metzler, D. E. *J. Am. Chem. Soc.* **1965**, *87*, 4181–4184.
24. By the procedure based on Ref. 23, **LC** was obtained at 51% yield.
25. Smith, E. C.; Metzler, D. E. *J. Am. Chem. Soc.* **1963**, *85*, 3285–3288.
26. The synthesis of impure **LF**: As well as the procedure in Ref. 17, **FMF** was used without isolation. While stirring, sodium hydroxide (16 g) was added to the suspension (200 ml) containing **FMF**. The suspension immediately changed to a dark wine red solution, and was stirred for 0.5 h at room temperature. The impure precipitation formed by addition of conc. HCl (60 ml) was removed by filtration. Then conc. NH_3 was added to the filtrate until the orange solution changed to a dark green suspension. The precipitate (1.09 g) was obtained, but the obtained **LF** was impure. The ratio of **LF** and **LC** was 86:14.
27. Purification of **LF**: The precipitation obtained in Ref. 26 was redissolved with conc. HCl (200 ml), and then the solution was concentrated to half of its original volume by heating. The impure solid precipitated during cooling was removed by filtration. The filtrate (100 ml) was further concentrated to a volume of 20 ml by heating. An orange solid was precipitated by cooling, isolated by filtration, washed with water and methanol, and dried. The pure product **LF** was prepared in 7.7% total yield from **RF** (157 mg, 0.616 mmol).
28. Posthuma, J.; Berends, W. *Biochim. Biophys. Acta* **1966**, *112*, 422–435.
29. Penzer, G. R.; Radda, G. K. *Quarterly Rev. Chem. Soc.* **1967**, *21*, 43–65.
30. Damages such as spirohydantoin can be little detected at 254 nm. Therefore, generation of damages, other than Iz, can explain the reason why the amount of guanine oxidation was different from that of Iz generation.
31. The molar absorption coefficients (L mol^{-1} cm^{-1}) (H_2O) were as follows: **RF**, ε_{366} = 9800 and ε_{440} = 12000; **LC**, ε_{366} = 6500 and ε_{440} = 160.
32. Kino, K.; Sugiyama, H.; Miyazawa, H. In *Progress in DNA Damage Research*; Miura, S., Nakano, S., Eds.; Nova Science: Hauppauge, NY, 2008; pp 271–276.
33. Sato, K.; Minami, H.; Taguchi, H.; Maeda, T.; Yoshikawa, K.; Tsuji, T. *Photomed. Photobiol.* **1995**, *17*, 125–126.
34. Sato, K.; Taguchi, H.; Maeda, T.; Minami, H.; Asada, Y.; Watanabe, Y.; Yoshikawa, K. *J. Invest. Dermatol.* **1995**, *105*, 608–612.
35. Martin, C. B.; Wilfong, E.; Ruane, P.; Goodrich, R.; Platz, M. *Photochem. Photobiol.* **2005**, *81*, 474–480.