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*Original Contribution*

# IN VITRO STUDIES ON THE PHOTOBIOLOGICAL PROPERTIES OF ALOE EMODIN AND ALOIN A

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**Abstract**—Plants containing aloin A, aloe emodin, and structurally related anthraquinones have long been used as traditional medicines and in the formulation of retail products such as laxatives, dietary supplements, and cosmetics. Since a recent study indicated that topically applied aloe emodin increases the sensitivity of skin to UV light, we examined the events following photoexcitation of aloin A and aloe emodin. We determined that incubation of human skin fibroblasts with 20 $\mu$M aloe emodin for 18 h followed by irradiation with UV or visible light resulted in significant photocytotoxicity. This photocytotoxicity was accompanied by oxidative damage in both cellular DNA and RNA. In contrast, no photocytotoxicity was observed following incubation with up to 500 $\mu$M aloin A and irradiation with UVA light. In an attempt to explain the different photobiological properties of aloin A and aloe emodin, laser flash photolysis experiments were performed. We determined that the triplet state of aloe emodin was readily formed following photoexcitation. However, no transient intermediates were formed following photoexcitation of aloin A. Therefore, generation of reactive oxygen species and oxidative damage after irradiation of aloin A is unlikely. Although aloin A was not directly photocytotoxic, we found that human skin fibroblasts can metabolize aloin A to aloe emodin. © 2003 Elsevier Science Inc.

**Keywords**—Aloe emodin, Aloin, Metabolism, Oxidation, Phototoxicity, UV, Free radicals

## INTRODUCTION

Aloin, the C-glucoside of aloe emodin anthrone, occurs in aloe, a plant having substantial commercial importance [1,2]. The levels of aloin in aloe are highly variable and appear to depend on the species and strain of aloe as well as growing conditions [1]. Aloin, which is localized in the outer rind of the aloe plant, has been reported to constitute up to 30% of the aloe plant's dried leaf exudates [1]. Aloin occurs naturally as a mixture of diastereomers. Studies of aloin's biosynthesis indicate that aloin B (the $C_{10}$,$C_{1'}$:R,S diastereomer of aloin) is preferentially formed [3]. Nonenzymatic conversion to aloin A, the $C_{10}$,$C_{1'}$:S,S diastereomer of aloin (Fig. 1), is thought to result in the mixture of aloin A and aloin B observed for naturally derived aloin [3,4].

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Aloe emodin (Fig. 1) is present in the leaves and roots of a number of plants, usually occurring in combination with its glycosides or in a reduced (anthrone) form [2]. Aloe emodin is present in low levels in plants such as aloe and senna [2] and it is thought to arise through oxidative decomposition of its glycosides rather than through direct biosynthesis [3]. Although aloe emodin is a minor constituent of most botanical raw materials, studies have shown that aloe emodin is the pharmacologically active metabolite of aloin and sennosides, both botanical products used for their cathartic activity [5,6].

Because plants and botanical ingredients containing aloin and aloe emodin are widely used in traditional medicine and in retail products, such as over-the-counter drugs, dietary supplements, and cosmetics, the toxicological properties of these compounds have been examined in a number of studies. Aloin has been reported to be nonmutagenic using an in vitro assay [7]. In vivo studies have shown that orally administered aloin is poorly absorbed but is metabolized by intestinal microflora to aloe emodin, which is readily absorbed [8]. In

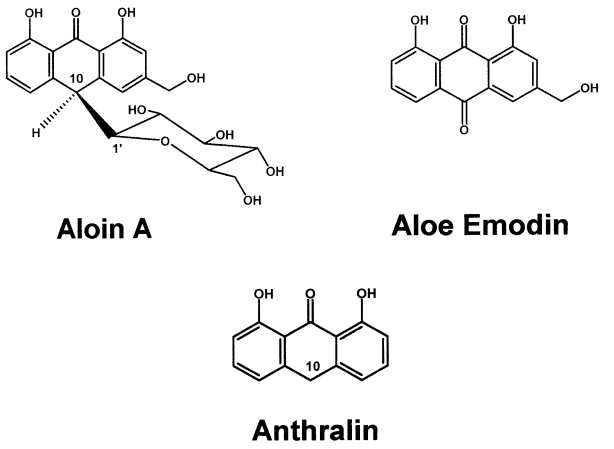


Fig. 1. Structures of aloin A, aloe emodin, and anthralin.

vitro studies have provided evidence of aloe emodin's toxicity and suggest that aloe emodin has preferential toxicity to carcinoma cells [9–11]. Furthermore, in vitro studies have demonstrated aloe emodin's genotoxicity [5,12] and ability to promote malignant transformation of cells [13]. Heidemann et al. have reported, however, that aloe emodin is not mutagenic when tested in vivo [12]. In addition, several investigators have demonstrated that UV and visible light potentiate the toxicity of aloe emodin and structurally related anthraquinones. Emodin, a structural isomer of aloe emodin, has been reported to be toxic to leukemia cells after exposure to visible light [14]. Kersten et al. determined that emodin was phototoxic, but not photogenotoxic, when excited by UV light [15]. Recently, we reported that exposure of human skin fibroblasts to aloe emodin and UVA light (320–400 nm) elicited phototoxicity [16]. In addition to these in vitro investigations, Strickland et al. have reported that topically applied aloe emodin enhances the formation of UV light-induced malignant melanoma in C3H mice [17].

While there is emerging evidence that aloe emodin can enhance the damaging effects of light, little is known about the mechanism of photosensitization. In the present study, we have examined the effects of aloin A and aloe emodin on the sensitivity of human skin fibroblasts to light. Previously, we have shown that photoexcitation of aloe emodin results in the formation of singlet oxygen ($^1O_2$) [16]. Therefore, oxidative damage may play a significant role in photosensitization by aloe emodin. Investigators have found that 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxodG) and 8-oxo-7, 8-dihydroguanosine (8-oxoG) are sensitive markers for oxidative damage produced by a number of reactive oxygen species, such as $^1O_2$, $^{\bullet}OH$, and $O_2^{\bullet -}$ [18–21]. In the current study, we have measured the effects of photoexcited aloe emodin and aloin on the levels of guanine hydroxylation in cellular DNA and RNA, leading to the formation of 8-oxodG and 8-oxoG, respectively.

## MATERIALS AND METHODS

### *Cell culture*

Human skin fibroblasts (ATCC CRL-1634) were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in Dulbecco's modified Eagle medium, without phenol red, containing 10% fetal bovine serum (Life Technologies Inc., Rockville, MD, USA), 50 $\mu$g/ml gentamicin, 4.5 mg/ml glucose, and 4 mM L-glutamine. Cultures were incubated at 37°C in a humidified atmosphere containing 5% $CO_2$.

### *Photocytotoxicity assay*

For assessing the photocytotoxicity of aloe emodin, subconfluent monolayers of human skin fibroblasts in 60 mm dishes were incubated for 18 h with media containing 20 $\mu$M aloe emodin (Sigma Chemical Co., St. Louis, MO, USA). For examining the photosensitizing effects of aloin A, additional groups of dishes were similarly incubated for 18 h with media containing 50, 100, 200, 300, 400, or 500 $\mu$M aloin A (Indofine Chemical Co. Inc., Somerville, NJ, USA). Monolayers were then washed twice with phosphate-buffered saline (PBS) and fresh PBS was added to cover the fibroblasts. The cells were then irradiated through the PBS. Since aloin A absorbs predominately in the UVA spectral region (Fig. 2A), only UVA light was used to assess the photocytotoxicity of aloin A. Because aloe emodin absorbs both UVA light (320–400 nm) and visible light (320–700 nm) (Fig. 2B), separate photocytotoxicity experiments were performed using each spectral region.

The source of UVA light consisted of two GE F40BL bulbs (General Electric, Freemont, CA, USA) filtered through 3 mm of soft glass. Visible (400–700 nm) radiation was provided by two 40 W GE watt-miser bulbs (General Electric) filtered through Plexiglas UF3 (Rohm and Haas Co., Bristol, PA, USA). The spectral irradiances of the sources of UVA light and visible light were $2.82 \times 10^{-2}$ KW/m$^2$ and $2.40 \times 10^{-2}$ KW/m$^2$, respectively. All irradiations were performed at 25 $\pm$ 2°C and typically lasted no longer than 30 min. Sham-irradiated samples were maintained at 25 $\pm$ 2°C in the dark. After irradiation, cells were removed from the dishes by trypsinization and seeded into 60 mm dishes. After incubation for 7–10 d, cells were fixed with methanol, stained with crystal violet, and cell colonies were counted.

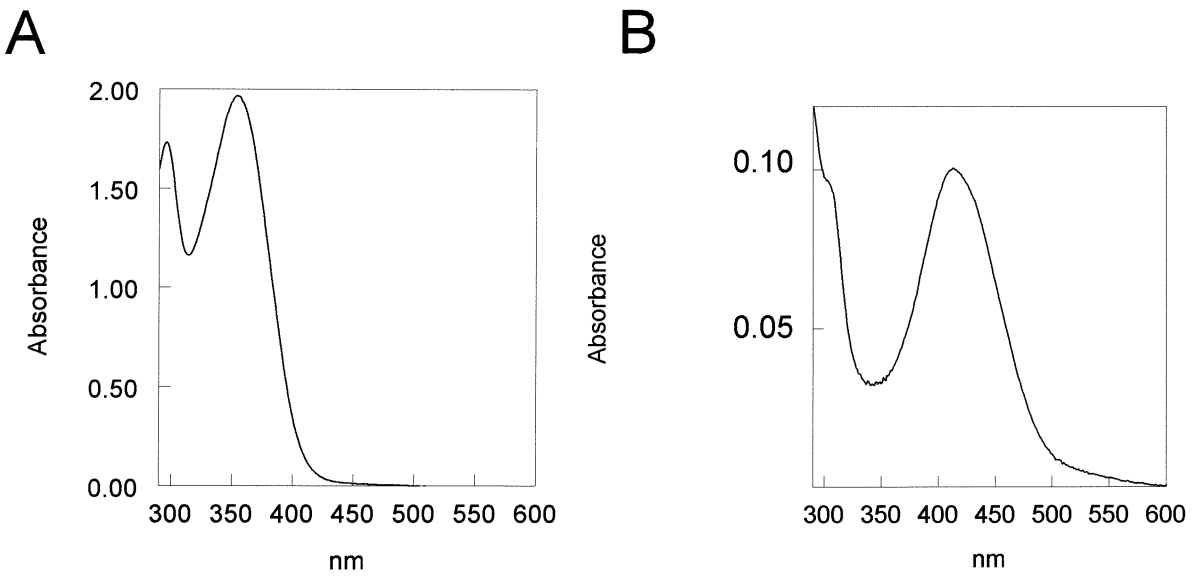


Fig. 2. Absorption spectra of 300 $\mu$M aloin A (A) and 20 $\mu$M aloe emodin (B). Both aloin A and aloe emodin were dissolved in phosphate-buffered saline.

*Photo-oxidative damage to cellular RNA and DNA sensitized by aloe emodin*

Subconfluent monolayers of human skin fibroblasts in 150 mm dishes were incubated with medium containing 20 $\mu$M aloe emodin for 18 h. Monolayers were then washed twice with PBS. Fresh PBS was added to cover the cells, and cells were irradiated with UVA light or visible light as described above. Immediately after irradiation, monolayers of cells were washed once with PBS, and cells were then lysed by the addition of 15 ml of a solution containing 6 M guanidinium hydrochloride, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.5 M sodium acetate, 1.3% N-lauroylsarcosine, 1.5 mg proteinase K. The lysate was incubated for 1 h at 50°C. A mixture of cellular RNA and DNA was obtained by ethanol precipitation as previously described [22]. The mixture of RNA and DNA was resuspended in 100 $\mu$l of 10 mM tri(hydroxymethyl)-aminomethane hydrochloride (Tris-HCl), pH 7.4, 10 mM $MgCl_2$, 1 mM $ZnCl_2$, 240 units of Dnase 1, 20 units of nuclease P1, and 3.2 units of calf intestine alkaline phosphatase. Enzymatic hydrolysis was allowed to proceed for 6 h at 37°C. The nucleoside mixture obtained after hydrolysis was stored at −20°C prior to analysis by HPLC.

The levels of 8-oxodG and 8-oxoG in the nucleoside mixture derived from cellular DNA and RNA were quantified as a measure of oxidative damage. A 90 $\mu$l aliquot was injected onto an Adsorbosphere HS $C_{18}$ column (Alltech Associates, Deerfield, IL, USA). The column was eluted with 7% methanol in 0.05 M phosphate buffer at 0.7 ml/min. A Waters Model 990E detector (Millipore Corp., Milford, MA, USA) was used to quantify unmodified nucleosides by their absorbance at 254 nm. A Dionex pulsed amperometric detector (Dionex Corp., Sunnyvale, CA, USA), located downstream from the UV detector, was used to quantify 8-oxoG and 8-oxodG [22]. Standard solutions for quantifying 8-oxoG and 8-oxodG were prepared from 8-oxoG and 8-oxodG received from Calbiochem (San Diego, CA, USA). The results of the HPLC analysis are expressed as the fraction of hydroxylated guanine bases (i.e., 8-oxodG/$10^5$ dG for DNA, 8-oxoG/$10^5$ G for RNA).

*Photo-oxidative damage to nucleotides and calf thymus DNA sensitized by aloe emodin*

Deoxyribonucleotides and calf thymus DNA were obtained from Sigma Chemical Co. and were used without further purification. A solution containing dAMP, dCMP, dGMP, and TMP, each at a concentration of 0.24 mM, was prepared in 10 mM phosphate buffer, pH 7.4. A similar solution containing a mixture of these four 2'-deoxyribonucleotides was prepared in a phosphate buffer made with $D_2O$ (pD 7.4). A 300 $\mu$g/ml solution of calf thymus DNA (equivalent to 0.24 mM for each nucleotide base) was prepared in 10 mM phosphate buffer (pH 7.4). After addition of sufficient aloe emodin to yield a final concentration of 10 $\mu$M, solutions were exposed to UVA light as described above.

For analysis of photo-oxidative damage to deoxyribonucleotides, samples were dephosphorylated by the addition of 0.03 ml of 100 mM $MgCl_2$ and 20 units of calf intestine alkaline phosphatase to 0.3 ml aliquots of 2'-

deoxyribonucleotides. After incubation for 2 h at 37°C, levels of 8-oxodG were quantified by HPLC as described above. For analysis of photo-oxidative damage to calf thymus DNA, DNA was first precipitated by the addition of ethanol. The DNA was then resuspended in 100 $\mu$l of 10 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 1 mM $ZnCl_2$, 240 units of Dnase 1, 20 units of nuclease P1, and 3.2 units of calf alkaline intestine phosphatase. Enzymatic hydrolysis was allowed to proceed for 6 h at 37°C. Nucleoside mixtures obtained after hydrolysis were stored at −20°C prior to analysis by HPLC.

*In vitro metabolism of aloin A*

Confluent monolayers of human skin fibroblasts in 150 mm dishes were incubated for up to 4 d with media containing 300 $\mu$M aloin A. Fibroblasts incubated with media without aloin A, and dishes without cells but containing media supplemented with 300 $\mu$M aloin A, served as controls. Media were removed at selected times to determine the levels of aloe emodin. To extract aloe emodin from medium, 3 ml of medium was mixed with 3 ml of acetone; 6 ml of ethyl acetate was then added, followed by vigorous mixing. The organic layer was withdrawn and dried over 0.5 g of anhydrous sodium sulfate. The dried organic layer was evaporated under a stream of nitrogen and reconstituted in 1 ml of acetonitrile. The level of aloe emodin in each reconstituted sample was determined by HPLC. A 100 $\mu$l aliquot of the sample was injected onto a 150 × 4.6 mm $C_{18}$ Adsorbosphere column (Alltech Associates). The column was eluted at 1.5 ml/min with a linear gradient beginning with 15% acetonitrile/water and ending at 45 min with 85% acetonitrile/water. Aloe emodin was detected and quantified by its absorption at 430 nm.

*Detection of transient species formed after photoexcitation of aloin A and aloe emodin*

To identify transient intermediates formed after photoexcitation of aloin A and aloe emodin, laser flash photolysis experiments were performed using the third harmonic output (355 nm) from a Continuum II-10 Q-switched Nd (Continuum, Santa Clara, CA, USA): YAG laser as the excitation source. Transient absorption spectra were collected at 300 ns following the excitation pulse of the laser. Transient absorption spectra were monitored using a 350 W xenon lamp (Oriel Instruments, Stratford, CT, USA), which passed through the sample cell at a right angle to the excitation beam. Transient waveforms were captured using a Lecroy 9420 digital oscilloscope (Lecroy Corp., Chestnut Ridge, NY, USA). Samples were prepared in acetonitrile and had optical densities of 0.39 for aloe emodin and 0.94 for aloin A at 355 nm. Samples were purged for 15 min with $N_2$ prior to excitation for all laser flash photolysis experiments.

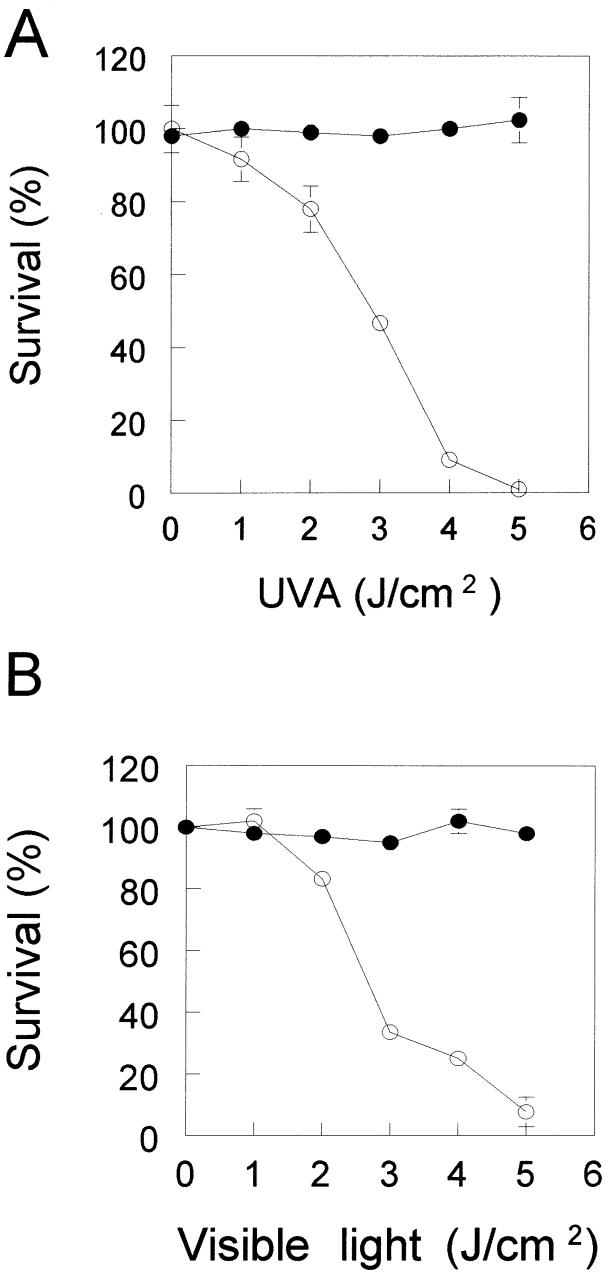


Fig. 3. Photocytotoxicity of aloe emodin. Human skin fibroblasts, incubated with 20 $\mu$M aloe emodin for 18 h, were irradiated with UVA light (A) or visible light (B) (○). Irradiated fibroblasts without previous exposure to aloe emodin served as controls (●). Photocytotoxicity was assessed as the inhibition of colony formation as described in Materials and Methods. Each point represents the mean of four determinations ± SD.

## RESULTS

Preincubation with 20 $\mu$M aloe emodin followed by exposure to UVA light diminished the survival of human

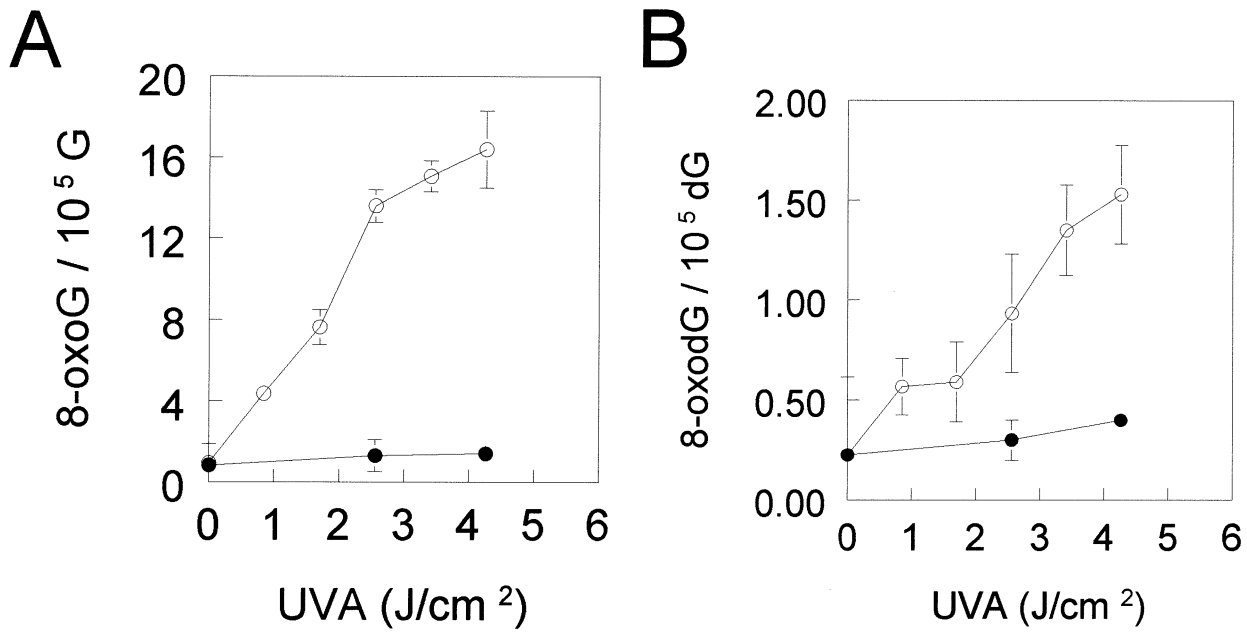


Fig. 4. Photo-oxidative damage to cellular RNA and DNA accompanying exposure to aloe emodin and UVA light. Human skin fibroblasts were exposed to 20 $\mu$M aloe emodin for 18 h and then irradiated with selected fluences of UVA light (○). Control samples were exposed to UVA light without preincubation with aloe emodin (●). Photo-oxidative damage was measured as the oxidation of guanosine in cellular RNA (A) and oxidation of 2'-deoxyguanosine in cellular DNA (B). The data represent the mean of four determinations ± SD.

skin fibroblasts, measured as the ability to form colonies (Fig. 3A). This effect on cell survival was dependent upon the incident level of UVA light. Exposure to UVA light, without preincubation with aloe emodin, had no significant effect on cell survival (Fig. 3A). In addition, treatment with aloe emodin alone had no effect on cell survival (data not shown). Similar results were obtained after preincubation of cells with 20 $\mu$M aloe emodin and irradiation with visible light (Fig. 3B). No photocytotoxicity was elicited by preincubating fibroblasts with up to 500 $\mu$M aloin A followed by irradiating with UVA light (data not shown).

The method used here for assessing photo-oxidative damage to cellular nucleic acids allows measurement of damage to both RNA and DNA in a single sample of irradiated cells. Significant levels of oxidative damage were found in the RNA (Fig. 4A) and DNA (Fig. 4B) of fibroblasts exposed to 20 $\mu$M aloe emodin and UVA light. Oxidative damage in RNA and DNA (measured as 8-oxoG and 8-oxodG, respectively) was observed after treatment with aloe emodin and levels of UVA light resulting in only low or moderate levels of toxicity. Levels of 8-oxoG in cellular RNA were generally about 10-fold higher than levels of 8-oxodG in DNA (Figs. 4A and 4B). Treatment with UVA light alone did not significantly increase oxidative damage to cellular nucleic acids. In addition, treatment with aloe emodin alone had no effect on oxidative damage to cellular nucleic acids (data not shown). Treatment with 20 $\mu$M aloe emodin and visible light resulted in photo-oxidative damage to cellular RNA and DNA (Figs. 5A and 5B). Exposure to visible light alone did not result in significant oxidative damage to cellular RNA and DNA (Figs. 5A and 5B).

To investigate potential mechanisms of photosensitization by aloe emodin, calf thymus DNA and 2'-deoxyribonucleotides were treated with aloe emodin and UVA light. No photo-oxidative damage was detected in calf thymus DNA after treatment with 10 $\mu$M aloe emodin and up to 10 J/cm$^2$ UVA light (data not shown). In contrast, significant photo-oxidative damage was found in dGMP contained in a mixture of 2'-deoxyribonucleotides treated with aloe emodin and UVA light (Fig. 6A). This observation, that aloe emodin photosensitizes oxidative damage to dGMP (contained in a mixture of 2'-deoxyribonucleotides) but not to guanine bases in DNA, suggests that binding of aloe emodin to DNA results in the loss of photosensitizing activity.

To further investigate the mechanism underlying the oxidative damage to 2'-deoxyribonucleotide photosensitized by aloe emodin, $D_2O$ was used as the solvent for sample preparation. We observed that replacing $H_2O$ with $D_2O$ results in a dramatic increase in photo-oxidative damage (Fig. 6B). Since the lifetime of singlet oxygen ($^1O_2$) is approximately 14 times longer in $D_2O$

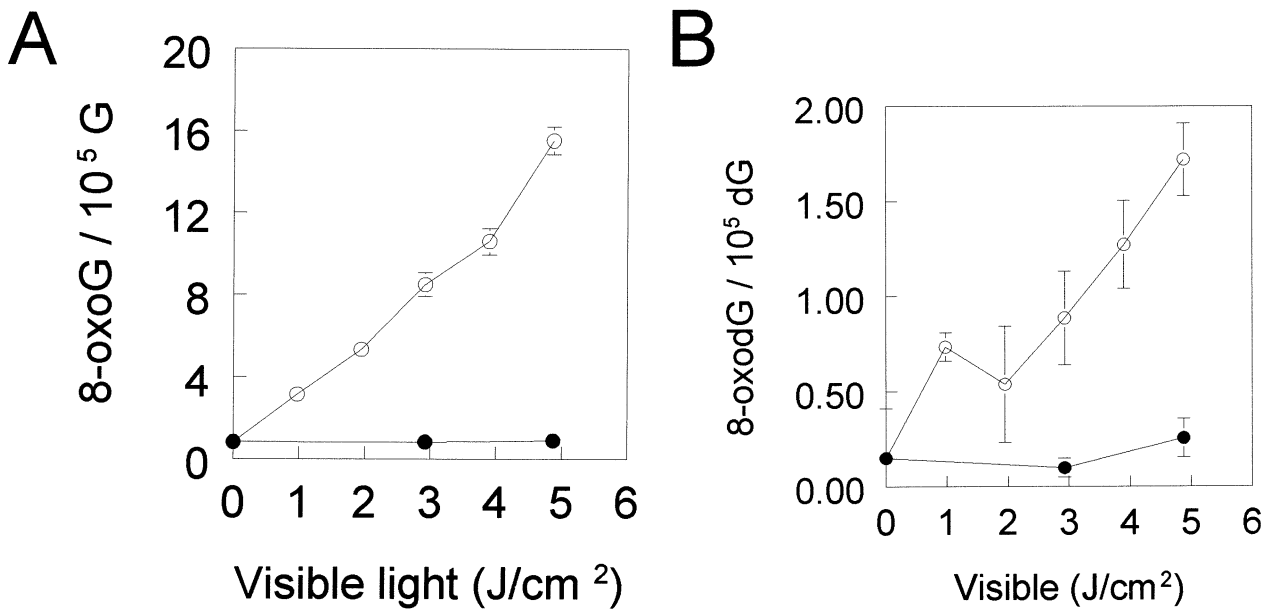


Fig. 5. Photo-oxidative damage to cellular RNA and DNA accompanying exposure to aloe emodin and visible light. Human skin fibroblasts were exposed to 20 $\mu$M aloe emodin for 18 h and then irradiated with selected fluences of visible light (○). Control samples were exposed to visible light without preincubation with aloe emodin (●). Photo-oxidative damage was measured as the oxidation of guanosine in cellular RNA (A) and oxidation of 2'-deoxyguanosine in cellular DNA (B). The data represent the mean of four determinations ± SD.

than in $H_2O$ [23], the increase in the formation of 8-oxodG we observed in buffers containing $D_2O$ suggests that $^1O_2$, formed following photoexcitation of aloe emodin, plays a role in the oxidative damage photosensitized by aloe emodin.

We found that human skin fibroblasts were able to metabolize aloin A to aloe emodin (Fig. 7). Aloin A, incubated in culture medium with fibroblasts for 4 d, was partially (4.7%) converted to aloe emodin. No transformation of aloin A to aloe emodin was observed when 300 $\mu$M aloin A was incubated in flasks without fibroblasts. While it has been firmly established that intestinal bacteria can transform aloin to aloe emodin [5,7,8], this is the first demonstration that aloe emodin can be formed from aloin by mammalian cells. One implication of these results is that if topically applied aloin penetrates into the viable cells of the skin, the photosensitizer, aloe emodin, could be formed.

As we have previously reported, the transient absorption spectrum of aloe emodin, obtained 300 ns after photoexcitation, contains absorption maxima at 410, 480, and 670 nm. Our previous work suggests that all three absorption maxima arise due to population of a single triplet state species after photoexcitation of aloe emodin [16]. While the triplet state plays a central role in the photophysics of aloe emodin [16], the transient absorption spectrum of aloin A indicates that photoexcitation of aloin A does not lead to the formation of aloin A's triplet state or other intermediates absorbing light with wavelengths longer than 400 nm (Fig. 8).

## DISCUSSION

Because of the increasing demand for retail products containing natural ingredients, the use of botanical ingredients in products applied to sun-exposed skin has increased in the last decade [24]. The current study was initiated as part of a project intended to identify phototoxic phytochemicals in topically applied products, such as cosmetics.

Plants biosynthesize an array of secondary metabolites as a defense against environmental insults. The biosynthesis of these compounds, known as phytoalexins, is elicited by plant pathogens, insects, and other environmental factors [25]. Studies have shown that a number of phytoalexins are protective only after they are excited by light. [26]. Light-activated phytoalexins are structurally diverse and include $\beta$-carbolines, furanocoumarins, hypericin and other extended quinones, isoquinolines, polyacetylenes, and thiopenes [26]. Clearly, the increased use of botanical ingredients in retail products increases the probability that consumers will be exposed to light-activated phytoalexins and potential phototoxicity.

Evidence suggests that aloin occurs in aloes as a phytoalexin. Occurring exclusively in the outer layer of

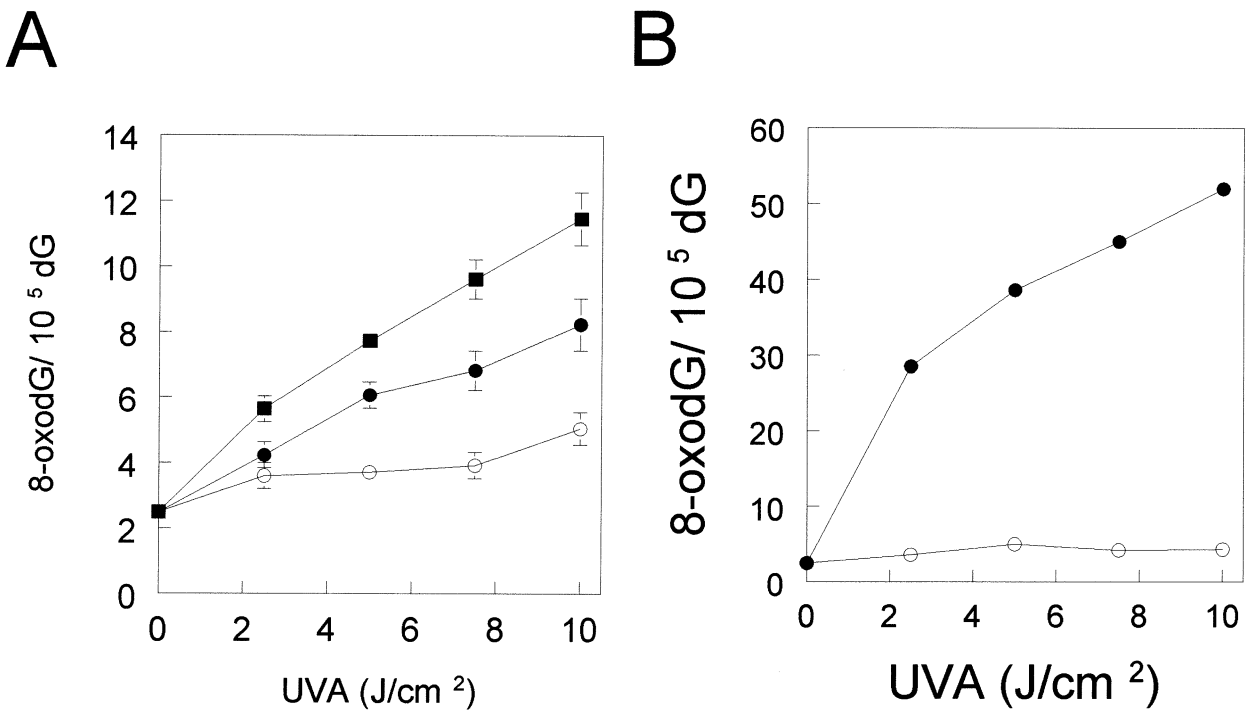


Fig. 6. Photo-oxidative damage to dGMP sensitized by aloe emodin. (A) Mixtures of dAMP, dCMP, dGMP, and TMP (each 0.24 mM) were treated with 0 $\mu$M (○), 1 $\mu$M (●), or 10 $\mu$M (■) aloe emodin. Samples were then irradiated with selected levels of UVA light. After enzymatic dephosphorylation of 2'-deoxyribonucleotides, the extent of photo-oxidative damage was assessed by measuring the amount of 8-oxodG (see Materials and Methods). (B) Analogously, a mixture of 2'-deoxynucleotides in phosphate-buffered saline prepared with $D_2O$ was treated with 0 $\mu$M (○) or 1 $\mu$M (●) aloe emodin. Photo-oxidative damage to dGMP was determined as described for (A). Data represent the mean of three determinations ± SD.

the aloe leaf where a defensive role is most vitally required, aloin is unevenly distributed in the leaves of plants such as Aloe arborescens [27]. The highest concentrations of aloin are found to occur in areas of leaves most accessible for consumption by insects or animals [27]. Storage of aloe emodin, the defensively active phytochemical, as an inactive glycoside (aloin) is a defensive strategy frequently found in plants [28]. Ostensibly, pests experience the toxic and phototoxic effects of aloe emodin after ingesting aloin in plant leaves and metabolizing aloin to aloe emodin.

We observed that human skin fibroblasts, preincubated with aloe emodin, show increased sensitivity to both UVA light and visible light. The levels of light used in these in vitro studies are similar to levels of exposure expected during a day's activities in sunlight. If similar changes in sensitivity to UVA light and visible light are observed in humans exposed topically to aloe emodin, a dramatic increase in sensitivity to sunlight may be observed. In addition, the usual strategies for protection against sun-induced skin damage would be marginally effective, since most sunscreens offer little protection from UVA light and no protection from visible light. Oxidative damage to both RNA and DNA was associated with the phototoxicity induced by aloe emodin. Significant photo-oxidative damage in RNA and DNA occurred even at low levels of photocytotoxicity, suggesting that photo-oxidative damage may cause, rather than result from, the cellular death photosensitized by aloe emodin.

Levels of oxidative damage in RNA were consistently higher than in DNA. In general, oxidative damage in RNA has been found to exceed oxidative damage in DNA in both in vivo and in vitro studies [29–31]. This difference may be due to rapid enzymatic repair of oxidative damage in DNA [32], or protection of DNA from oxidative damage by compartmentalization in the nucleus and by chromatin [33]. In addition, we have previously determined that aloe emodin efficiently binds to DNA, and that binding to DNA quenches aloe emodin's fluorescence [16]. Therefore, aloe emodin bound to DNA is expected to be less photoreactive than unbound aloe emodin. Indeed, in the current study we have found that no photo-oxidative damage results after irradiation of calf thymus DNA treated with stoichiometrically lower levels of aloe emodin. These results suggest that the photo-oxidative damage we observed in cellular DNA was sensitized by unbound aloe emodin located in the cytoplasm or nucleus of the skin fibroblasts. Moreover, these results imply that reactive intermediates, formed

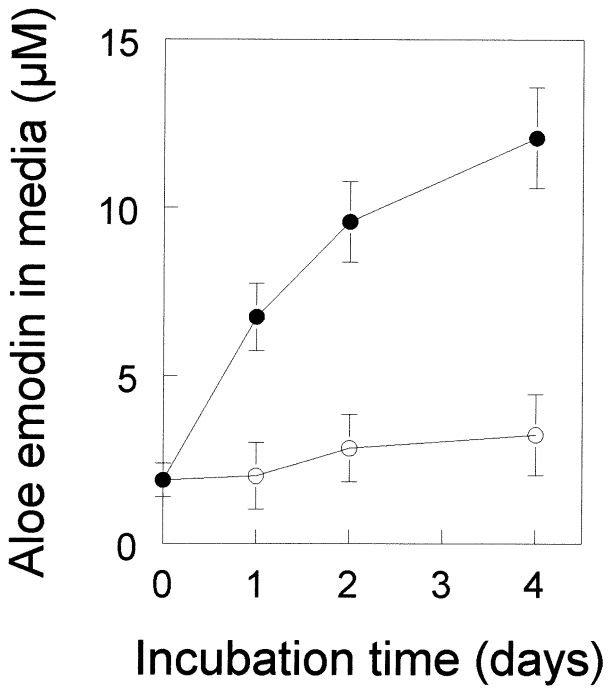


Fig. 7. In vitro metabolism of aloin A. Tissue culture medium containing 300 $\mu$M aloin A was incubated with confluent monolayers of human skin fibroblasts (●) or in flasks without fibroblasts (○). At selected times, the level of aloe emodin was determined as described in Materials and Methods. Data represent the mean of three determinations ± SD.

following photoexcitation of aloe emodin, are involved in photo-oxidation of cellular DNA.

The biological significance of photo-oxidative damage to cellular RNA and DNA, measured as the formation of 8-oxoG and 8-oxodG, is dual. Firstly, 8-oxoG and 8-oxodG are sensitive biomarkers for oxidative stress in the cell. Our results demonstrate that aloe emodin increases the level of oxidative stress in both the cytoplasmic and nuclear compartments of the cell. However, while we observed that oxidative damage to RNA and DNA increases concomitantly with photocytotoxicicty, we can not definitively identify RNA and DNA as the sole or essential cellular targets damaged by photoexcited aloe emodin. Secondly, the formation of 8-oxodG is biologically significant since this lesion in DNA has been shown to be mutagenic [34] and can result in morphological transformation of cells [35]. While our current knowledge of the role that oxidative damage to DNA plays in carcinogenesis is incomplete, our results indicate that the photogenotoxicity of aloe emodin must be considered as a mechanism for aloe emodin's reported photocarcinogenicity [17].

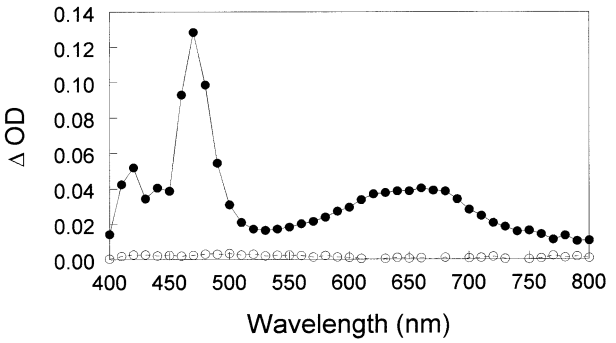


Fig. 8. Transient absorption spectra following photoexcitation of aloin A and aloe emodin. Solutions of aloe emodin (●) or aloin A (○) in acetonitrile were excited at 355 nm with a laser pulse. Transient absorption spectra were collected 300 ns after the excitation pulse.

While we can not derive a detailed photochemical mechanism for aloe emodin's photosensitizing activity from our studies, previously published works on the photochemistry of anthraquinones suggest several potential mechanisms. Photoexcitation of anthraquinones leads to efficient production of the anthraquinones' excited triplet state [36]. Broadly speaking, two types of photochemical reactions are known to proceed from the excited triplet state of anthraquinones. In the first type of photochemical reaction, electron transfer to anthraquinones, such as anthraquinone-2-sulfonic acid and various cationic anthraquinones, in their excited triplet states has been reported to result in oxidative damage to bases in DNA [37]. However, we have previously shown that electron transfer from bases in DNA or RNA to photoexcited aloe emodin is not energetically favorable [16]. Therefore, the photo-oxidative damage we have observed in cellular RNA and DNA can not result from direct electron transfer from guanine in RNA or DNA to aloe emodin. In addition, it has been shown that electron transfer can occur from cellular components, such as NADH, to excited states of anthraquinones to yield anthraquinone radical anions [38]. Subsequent electron transfers have been shown to result in the formation of superoxide radical anion and hydrogen peroxide [38]. These reactive oxygen species may have contributed to the oxidative damage we observed in cellular RNA and DNA. A second photochemical pathway involves direct transfer of energy from the excited triplet state of the anthraquinone to oxygen to produce $^1O_2$. Our previous studies have shown that aloe emodin efficiently photosensitizes the production of $^1O_2$ [16]. In addition, we have demonstrated that the photocytotoxicity of aloe emodin is substantially greater when $D_2O$, rather than $H_2O$, is used as the solvent [16]. This enhancement of photocytotoxicity by $D_2O$ provides additional evidence that $^1O_2$ plays a role in the photobiological activity of aloe emodin. Furthermore, we determined in the current study that oxidation of nucleotides photosensitized by aloe emodin is more efficient when the buffer is prepared with $D_2O$ rather than with $H_2O$. It therefore appears that

$^1O_2$ may play a significant role in the photocytotoxicity and photo-oxidiative cellular damage sensitized by aloe emodin.

We have determined that photoexcitation of aloin A does not produce excited state intermediates that can be identified by laser flash photolysis. In addition, irradiation of aloin A does not lead to photocytotoxicity. These results are consistent with what is known about structurally similar anthrones, such as anthralin (Fig. 1). Bruce et al. were unable to detect the triplet state of anthralin after excitation at 355 nm [39]. Furthermore, Dabestani et al. have reported that neither $^1O_2$ nor other reactive species, such as superoxide radical anion, are formed when anthralin is irradiated using a xenon arc lamp [40]. These investigators present evidence that the photosensitizing activity occasionally associated with the therapeutic use of anthralin for treating psoriasis is actually due to the anthralin anion or 1,8-dihydroxyanthraquinone, a product of anthralin oxidation [37]. Analogously, we have found that while the anthrone, aloin A, is photobiologically inactive, oxidative cleavage of aloin A results in the formation of aloe emodin, which has significant in vitro photosensitizing activity.

In summary, excitation of aloe emodin by UV or visible light results in cytotoxicity and oxidative damage to both cellular RNA and DNA. Although we have not identified the cellular targets essential for aloe emodin's photosensitizing activity, our results do suggest that oxidative damage plays a significant role in the photocytotoxicity elicited by aloe emodin. In addition, while aloin A was not photocytotoxic, skin fibroblasts were able to metabolize aloin A to aloe emodin. These results suggest exposure to products containing aloe emodin, or aloin A under conditions where it is metabolized or otherwise converted to aloe emodin, could increase an individual's sensitivity to sunlight. Additional studies are needed to establish whether these results, obtained in an in vitro model system, correctly predict the photosensitizing potential of aloe emodin and aloin A in vivo.

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