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UV protection and singlet-oxygen quenching activity of intramolecularly hydrogen-bonded hydroxyanthraquinone derivatives found in aloe

Shin-ichi Nagaoka [a,*], Keishi Ohara [a], Miki Takei [a], Mari Nakamura [a], Mariko Mishima [a], Umpei Nagashima [b]

[a] Department of Chemistry, Faculty of Science and Graduate School of Science and Engineering, Ehime University, Matsuyama 790-8577, Japan
[b] Nanosystem Research Institute (NRI), National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Umezono, Tsukuba 305-8568, Japan

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This paper is dedicated to Professor Paul F. Barbara of University of Texas at Austin who passed away on October 31, 2010 at the age of 57 years from complications following a cardiac arrest.

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ABSTRACT

The UV protection and singlet-oxygen ($^1O_2$) quenching activity of intramolecularly hydrogen-bonded hydroxyanthraquinone derivatives found in aloe have been studied by means of laser spectroscopy. The UV protective activity provided by excited-state intramolecular proton-transfer (ESIPT) in these molecules correlates with their $^1O_2$ quenching activity, and the UV protective molecules have high $^1O_2$-quenching function. The reason for this correlation can be understood by considering ESIPT-induced distortion of ground-state potential surfaces in encounter complexes with $^1O_2$.

1. Introduction

Aloe is a flowering fleshy plant with succulent sharp leaves. The cosmetic and healing properties of aloe have been highly valued by a lot of people (Cleopatra, Aristotle, Alexander the Great and so on) for thousands of years [1–6]. For example, legend says that aloe was one of Cleopatra's beauty secrets. In the present age, aloe gel is an active ingredient in hundreds of cosmetics, skin lotions and sun blocks for UV protection. Accordingly, it can be expected that excited-state intramolecular proton-transfer (ESIPT [7–11]) in aloe species, as well as in various skin sun-blocks [12], provides the UV protection on skin, and aloe species also quench harmful singlet oxygen ($^1O_2$, $^1\Delta_g$ state [13]) generated on UV-irradiated skin [14].

In a previous paper [15], we reported the UV protection and $^1O_2$ quenching of aloesaponarin I (methyl 3,8-dihydroxy-1-methyl-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate, abbreviated as AS1, Fig. 1) found in aloe [16]. In AS1, the ESIPT along one of the intramolecular hydrogen-bonds (C_8-O-H$\cdots$O=C_9) provides the UV protection, and the functional groups participating in the ESIPT play important roles also in the $^1O_2$ quenching. AS1 has a $^1O_2$-quenching rate-constant larger than vitamin E that is well-known as an efficient $^1O_2$ quencher [13,17], and AS1 has a long duration of action due to its resistance to UV degradation and chemical attacks by $^1O_2$ and free radicals.

Aloe contains various intramolecularly hydrogen-bonded hydroxyanthraquinone derivatives such as AS1 [16], and it is expected that those molecules can also be UV protective and have $^1O_2$ quenching activity. The reaction in which $^1O_2$ and anthraquinone participate is also a topic of interest [18]. Accordingly, in the work presented here, we have studied the ESIPT-based UV-protective and $^1O_2$-quenching functions of intramolecularly hydrogen-bonded hydroxyanthraquinone derivatives found in aloe [16] by means of laser spectroscopy.

2. Experimental

Fig. 1 shows the ground-state (S_0-state) structures of the molecules used in the present work. Each of these molecules

* Corresponding author. Tel.: +81 89 927 9592; fax: +81 89 927 9590.
E-mail address: nagaoka@ehime-u.ac.jp (S. Nagaoka).

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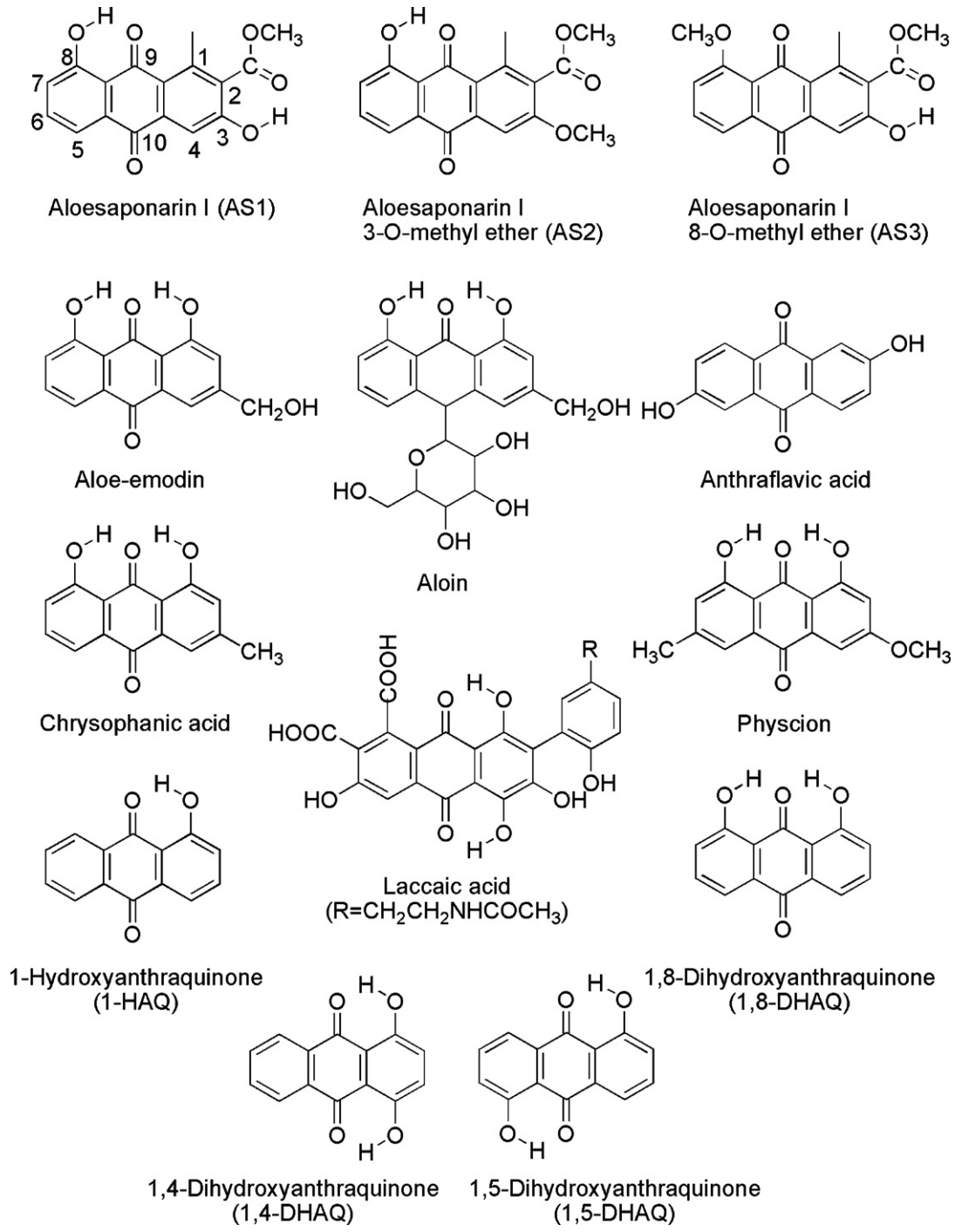

Fig. 1. Structure of molecules used in the present work.

except aloin is a hydroxyanthraquinone (HAQ). The UV protection and $^1O_2$ quenching activity of AS1, aloesaponarin I 3-O-methyl ether (methyl 8-hydroxy-3-methoxy-1-methyl-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate, AS2) and aloesaponarin I 8-O-methyl ether (methyl 3-hydroxy-8-methoxy-1-methyl-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate, AS3) were studied in a previous work [15]. AS1, aloe-emodin, aloin and chrysophanic acid are chemical constituents characteristics of aloe [16]. Some molecules similar in molecular structure to laccaic acid or physcion are also found in aloe [16]. Originally thought to be one compound, laccaic acid has been separated into four compounds [19], but in the present study, the structure of the major compound shown in Fig. 1 [19] is used in estimation of the molar concentration. Reported contents of the above-mentioned components present in aloe are given in Table 1 [20–23]. Although anthraflavic acid is not a chemical constituent characteristics of aloe [16], it is found in rhubarb, which is one of ancient and well-known Chinese herbal medicines and also contains various HAQs [24]. Since the above-mentioned molecules except aloin have a common skeletal moiety and are different in the side group(s) attached to it, we have also studied the skeletal molecule (1-hydroxyanthraquinone, 1-HAQ) and its derivatives; 1,4-dihydroxyanthraquinone (quinizarin, 1,4-DHAQ), 1,5-dihydroxyanthraquinone (anthrarufin, 1,5-DHAQ) and 1,8-dihydroxyanthraquinone (chrysazin, 1,8-DHAQ). The compounds

Table 1
Fluorescence quantum yields ($\phi_{non\text{-}PT}$, ϕ_{SWE} and ϕ_{LWE}) and k_Q of aloin and HAQs.

Molecule	Content in aloe (wt%)	$\phi_{non\text{-}PT}$	ϕ_{SWE}	ϕ_{LWE}	ϕ_{LWE}/ϕ_{SWE}	k_Q (M^{-1} s^{-1})
Group A						
AS1 [15]	0.01[a]		Negligible	9.8×10^{-3}	Large	2.8×10^{8}
AS2 [15]			Negligible	9.2×10^{-3}	Large	3.1×10^{8}
1-HAQ			2.3×10^{-3}	1.5×10^{-2}	6.5	1.8×10^{8}
1,5-DHAQ			3.0×10^{-3}	2.5×10^{-2}	8.3	2.6×10^{8}
Group B						
Aloe-emodin	0.03[b]		6.8×10^{-3}	2.0×10^{-2}	2.9	0.5×10^{8}
Chrysophanic acid	–[c]		8.8×10^{-3}	2.1×10^{-2}	2.4	0.7×10^{8}
Physcion			2.2×10^{-2}	4.9×10^{-2}	2.2	0.7×10^{8}
1,8-DHAQ			6.4×10^{-3}	1.7×10^{-2}	2.7	1.2×10^{8}
Group C						
Anthraflavic acid		Negligible	Negligible	Negligible		Negligible
AS3 [15]		Negligible	Negligible	Negligible		Negligible
Laccaic acid	–[c]	4.0×10^{-3}				Negligible
1,4-DHAQ		0.20				Negligible
Miscellaneous						
Aloin	9[d]		1.7×10^{-4}	2.2×10^{-3}	13	Negligible
β-Carotene [27]						1.2×10^{10}
Vitamin E[e] [15]						1.2×10^{8}

[a] Homogenate of fresh subterranean stem of *Aloe saponaria* Haworth [20].
[b] Freeze-dried methanol-extract of leaves of *Aloe elgonica* Bullock [21].
[c] Chrysophanic acid is a chemical constituent characteristic of aloe, and a molecule similar in molecular structure to laccaic acid is also found in aloe [16]. However, their contents present in aloe have not been reported [20,22].
[d] Inspissated juice of *Aloe ferox* Miller and *Aloe perryi* Baker [23].
[e] α-Tocopherol.

shown in Fig. 1, except AS1, AS2 and AS3, were commercially available and used as received.

The setup and experimental procedures were described in a previous paper [15]. Briefly, the absorption spectra of the molecules shown in Fig. 1 (HAQs and aloin) were measured in ethanol with a Shimadzu UV mini-1240 spectrophotometer. The fluorescence spectra were measured in ethanol with a Shimadzu RF-5000 spectrofluorophotometer and analyzed by using curve-fitting [25]. The fluorescence quantum yields (ϕ's) were determined by comparing the fluorescence-emission spectra of the samples with that of 9,10-diphenylanthracene in ethanol (ϕ = 1.0 [26]) after they had been corrected for the spectral sensitivity of the detector.

When a quencher (Q) is added to a solution, $^1O_2$ is quenched in the following way.

$$^1O_2 + Q \xrightarrow{k_Q} {}^3O_2 + \text{quenching} \quad \text{product} \tag{1}$$

The second-order rate-constant of the $^1O_2$ quenching by Q (k_Q) was determined by measuring the phosphorescence-decay rate-constant of $^1O_2$ (k) in an air-saturated ethanol solution including rose bengal (sensitizer) and Q. The k values were measured by a Hamamatsu C7990-01 near-infrared fluorescence-lifetime measurement system in which the second harmonic of a Nd:YAG laser (532 nm) was used as the excitation light. According to Merkel and Kearns (see the explanation given in Ref. [27]), the k value is given by

$$k = k_0 + k_Q[Q] \tag{2}$$

where k_0 and [Q] denote the natural-decay rate-constant of $^1O_2$ and the molar concentration of Q, respectively.

3. Results and discussion

3.1. UV protection provided by ESIPT

Photosensitization causes considerable degradation of polymer substrate etc. [28], and is efficient when intersystem crossing from the lowest excited singlet-state (S_1 state) is fast [29a]. The intersystem crossing is generally very fast in quinones [30,31], but internal conversion from the S_1 state is very slow in most molecules (Ermolev's rule) [29b]. However, there are some exceptions to these rules, and when the harmful photosensitization through the intersystem crossing etc. is prevented by very fast internal conversion from the S_1 state, those molecules may be used as UV protectors.

Various intramolecularly hydrogen-bonded molecules susceptible to ESIPT are widely used as UV protectors (see Ref. [12] and many references cited in Ref. [32]). The UV protection mechanism of the ESIPT-active molecules is based essentially on absorbing harmful UV radiation and its dissipation as innocuous thermal energy [33] through the very fast internal conversion [34], so that the UV radiation does not lead to the harmful photosensitization [33] through the intersystem crossing etc. The very fast internal conversion at room temperature would come from out-of-plane bending and/or torsional motion as suggested previously [35,36]. When the ESIPT is blocked, the photochemical stability is greatly reduced [37] by enhancement of the photosensitization through the intersystem crossing etc. In this section, we examine the UV protection provided by ESIPT of intramolecularly hydrogen-bonded HAQs found in aloe.

The fluorescence-emission of anthraflavic acid is negligibly weak as well as that of anthraquinone is, because the S_1 state has a character of $^1(n,\pi^*)$ and the $^1(n,\pi^*) \rightarrow S_0$ radiative decay-rate is much less than the intersystem-crossing rate from $^1(n,\pi^*)$ [29c]. In contrast, in aloin and HAQs except anthraflavic acid, the intramolecular hydrogen-bonding blue-shifts the $^1(n,\pi^*)$ state, and the S_1 state has a character of $^1(\pi,\pi^*)$. As a result, non-negligible fluorescence-emission is observed in these molecules [29c].

Fig. 2 shows the absorption and fluorescence spectra of AS1, laccaic acid and chrysophanic acid in ethanol. AS1 shows the fluorescence-emission around 600 nm with an unusually large red-shift (Stokes-shift) from the absorption peak (Fig. 2a) [15]. As in many previous reports [7–11], this observation can be explained in terms of ESIPT (see Fig. 3 of Ref. [15]): photoexcitation of the normal form (stable S_0-state species) produces the lowest-excited $^1(\pi,\pi^*)$ state (S_1 state), in which ESIPT along an intramolecular hydrogen-bond takes place rapidly, stabilizing the S_1 state. The S_1 state of the proton-transferred form decays to the S_0 state through the fluorescence-emission or the internal conversion, which is so fast as to dominate over the photosensitization at room temperature. Then the reverse proton transfer takes place in the S_0 state and the stable normal form is regenerated. Because a significant

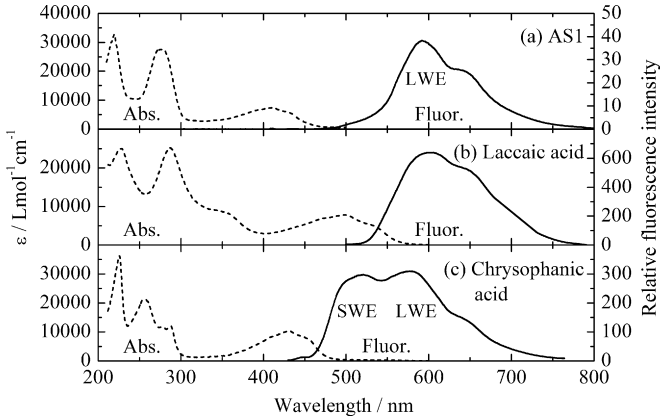

Fig. 2. Absorption (Abs.) and fluorescence-emission (Fluor.) spectra of (a) AS1 [15], (b) laccaic acid and (c) chrysophanic acid in ethanol. ε denotes the molar absorption coefficient. SWE and LWE stand for short and long wavelength emissions, respectively. The fluorescence-emission spectra of AS1, laccaic acid and chrysophanic acid were obtained by photoexcitation at 400, 350 and 390 nm, respectively, and have not been corrected for the spectral sensitivity of the detector.

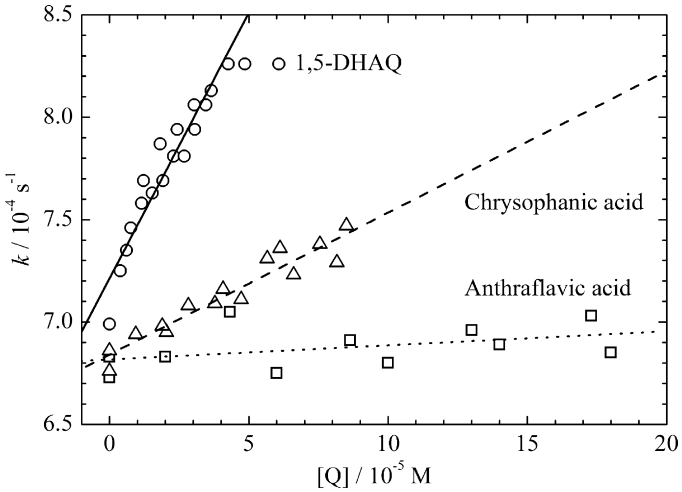

Fig. 4. Dependence of $^1O_2$-quenching k on [1,5-DHAQ] (circle), [chrysophanic acid] (triangle), and [anthraflavic acid] (square). The k values were obtained by monitoring the phosphorescence decay of $^1O_2$ at 1273 nm in ethanol.

amount of absorption photon energy is dissipated as heat in this cycle, the proton-transferred form fluoresces at a lower energy with an unusually large Stokes-shift. The Stokes-shifted fluorescence-emission is thus an indication of ESIPT, which causes distortion and displacement of the potential surfaces of the S_0 and S_1 states. Note that the distortion reduces the potential surface curvature of the S_0 state (see Fig. 3 of Ref. [15]). The whole energy dissipation caused by ESIPT prevents the harmful photosensitization and provides the UV protection. The regenerated normal form can contribute again to the ESIPT-based UV protection.

The spectra of laccaic acid (Fig. 2b), however, differ from those of AS1 (Fig. 2a). The Stokes-shift of laccaic acid is much less than that of AS1, although a large Stokes-shift is an indication of ESIPT. Laccaic acid in the S_1 state thus does not seem to be susceptible to ESIPT, even though the hydroxy groups at the 1- and 4-positions are intramolecularly hydrogen-bonded to the carbonyl oxygens at the 9- and 10-positions, respectively. The skeletal moiety of laccaic acid is 1,4-DHAQ, which is not susceptible to ESIPT, either [38]. The

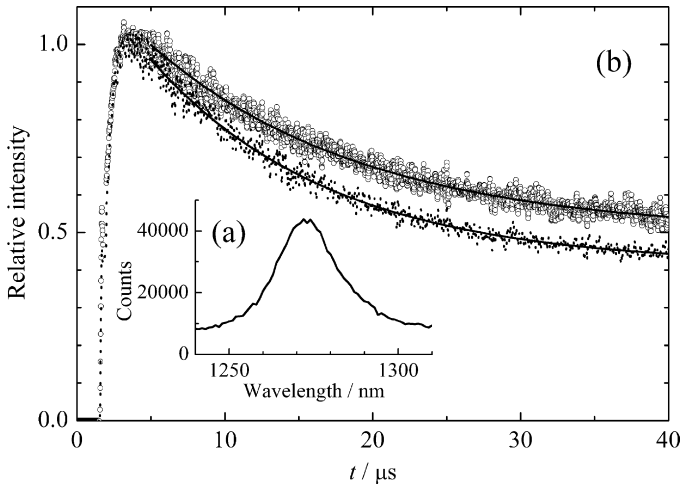

Fig. 3. (a) Phosphorescence-emission spectrum from $^1O_2$ produced through photosensitization from rose bengal in ethanol. (b) Phosphorescence decay curves of $^1O_2$ in the absence (open circles) and presence (dots) of 1,5-DHAQ (3.45×10^{-5} M) in ethanol. The fitted curves (solid lines) are added to confirm that the phosphorescence decays after subtraction of background counts due to dark current are well-characterized by single exponential decays. Because of specifications of the photon-counting circuit-board used, the background counts are seemingly very low around $t = 0$.

absence of ESIPT in 1,4-DHAQ, together with the presence in 1,5-DHAQ and 1,8-DHAQ, can be understood by considering the nodal-pattern of the wave function in the S_1 state [11,38]. The distortion and displacement of the potential surfaces in ESIPT-inactive HAQs are much less than those in ESIPT-active HAQs. The fluorescence quantum yield of an ESIPT-inactive HAQ is denoted as $\phi_{\text{non-PT}}$ and given in Table 1.

Chrysophanic acid shows dual fluorescence-emission in short and long wavelength regions (Fig. 2c). The Stokes-shift of the long wavelength emission (LWE) is large and indicates ESIPT along an intramolecular hydrogen-bond of chrysophanic acid. The short wavelength emission (SWE), like that of salicylaldehyde [39], originates from the open conformer with the hydroxy groups not intramolecularly hydrogen-bonded to the carbonyl oxygen. In AS1, LWE dominates over SWE (Fig. 2a).

The quantum yields of SWE and LWE (ϕ_{SWE} and ϕ_{LWE}, respectively) of aloin and ESIPT-active HAQs in ethanol are given in Table 1 together with the intensity ratio $\phi_{\text{LWE}}/\phi_{\text{SWE}}$. The greater the $\phi_{\text{LWE}}/\phi_{\text{SWE}}$ value is, the more favorable the molecule is for ESIPT and its-based UV-protection. Since ϕ_{LWE} is much greater than ϕ_{SWE} in 1-HAQ that is the skeletal molecule as noted in Section 2, intramolecular hydrogen-bonds of HAQs would basically be strong and less likely to be easily broken in ethanol [15].

3.2. $^1O_2$ quenching

Fig. 3a shows the phosphorescence-emission spectrum of $^1O_2$ produced through photosensitization from rose bengal in ethanol, and Fig. 3b shows the decay curves obtained by monitoring the phosphorescence-emission at 1273 nm in the presence and absence of 1,5-DHAQ. The phosphorescence decay after subtraction of background counts due to dark current is well-characterized by a single exponential decay, and the decay rate-constant k increases when 1,5-DHAQ is added to the solution (Fig. 3b), showing that 1,5-DHAQ can quench $^1O_2$ (reaction (1)). Some examples of the dependence of the rate constant k on the concentration of the quencher Q (1,5-DHAQ, chrysophanic acid or anthraflavic acid) are shown in Fig. 4. The saturation seen in the plot for 1,5-DHAQ seems to be due to self-association [40], and similar results are also obtained in AS2 [15] and 1-HAQ. By using Eq. (2) the k_Q values of the molecules given in Fig. 1 have been determined from the slopes of the k versus [Q] plots, and are listed in Table 1.

The k_Q value of 1,5-DHAQ is much larger than that of anthraflavic acid (Fig. 4 and Table 1), although 1,5-DHAQ and anthraflavic acid

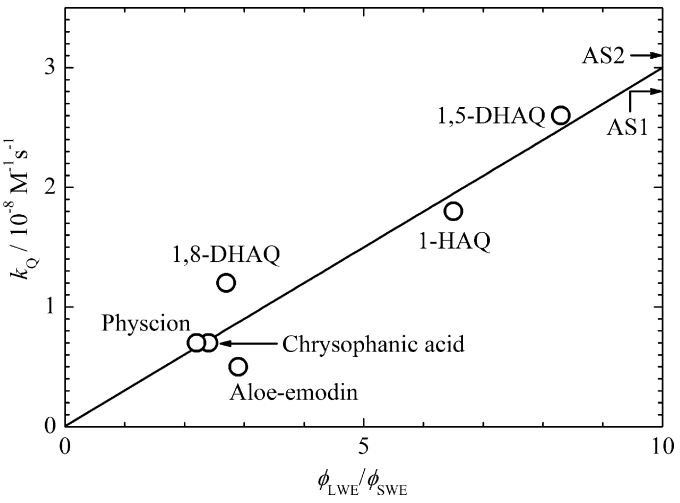

Fig. 5. Plot of k_Q versus ϕ_{LWE}/ϕ_{SWE}. Arrows at the right vertical axis indicate the k_Q values of AS1 and AS2 [15].

(2,6-dihydroxyanthraquinone) are isomers differing in the positions of the hydroxy groups and belonging to the same point group C_{2h} (Fig. 1). Note that LWE dominates over SWE in 1,5-DHAQ like AS1 (Table 1) and 1,5-DHAQ is susceptible to ESIPT. As in the case of AS1 [15], 1,5-DHAQ is thought to form stable encounter complexes with $^1O_2$, which is attached to the functional groups participating in the ESIPT. Then, the encounter complex is favorable for $^1O_2$ quenching and shows a great k_Q value [15]. Thus, the formation of the encounter complex related to ESIPT is an accelerator of $^1O_2$ quenching. On the other hand, the fluorescence-emission of anthraflavic acid is negligibly weak (Section 3.1) and it is not susceptible to ESIPT. Accordingly, the $^1O_2$-quenching accelerator present in 1,5-DHAQ is absent in anthraflavic acid, and the k_Q value of anthraflavic acid is much less than that of 1,5-DHAQ. Chrysophanic acid showing dual fluorescence-emission (SWE and LWE comparable to each other, see Fig. 2c) is roughly intermediate, in $^1O_2$ quenching, between 1,5-DHAQ and anthraflavic acid (Fig. 4).

3.3. Correlation between UV protection and $^1O_2$ quenching activity

As shown in Table 1, the UV protective activity provided by ESIPT in HAQs (our sample molecules except aloin) positively correlates with their $^1O_2$ quenching activity. AS1, AS2 [15], 1-HAQ and 1,5-DHAQ (A group) have large ϕ_{LWE}/ϕ_{SWE} values providing efficient UV protection by ESIPT, and at the same time they show great k_Q values causing large $^1O_2$-quenching. In contrast, AS3 [15], anthraflavic acid, laccaic acid and 1,4-DHAQ (C group) are not UV protective (negligible ϕ_{LWE} showing lack of ESIPT), and do not efficiently quench $^1O_2$ (negligible k_Q), either. These molecules (C group) may induce photosensitization through efficient intersystem crossing etc. leading to degradation and also induce oxidative action by $^1O_2$. Aloe-emodin, chrysophanic acid, physcion, and 1,8-DHAQ (B group) are intermediate between A and C groups, and show intermediate ESIPT-based UV-protection and intermediate $^1O_2$ quenching activity (Table 1). The plot of k_Q versus ϕ_{LWE}/ϕ_{SWE} for A and B molecule groups is found to give a fair linear fit with a positive slope of 3.0×10^7 M^{-1} s^{-1}, an intercept of 5.3×10^5 M^{-1} s^{-1} and a correlation coefficient squared of 0.88 (Fig. 5).

The reason for this correlation could be qualitatively explained in the following way. As shown previously [15], the S_0-state species of ESIPT-active and $^1O_2$-quenchable HAQs such as AS1 form stable encounter complexes with $^1O_2$ ($\{HAQ\cdots{}^1O_2\}$), and the OH and C=O groups participating in ESIPT play important roles also in $^1O_2$ quenching through the complex formation. Accordingly, it is expected that the quenching-reaction coordinate from $\{HAQ\cdots{}^1O_2\}$ to $\{HAQ\cdots{}^3O_2\}$ has a component parallel to the reaction coordinate of ESIPT. If the two coordinates were perpendicular to each other, the correlation between k_Q and ϕ_{LWE}/ϕ_{SWE} would not be evident. Therefore, in ESIPT-active HAQs it is thought that the potential curves of $\{HAQ\cdots{}^1O_2\}$ and $\{HAQ\cdots{}^3O_2\}$ projected on the ESIPT coordinate system are located apart from each other, $\{HAQ\cdots{}^3O_2\}$ is lower in energy than $\{HAQ\cdots{}^1O_2\}$, and the crossing of the two potential curves puts a barrier between them; that is, an activation energy is necessary to the $\{HAQ\cdots{}^1O_2\} \rightarrow \{HAQ\cdots{}^3O_2\}$ reaction. Note that a decrease in potential curvature along the ESIPT coordinate, then, reduces the activation energy. As described in Section 3.1, ESIPT really decreases the potential surface curvature in the S_0 state of HAQ (see Fig. 3 of Ref. [15]) [7–11]. This decrease in the S_0-curvature of the ESIPT-active HAQ makes the activation energy of the corresponding $\{HAQ\cdots{}^1O_2\} \rightarrow \{HAQ\cdots{}^3O_2\}$ less than in the case that an ESIPT-inactive HAQ forms an encounter complex without the above-mentioned decrease in S_0-curvature. The activation energy for the ESIPT-active HAQ is also less than in the case that the S_0-state species of a HAQ does not form a stable complex with $^1O_2$. As a result, ESIPT-active and UV-protective HAQs are considered to have high $^1O_2$-quenching activity. However, further investigation is needed on the reason why the nearly *linear* correlation is revealed in Fig. 5.

In aloin, the anthraquinone skeleton of aloe-emodin is modified by the addition of a sugar molecule at the 10-position, and aloin is no longer a HAQ (Fig. 1). Although both of aloin and aloe-emodin have large ϕ_{LWE}/ϕ_{SWE} values and are susceptible to ESIPT, the k_Q value of aloin is much less than that of aloe-emodin and negligibly small (Table 1). It seems that the addition of the sugar molecule at the 10-position suppresses the $^1O_2$ quenching activity and the anthraquinone moiety in HAQ plays an important role in $^1O_2$ quenching.

3.4. Advantages as UV protective and $^1O_2$ quenching agents

In this section, on the basis of the results obtained here and previously [15], we discuss the advantages of HAQs contained in aloe, as UV protective and $^1O_2$ quenching agents. In intramolecularly hydrogen-bonded HAQs belonging to A and B molecule groups, the normal form regenerated through ESIPT cycle can contribute again to the UV protection provided by the ESIPT (see Fig. 3 of Ref. [15]). Accordingly, those HAQs found in aloe have the advantage of being recyclable UV-protective agents.

Those HAQs (A and B groups) also have great k_Q values as well as AS1 does, and quench $^1O_2$ through non-destructive charge–transfer before being destroyed by irreversible chemical reactions with $^1O_2$ [15]. Accordingly, the UV protective and $^1O_2$ quenching functions of those HAQs are not easily deactivated through the irreversible chemical attacks by $^1O_2$ generated upon UV irradiation [14], and therefore those HAQs have the advantage of retaining their UV protection and $^1O_2$ quenching for a long time. In contrast, carotenoids such as β-carotene quench $^1O_2$, through electronic energy transfer [13], much more efficiently than HAQs belonging to A and B groups (Table 1), but most of the carotenoids deteriorate under irradiation and need to be stored protected from light at low temperature [19].

Since vitamin E has no intramolecular hydrogen-bonding, it shows no UV protective function provided by ESIPT. However, as well known, vitamin E efficiently quenches $^1O_2$ through charge transfer [13,17] as well as AS1 does [15], and has a $^1O_2$-quenching rate-constant [15] similar to those of HAQs belonging to A and B groups (Table 1). In vitamin E derivatives, the $^1O_2$-quenching rate-constant was found to linearly correlate with the rate constant of the free-radical scavenging reaction [17], in which the OH bond

of vitamin E dissociates and vitamin-E radical is produced. That is, the more active the vitamin E derivative is in $^1O_2$ quenching, the more easily it is destroyed through chemical attacks by free radicals. However, such a correlation was not found in intramolecularly hydrogen-bonded HAQs such as AS1; the free radicals do not efficiently react with HAQs having high k_Q [15]. It is thought that the intramolecular hydrogen-bond of those HAQs (OH···O) suppresses the OH dissociation and reduces the activity of the destructive reactions with the free-radicals. Accordingly, the UV protective and $^1O_2$ quenching functions of those HAQs are not easily deactivated through the chemical attacks by the free radicals that are frequently produced in biological systems [41], and therefore those HAQs have the advantage of being long-lasting UV protective and $^1O_2$-quenching agents.

Next we will examine contents of HAQ components present in aloe and discuss UV protection and $^1O_2$ quenching activity of aloe itself on the basis of the data obtained here and reported previously [15]. The content of aloe-emodin (mol wt = 270.24) in aloe was found to be 0.03 wt% (Table 1) and the molar concentration in the solution extracted from aloe was about 1×10^{-4} M [21]. Since the molar absorption coefficient (ε) in ethanol is 1000–9000 L mol^{-1} cm^{-1} in UVA region, the absorbance for 1 cm is 0.1–0.9, and thus fair UV protection is expected even for aloe-emodin alone in the solution extracted from aloe. The molar concentration of aloe-emodin in the extracted solution is about 5 times as much as that of vitamin E in human plasma (2.2×10^{-5} M) [42], and the k_Q value of aloe-emodin is about half of that of vitamin E (Table 1). Accordingly, aloe-emodin in the extracted solution is expected to show $^1O_2$ quenching activity similar to that for vitamin E in human plasma. However, since aloe-emodin is phototoxic as mentioned previously [15], the other intramolecularly hydrogen-bonded HAQs whose contents in aloe have not been reported in detail may contribute to the sunlight protection and healthcare for which aloe has been used since ancient times.

If the molar concentration of AS1 in the solution extracted from aloe is similar to that of aloe-emodin, AS1 can also contribute to the UV protection and $^1O_2$ quenching. Furthermore, the content of AS1 (mol wt = 312.063) in aloe (0.01 wt%, Table 1) is about 15 times as much as that of vitamin E (mol wt = 430.71) in rat liver (0.00065 wt% [43]), and the k_Q value of AS1 is 2.3 times as much as that of vitamin E (Table 1). Accordingly, AS1 in aloe can quench $^1O_2$ much more efficiently than vitamin E in rat liver. Like this, various HAQ components present in aloe would jointly provide the UV protective and $^1O_2$ quenching functions of aloe itself.

4. Conclusion

The UV protection and $^1O_2$ quenching activity of intramolecularly hydrogen-bonded HAQs found in aloe have been studied by means of laser spectroscopy. The UV protective activity provided by ESIPT in these molecules correlates with their $^1O_2$ quenching activity, and the UV protective molecules have high $^1O_2$-quenching function. The reason for this correlation can be understood by considering ESIPT-induced distortion of the S_0-state potential surface in the encounter complex $\{HAQ\cdots{}^1O_2\}$.

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