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Riboflavin-Photosensitized Oxidation Is Enhanced by Conjugation in Unsaturated Lipids

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ABSTRACT: Methyl esters of polyunsaturated fatty acids were found to quench triplet-excited riboflavin (^{3}Rib) in efficient bimolecular reactions with rate constants, as determined by laser flash photolysis, linearly depending upon the number of bis-allylic methylene (from 1 to 5). Deactivation of ^{3}Rib is predicted by combining the experimental second-order rate constants k_2 determined for acetonitrile/water (8:2, v/v) at 25 °C with density functional theory (DFT) calculations of bond dissociation energy to have an upper limiting value of 1.22×10^7 L mol^{-1} s^{-1} for hydrogen abstraction from bis-allylic methylene groups in unsaturated lipid by ^{3}Rib. Still, ergosterol was found to deactivate ^{3}Rib with $k_2 = 6.2 \times 10^8$ L mol^{-1} s^{-1}, which is more efficient than cholesterol, with 6.9×10^7 L mol^{-1} s^{-1}. Likewise conjugated (9*E*,11*E*) methyl linoleate (CLA) reacts with 3.3×10^7 L mol^{-1} s^{-1}, 30 times more efficient than previously found for methyl α-linolenate. Conjugation as in CLA and ergosterol is concluded to enhance ^{3}Rib deactivation, and dietary plant sterols and CLA may accordingly be important macronutrients for eye and skin health, protecting against light exposure through efficient deactivation of ^{3}Rib.

KEYWORDS: *Riboflavin, laser flash photolysis, photooxidation, conjugated linoleic acid, eicosapentaenoic acid*

INTRODUCTION

Flavins, a family of N-heterocyclic compounds sharing a common isoalloxazine ring, are ubiquitous in both the plant and animal kingdom. Riboflavin, an isoalloxazine derivative generally referred to as vitamin B_2 (see Scheme 1), is the main constituent of the prosthetic groups flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) in many flavoproteins and plays as such various roles ranging from yellow pigmentation to being enzymatic co-factors for numerous redox reactions.[1,2] Interest in the photochemistry and photophysics of vitamin B_2 has been increasing during the past decade, because of the implication of B_2 vitamin in various photobiological processes in human skin and eyes and also for its crucial role in the redox stability of food and beverages under light exposure.[3−6] Following ultraviolet (UV) and visible light excitation, lower singlet-excited states of flavins (τ = 5 ns) are populated and subsequently converted to the lowest triplet-excited state by efficient intersystem crossing (Φ_{ISC} = 0.67).[1,3] The long-lived flavin triplet-excited state (τ = 15 μs in water) is a powerful oxidant [$E°$ = 1.77 V versus normal hydrogen electrode (NHE)] capable of oxidizing sensitive biomolecules (type I photooxidation) or transferring the excitation energy to ground-state oxygen in an efficient spin-allowed physical quenching process to yield singlet oxygen (Φ_Δ = 0.68), a reactive electrophile, leading to type II photooxidation of a large number of biomolecules.[3,7−9]

An increasing amount of evidence over the past few years supports the nutritional and health benefits of increasing the content of long-chain ω-3 polyunsaturated fatty acids and phytosterols in the diet. ω-3 fatty acids are further recognized as photoprotective macronutrients in the skin and eyes, protecting against sunlight-induced skin inflammation,[10,11] photoaging,[12] photocarcinogenesis,[13,14] and eye age-related macular degeneration (AMD).[15] Some flavonoids and plant sterols seem to have similar beneficial effects.[16,17]

In this connection, dietary recommendations for better eye and skin health will clearly benefit from a better understanding at the molecular level of the interaction between skin and eye photosensitizers, especially riboflavin and potential oxidation substrates, such as sensitive proteins and unsaturated lipids, including sterols, which, under some conditions, may serve as antioxidants, protecting the proteins and sensitive structures. The present study was accordingly undertaken to contribute to kinetic modeling of this complex interaction by investigating the rate of reductive quenching of singlet- and triplet-excited riboflavin by relevant long-chain ω-3 and ω-6 fatty acids together with sterols present in food, skin, and eyes. Notably, such knowledge may also be helpful for the formulation of dairy products enriched with fish oil or plant sterols. Dairy products, such as dairy spreads, have been recognized as good delivery systems for ω-3 polysaturated fatty acids and phytosterols. However, dairy products are known to be rich in B_2 vitamin, and therefore, because of their high degree of unsaturation of

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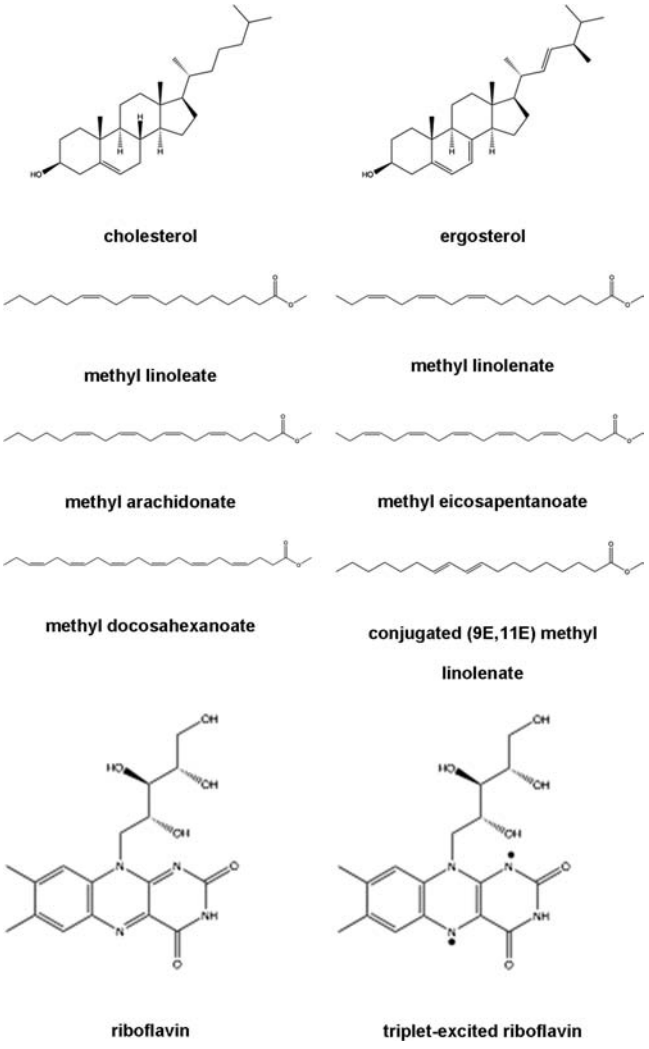
Scheme 1. Structures of Quenchers and Riboflavin and Triplet-Excited Riboflavin



ω-3 fatty acids, the enrichment of dairy products with fish lipids may cause rapid photooxidative deterioration of such functional food.[7,18,19]

EXPERIMENTAL SECTION

Materials. Riboflavin (Rib), cholesterol (COL), ergosterol (ERL), and methyl esters of arachidonic acid (ARA), conjugated (9*E*,11*E*)-linoleic acid (CLA), eicosapentaenoic acid (EPA), and docohexaenoic acid (DHA) were obtained from Sigma-Aldrich (St. Louis, MO) and used without further purification. Acetonitrile (Lab-Scan, Dublin, Ireland) were of spectroscopic grade. Ultrapurified water was obtained using a Milli-Q purification system (Millipore, Bedford, MA). Scheme 1 shows the structure of the compounds under investigation.

Techniques. *Flash Photolysis.* Laser flash photolysis with real-time transient absorption spectroscopy was carried out with a LKS.50 spectrometer from Applied Photophysics, Ltd. (Leatherhead, U.K.) using the third harmonic of a pulsed Q-switched Nd:YAG laser with 8 ns resolution (Spectron Laser System, Rugby, U.K.), attenuated to 10 mJ cm^{-2} as the excitation source. The transient time traces for absorption difference were recorded by a R928 photomultiplier tube from Hamamatsu Photonics (Hamamatsu City, Japan) tuned at 720 nm by a Czerney–Turner monochromator (Hamamatsu City, Japan). Appropriated UV cutoff filters were used to minimize sample deterioration by monitoring light (xenon arc lamp from Applied Photophysics, Ltd.). Samples were excited in 0.5 × 1.0 cm fluorescence cuvettes from Hellma (Mulheim, Germany). Flash photolysis experiments were also conducted using the third harmonic of a pulsed Q-switched Nd:YAG laser (Spectron Laser Systems, U.K.), attenuated to 10 mJ cm^{-2} as the excitation source. Transient time traces were collected using a mLFP-111 Luzchem (Ottawa, Ontario, Canada) miniaturized equipment. The signal from the monochromator/photomultiplier detection system was captured by a Tektronix TDS 2012 digitizer (Beaverton, OR). The laser system and the digitizer were connected to a personal computer via General Purpose Instrumentation Bus (GPIB) and serial interfaces that control all of the experimental parameters and provide suitable processing and data storage capabilities. The software package was developed in the LabVEW environment from National Instruments (Austin, TX) and compiled as a stand-alone application. Each kinetic trace was averaged 32 times, and observed rate constants were determined by nonlinear fitting with MatLabR2009 (The MathWorks, Natick, MA). All measurements were performed on fresh solutions thermostatted at 25.0 ± 0.5 °C and purged with high-purity nitrogen for at least 30 min before the experiment.

Time-Resolved Fluorescence Spectroscopy. Time-resolved fluorescence of the sample were measured by time-correlated single-photon counting using a picosecond spectrometer equipped with Glan-Laser polarizers (Newport, Irvine, CA), a Peltier-cooled PMTMCP from Hamamatsu model R3809U-50 (Hamamatsu City, Japan) as the photon detector, and Tennelec-Oxford (Oxford, Abingdon, U.K.) counting electronics. The light pulse was provided by frequency doubling the 200 fs laser pulse of a Mira 900 Ti-Sapphire laser pumped by a Verdi 5 W coherent laser (Santa Clara, CA), and the pulse frequency was reduced to 800 kHz using a Conoptics pulse picker. The fluorescence decays were taken in magic angle (λ_{exc} = 400 nm) and analyzed by a reconvolution procedure with instrument response function (irf) with exponential decay models, and the goodness of the fit was evaluated by the statistical parameters χ^2. Steady-state fluorescence was measured on a Hitachi F-7000 spectrofluorometer (Hitachi Hi-Tech, Mito, Japan) at 25 °C in acetonitrile/water (8:2, v/v).

Computational Methods. The density functional theory (DFT) calculations are as follows. Structures were first optimized by the DFT method with the B3LYP functional and the 6-311G(d) basis set in the gas phase. The absence of imaginary frequencies was used as the criterion to ensure that optimized structures are that of a minimum potential energy surface. Bond dissociation energy (BDE) was calculated by use of the expressions reported by DiLabio et al.[20] All calculations were performed with the software Gaussian 03 code.

RESULTS AND DISCUSSION

Excitation of acetonitrile/water (8:2, v/v) air-saturated solutions of riboflavin with 440 nm light resulted in fluorescence emission with a maximum at 517 nm. This fluorescence was not quenched by any of the fatty acid methyl esters under consideration for reaction with excited-state riboflavin (results not shown). In contrast, cholesterol and ergosterol both reduced the fluorescence intensity accordingly to the Stern–Volmer equation (Figure 1A).

$$I_0/I = 1 + k_q\tau_0[\text{quencher}] \quad (1)$$

The time constant for fluorescence decay was, however, not affected by the presence of cholesterol and ergosterol, as may be seen from Figure 1B, indicating static rather than dynamic quenching of the singlet-excited state of riboflavin by each of the two sterols. The lifetime of the singlet-excited state of riboflavin was found to be $\tau_0 = 4.86 \pm 0.01 \times 10^{-9}$ s at 25 °C in agreement with previous reports for similar conditions in methanol (Figure 1B).[21] The rate constant for singlet-excited quenching was found to have a value of $k_q = 1.11 \pm 0.01 \times 10^{13}$ L mol^{-1} s^{-1} for cholesterol and $k_q = 7.30 \pm 0.01 \times 10^{12}$ L mol^{-1} s^{-1} for ergosterol. The high numerical values rate constants for singlet-excited quenching, well above the diffusion limit, further

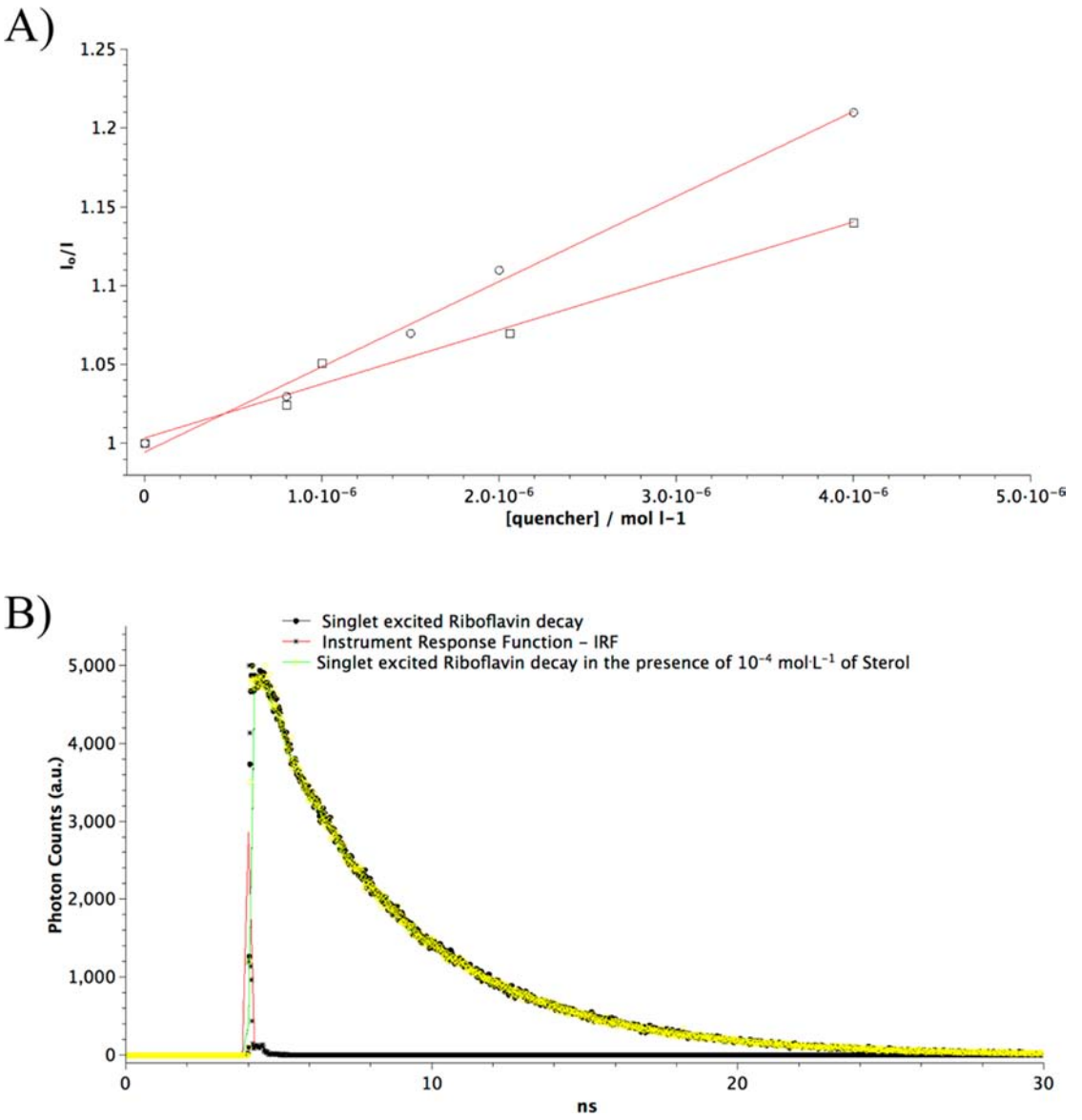


Figure 1. (A) Stern–Volmer plot for fluorescence quenching of singlet-excited riboflavin for increasing the concentration of cholesterol (circle) or ergosterol (square) in acetonitrile/water (8:2, v/v) at 25 °C. [Riboflavin] = 1×10^{-4} mol L^{-1}. (B) Fluorescent decay (λ_{exc} = 400 nm, decay monitored at 517 nm) of singlet-excited riboflavin in acetonitrile/water (8:2, v/v) at 25.0 °C in the absence (black; circle) and presence of 1×10^{-4} L mol^{-1} s^{-1} cholesterol (yellow; ellipse) or ergosterol (black, square). Instrument response function (IRF) (black, star). [Riboflavin] = 1×10^{-5} mol L^{-1}.

confirm a strong static quenching, which could be explained by stacking of the rather planar cholesterol or ergosterol with the likewise planar riboflavin structure in the ground state (compare to the formula in Scheme 1). Such molecular stacking by hydrophobic interaction apparently is not possible for the methyl esters of the unsaturated fatty acids, and fluorescence of riboflavin becomes unaffected by their presence.

The population of the strongly oxidizing triplet-excited state of riboflavin, following excitation of riboflavin with 355 nm light pulses of 8 ns and ~10 mJ/cm^2 under anaerobic conditions, resulted in transient spectral changes (Figure 2A) similar to those previously observed for aqueous solution of riboflavin and characteristic for the formation and decay of triplet-excited riboflavin.[3,7,18,22] The transient absorption spectra T–T for triplet-excited riboflavin, as shown in Figure 2A, are characterized by maxima centered around 310, 380, 550, and 710 nm with a characteristic ^{3}Rib* exponential decay with a rate constant of $k = 5.4 \times 10^4$ s^{-1}, corresponding to a natural lifetime of 13 μs. The decay of ^{3}Rib* in the presence of polyunsaturated fatty acid methyl esters or sterols, as monitored at 710 nm, remained exponential but was accelerated with a first-order rate constant being proportional to the substrate concentration, as seen in Figure 3A for EPA methyl ester and Figure 3B for CLA methyl ester. The observed pseudo-first-order rate constants, extracted from exponential decay traces at 710 nm, were likewise found for each of the other investigated lipids to depend linearly upon the concentration of excess of methyl ester or sterol (between 0.01 and 0.06 mol L^{-1}), confirming a bimolecular reductive quenching of triplet-excited riboflavin in each case. The second-order rate constants (Table 1) were extracted from the slope of the observed linear dependence of the pseudo-first-order rate constant (k_{obs}) as depending upon the quencher concentration according to

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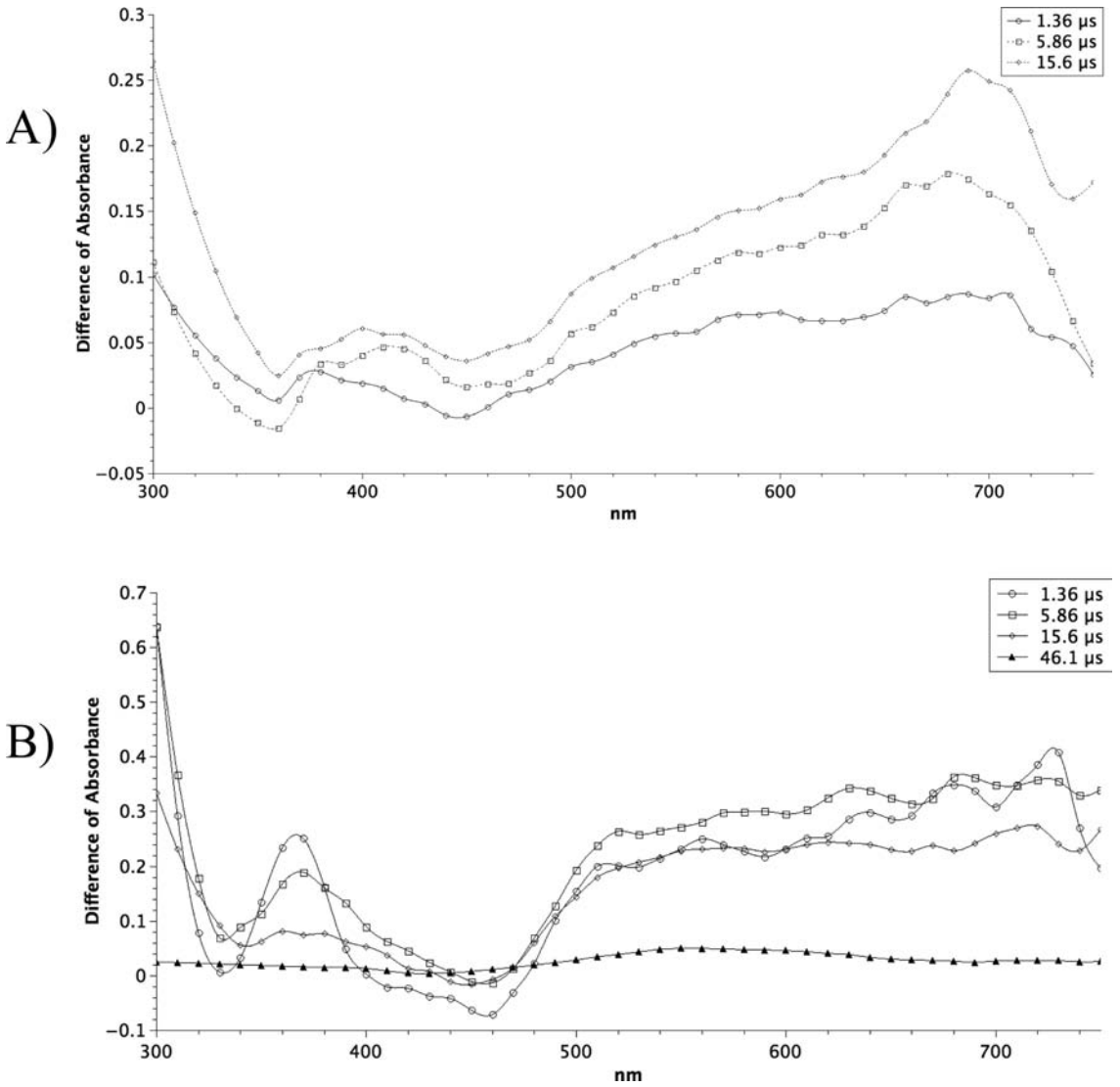


Figure 2. Transient spectra of (A) triplet-excited riboflavin and (B) triplet-excited riboflavin in the presence of cholesterol obtained with different time delays after the 8 ns laser pulse at 355 nm (15 mJ cm^2). [Riboflavin] = 3×10^{-5} mol L^{-1}, and [cholesterol] = 1×10^{-4} mol L^{-1}, in anaerobic acetonitrile/water (8:2, v/v).

$$k_{obs} = k_o + k_2[\text{quencher}] \quad (2)$$

where k_o is the rate constant for the triplet-state self-decay and k_2 is the second-order rate constant for triplet-excited riboflavin deactivation by the quencher, as presented in Table 1.

In the presence of a lipid, the triplet-excited state of riboflavin is quenched simultaneously with an increase of the absorption centered around 560 and 360 nm (Figure 2B). This D−D absorption band has been ascribed to riboflavin neutral radical (RibH•), which is formed by an hydrogen atom transfer (HAT) from the weakest C−H bond of the unsaturated lipid to the triplet-excited riboflavin (^{3}Rib).[18,22] Unfortunately, it is not possible to observe the D−D absorption band for the lipid radical (D−D absorption maxima are at around 280 nm for unsaturated lipid radical and centered around 316 nm for sterols), because this absorption overlaps with the D−D absorption of the neutral radical of riboflavin, however, with a much smaller absorption coefficient.[23]

The presence of the different lipids: COL, ERG, and methyl esters of ARA, EPA, DHA, and CLA result in each case in the same quenching behavior as seen in Figure 3 for EPA and CLA. The DHA methyl ester was found to quench triplet-excited riboflavin more efficiently than both the EPA and ARA methyl esters, as seen from the second-order rate constant reported in Table 1 in comparison to the rate constants previously reported for the methyl esters of linoleic acid and linolenic acid.[18] The value for k_2 for methyl linoleate was previously reported for *tert*-buthanol/water (7:3, v/v) as the solvent[18] but has now in the present study been redetermined for the acetonitrile/water (8:2, v/v) solvent used for the other lipids, resulting in only a minor correction of the value of k_2. The value found for k_2 for the methyl ester of CLA is significantly larger than the value for k_2 for the methyl ester of linoleic acid, a difference which could indicate a shift in the reaction mechanism. A similar difference for k_2 is seen by a comparison of cholesterol to a single double bond with ergosterol with the two conjugated double bonds. Notably, the efficient deactivation of triplet-excited riboflavin by cholesterol and ergosterol compared even to the highly unsaturated lipids points toward a mechanism where the hydrophobic interaction of ground-state riboflavin and the sterol become important for facile quenching upon excitation of riboflavin, because HAT occurs over very short distances, with

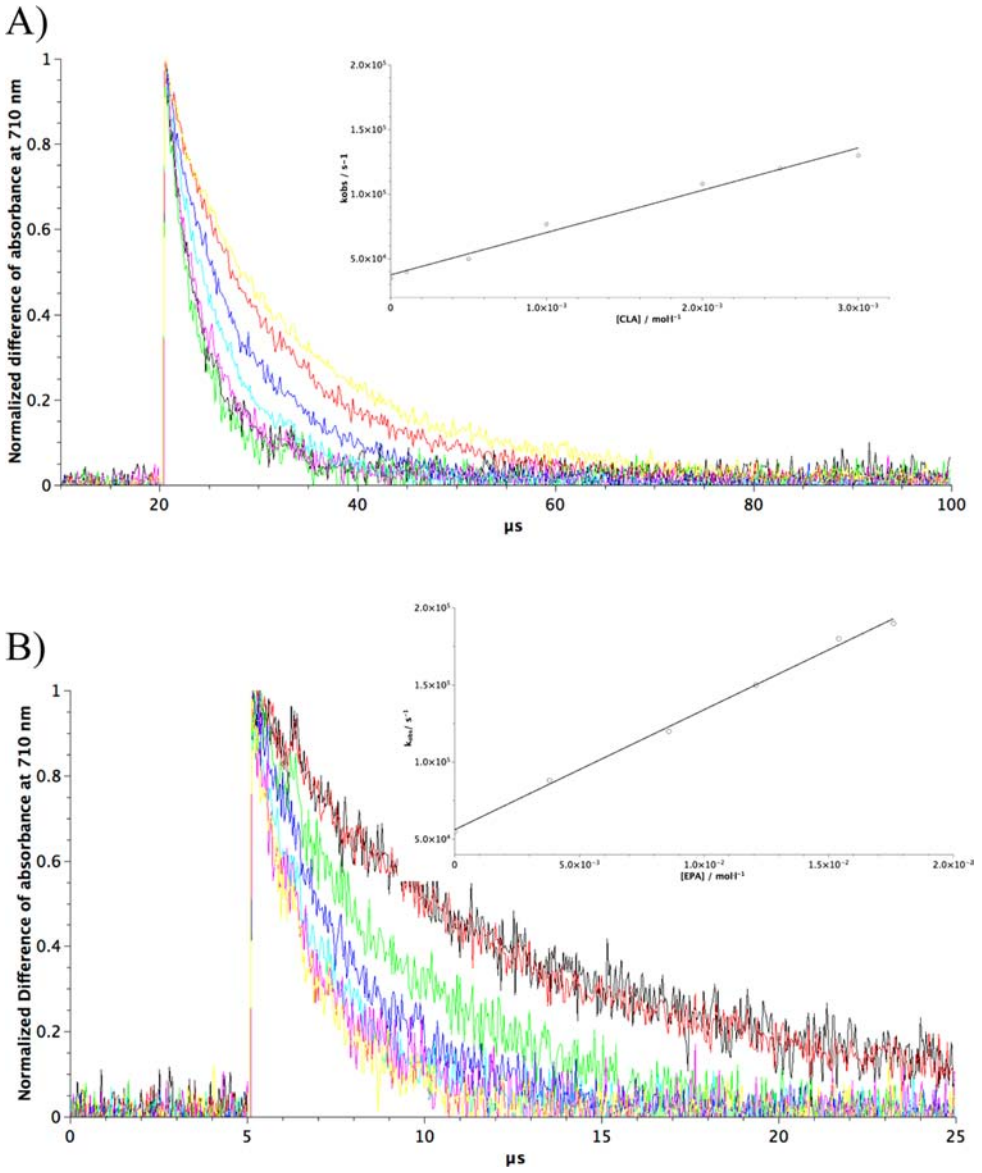


Figure 3. Time traces for decay of triplet-excited riboflavin probed as transient absorption difference at 720 nm, following 8 ns laser pulses of 10 mJ cm^2 at 355 nm for increasing concentrations of (A) EPA methyl ester or (B) CLA methyl ester, in acetonitrile/water (8:2, v/v) solution, under anaerobic conditions at 25.0 ± 0.5 °C. (Insets) Observed pseudo-first-order rate constant as a function of the lipid concentration. The second-order rate constant is calculated from the slope of the linear plot.

Table 1. Bimolecular Rate Constant at 25.0 ± 0.5 °C for Quenching of Triplet-Excited Riboflavin by Unsaturated Fatty Acid Methyl Esters and Sterols in Acetonitrile/Water (8:2, v/v), Theoretical Values (Obtained by DFT) for BDE as Determined for the Weakest C−H Bond in Unsaturated Fatty Acid Methyl Esters and Sterols, and Free-Energy Change Calculated for HAT from Fatty Acid Methyl Ester and Sterols to Triplet-Excited Riboflavin

quencher	number of bis-allylic hydrogens	k (L mol^{-1} s^{-1})	C−H BDE (kJ mol^{-1})	ΔG°_{HAT} (kJ mol^{-1})[a]
methyl oleate	0	no quenching[b]	C_8−H (330.7)[b]	−114.3
cholesterol	0	6.9 ± 0.7 × 10^7	C_7−H (328)[c]	−117
ergosterol	0	6.2 ± 0.1 × 10^8	C_{14}−H (296)[d]	−149
conjugated methyl linoleate (CLA)	0	3.3 ± 0.5 × 10^7	C_8−H (326)[d]	−119
methyl linoleate	1	8.4 ± 0.9 × 10^5 [b]	C_{11}−H (293.8)[b]	−151.2
methyl linolenate	2	3.1 ± 0.4 × 10^6 [b]	C_{11}−H (293.5)[b]	−151.2
methyl arachidonate (ARA)	3	6.2 ± 0.3 × 10^6	C_{10}−H (287.8)	−157.2
methyl eicosapentanoate (EPA)	4	7.9 ± 1.0 × 10^6	C_{10}−H (273)	−161.1
methyl docosahexanoate (DHA)	5	1.2 ± 0.5 × 10^7	C_{12}−H (267.2)	−177.8

[a]Calculated using theoretical values for BDE as determined for the weakest C−H bond in unsaturated lipid and the theoretical value for the HAA of the triplet-excited riboflavin.[18] ΔG°_{HAT} = BDE − HAA. HAA is 446.96 kJ mol^{-1} for riboflavin.[18] [b]From ref 18. [c]From ref 27. [d]From ref 26.

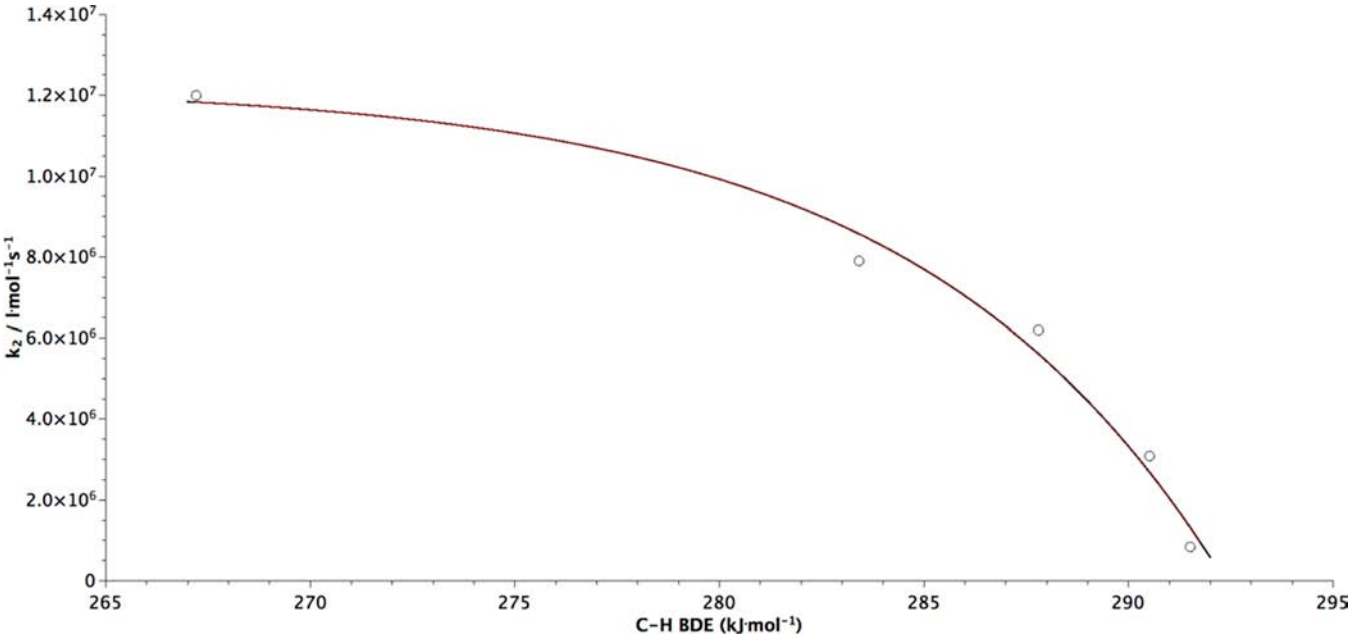


Figure 4. Second-order rate constant for deactivation of triplet-excited riboflavin by methyl esters of the natural non-conjugated unsaturated fatty acids linoleic acid, linolenic acid, ARA, EPA, and DHA versus values for BDE, as determined for the weakest C−H bond. The exponential regression yields a rate constant of 1.2×10^8 L mol^{-1} s^{-1} for BDE approaching zero, which may be considered as a upper limit for the rate of hydrogen atom abstraction from unsaturated fatty acid esters.

the formation of HAT precursor complexes becoming important.[24] In addition to the formation of the ground-state complex, the cyclic structure of sterols also facilitates that reactive hydrogen atoms are becoming spatially well-positioned for abstraction by triplet-excited riboflavin, with the dihedral angles of the carbon atoms at the cyclic structure being close to 90°. Similar effects in reactivity for cyclic structures is also seen for quenching of triplet-excited riboflavin by indene and allylbenzene, with indene being 60 times more reactive, despite that the reported BDE values predict the opposite ordering with 330 and 322 kJ mol^{-1} for BDE of indene and allylbenzene, respectively.[25]

The rate constants for lipid deactivation of triplet-excited riboflavin by unsaturated lipids, including sterols now collected, are important for both formulating functional foods enriched with ω-3 fatty acids and plant sterols and establishment of nutritional recommendations for improved skin and eye health.[3] The rate constants of Table 1 further invite theoretical considerations and generalizations. Because the bond energy of the C−H bond in the methylene group is critical for HAT, bond dissociation energy was calculated using DFT. Because of the complexity of the molecules, calculations for ARA, EPA, and DHA refer to the gas phase, and results for these fatty acids are in Table 1 compared to values for four other unsaturated fatty acids and for cholesterol and ergosterol, for which the results of similar calculations have been previously reported.[26,27] From the BDE values and the hydrogen atom affinity (HAA) for triplet-excited riboflavin, the reaction free energy for HAT from lipids to triplet-excited riboflavin was estimated and found very exergonic for all reactions (Table 1). Upon a comparison of the second-order rate constant for the unsaturated fatty acids collected in Table 1 with the theoretical calculated BDE values, it is evident that weak BDE is associated with fast HAT. For methyl oleate without any methylene hydrogens, the C−H bond is too strong and kinetically unfavorable compared to the natural decay of the triplet-excited state and no reaction is seen. For methyl linoleate, BDE comes below a critical threshold for the methylene hydrogen for reductive quenching by HAT. The rate increases for an increasing number of methylene groups and seems to approach a limiting rate for decreasing BDE, as evident from Figure 4, in which k_2 is considered to depend exponentially upon BDE for the methylene hydrogens. Upon extrapolation to a BDE value of zero, k_2 is expected to reach an upper limit of 1.22×10^7 L mol^{-1} s^{-1} for HAT for an hypothetical fatty acid with infinite unsaturation. It should be noted that the rate constant for hydrogen atom abstraction depends linearly upon the number of bis-allylic methylene groups (Figure 5) rather than the value of BDE, as may be seen by a comparison between Figures 4 and 5. For higher numbers of bis-allylic methylene groups where

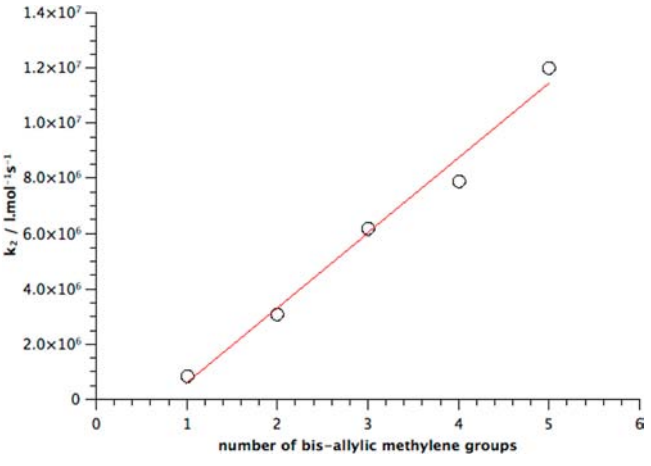


Figure 5. Plot of the second-order rate constant for deactivation of triplet-excited riboflavin by unsaturated fatty acid methyl esters at 25.0 ± 0.5 °C versus the number of oxidizable bis-allylic methylenic groups in the molecule.

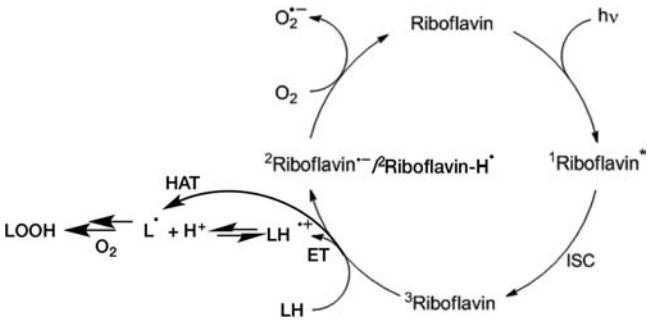
BDE approaches a limiting value, entropic effects related to the number of hydrogen atoms with comparable reactivity become more important.

The methyl ester of CLA quenches triplet-excited riboflavin with a rate similar to this upper limit, despite the absence of bis-allylic methylene groups and a BDE as high as 336 kJ mol^{-1} for the weakest C−H bond, which is comparable to the value for methyl oleate. A shift in the mechanism toward electron transfer (ET) should be considered, especially because 1,3-pentadiene mimicking CLA has an oxidation potential of 1.72 V[28] comparable to the value of triplet-excited riboflavin.

$$^3\text{Rib} + \text{CLA} \rightarrow {}^2\text{Rib}^{\bullet -} + \text{CLA}^{\bullet +} \quad (3)$$

Cholesterol and especially ergosterol also quench triplet-excited riboflavin faster than the estimated upper limit for polyunsaturated fatty acid esters. The ground-state hydrophobic interaction as demonstrated by fluorescence spectroscopy may account for this effect. Notably, ergosterol quenches ^{3}Rib with a rate as high as 6.2×10^8 L mol^{-1} s^{-1}, despite a BDE for the weakest C−H comparable to methyl linoleate, and again, ET from conjugated double bonds to ^{3}Rib should be considered as a parallel or alternative quenching mechanism to HAT. The rate of quenching ^{3}Rib by ergosterol is higher than the rate of ET from proteins, such as bovine serum albumine to ^{3}Rib (2.3×10^8 L mol^{-1} s^{-1}).[18] Unsaturated sterols and to a lesser degree CLA may accordingly, in contrast to carotenoids that do not quench triplet-excited riboflavin, protect proteins and vital eye and skin structures against photosensitized oxidation, otherwise leading to protein polymerization.[3,29] The results obtained herein suggest the mechanism for photooxidation of unsaturated lipids by riboflavin summarized in Scheme 2, resulting in lipid hydroperoxide as the major lipid oxidation product in food and biological systems under low oxygen pressure.

Scheme 2. Proposed Reaction Mechanism for the Photooxidation of Unsaturated Lipids by Riboflavin under Low Oxygen Pressure[a]



[a]HAT and ET denote hydrogen atom transfer and electron transfer mechanisms, respectively.

It is further evident from DFT quantum mechanical calculations that the BDE of the bis-allylic groups in fish oil fatty acid esters is significantly lower than the BDE of the bis-allylic groups in plant oil fatty acid esters, including arachidonic acid,[18] in effect making marine oils more reactive in lipid oxidation chain reactions compared to plant oils during oxidative stress, as may occur in food emulsions with added fish oil. However, conjugated fatty acid esters have been shown to quench triplet-excited riboflavin more efficient than both methyl esters of plant oil or fish oil fatty acids (ω-3 or ω-6) probably because of a shift in the mechanism from HAT to ET. Similar effects may be important for sterols, making plant sterols with conjugated double bonds, such as ergosterol or the pro-vitamin D (7-dehydrocholesterol), important for eye and skin health.[3] Likewise, a high CLA content may be protective against light-induced oxidation for dairy products with added fish oil.

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Notes

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REFERENCES

(1) Heelis, P. F. The photophysical and photochemical properties of flavinas (isoalloxazines). *Chem. Soc. Rev.* **1982**, *11*, 15−39.

(2) Senda, T.; Senda, M.; Kimura, S.; Ishida, T. Redox control of protein conformation in flavoproteins. *Antioxid. Redox Signaling* **2009**, *11*, 1741−1766.

(3) Cardoso, D. R.; Libardi, S. H.; Skibsted, L. H. Riboflavin as a photosensitizer. Effects on human health and food quality. *Food Funct.* **2012**, *3*, 487−502.

(4) Liebmann, J.; Born, M.; Kolb-Bachofen, V. Blue-light irradiation regulates proliferation and differentiation in human skin cells. *J. Invest. Dermatol.* **2010**, *130*, 259−269.

(5) Haywood, R.; Andrady, C.; Kassouf, N.; Sheppard, N. Intensity-dependent direct solar radiation- and UVA-induced radical damage to human skin and DNA, lipids and proteins. *Photochem. Photobiol.* **2011**, *87*, 117−130.

(6) Cho, K. S.; Lee, E. H.; Choi, J. S.; Joo, C. K. Reactive oxygen species-induced apoptosis and necrosis in bovine corneal endothelial cells. *Invest. Ophthalmol. Visual Sci.* **1999**, *40*, 911−919.

(7) Cardoso, D. R.; Franco, D. W.; Olsen, K.; Andersen, M. L.; Skibsted, L. H. Reactivity of bovine whey proteins, peptides, and amino acids toward triplet riboflavin as studied by laser flash photolysis. *J. Agric. Food Chem.* **2004**, *52*, 6602−6606.

(8) Huvaere, K.; Sinnaeve, B.; Van Bocxlaer, J.; Skibsted, L. H. Flavonoid deactivation of excited state flavins: reaction monitoring by mass spectrometry. *J. Agric. Food Chem.* **2012**, *60*, 9261−9272.

(9) Luo, W. C.; Muller, J. G.; Burrows., C. J. The pH-dependent role of superoxide in riboflavin-catalyzed photooxidation of 8-oxo-7,8-dihydroguanosine. *Org. Lett.* **2001**, *3*, 2801−2804.

(10) Nicolaou, A.; Masoodi, M.; Gledhill, K.; Haylett, A. K.; Thody, A. J.; Tobin, D. J.; Rhodes, L. E. The eicosanoid response to high dose UVR exposure of individuals prone and resistant to sunburn. *Photochem. Photobiol. Sci.* **2012**, *11*, 371−380.

(11) Pilkington, S. M.; Watson, R. R. B.; Nicolaou, A.; Rhodes, L. E. ω-3 polyunsaturated fatty acids: Photoprotective macronutrients. *Exp. Dermatol.* **2011**, *20*, 537−543.

(12) Rhodes, L. E.; Ofarrel, S.; Jackson, M. J.; Friedmann, P. S. Dietary fish-oil supplementation in humans reduces UVB-erythermal sensitivity but increases epidermal lipid-peroxidation. *J. Invest. Dermatol.* **1994**, *103*, 151−154.
(13) Black, H. S.; Rhodes., L. E. The potential of ω-3 fatty acids in the prevention of non-melanoma skin cancer. *Cancer Detect. Prev.* **2006**, *30*, 224−232.
(14) Rhodes, L. E.; Shahbakhti, H.; Azurdia, R. M.; Moison, R. M. W.; Steenwinkel, M.; Homburg, M. I.; McArdle, F.; Van Henegouwen, G.; Epe, B.; Vink, A. A. Effect of eicosapentaenoic acid, an ω-3 polyunsaturated fatty acid, on UVR-related cancer risk in humans. An assessment of early genotoxic markers. *Carcinogenesis* **2003**, *24*, 919−925.
(15) SanGiovanni, J. P.; Chew, E. Y.; Agron, E.; Clemons, T. E.; Ferris, F. L.; Gensler, G.; Lindblad, A. S.; Milton, R. C.; Seddon, J. M.; Klein, R.; Sperduto, R. D. The relationship of dietary ω-3 long-chain polyunsaturated fatty acid intake with incident age-related macular degeneration—AREDS report no. 23. *Arch. Ophthalmol.* **2008**, *126*, 1274−1279.
(16) Kalt, W.; Hanneken, A.; Milbury, P.; Tremblay, F. Recent research on polyphenolics in vision and eye health. *J. Agric. Food Chem.* **2010**, *58*, 4001−4007.
(17) Nichols, J. A.; Katiyar, S. K. Skin photoprotection by natural polyphenols: Anti-inflammatory, antioxidant and DNA repair mechanisms. *Arch. Dermatol. Res.* **2010**, *302*, 71−83.
(18) Huvaere, K.; Cardoso, D. R.; Homem-de-Mello, P.; Westermann, S.; Skibsted, L. H. Light-induced oxidation of unsaturated lipids as sensitized by flavins. *J. Phys. Chem. B* **2010**, *114*, 5583−5593.
(19) Garg, M. L.; Wood, L. G.; Singh, H.; Moughan, P. J. Means of delivering recommended levels of long chain ω-3 polyunsaturated fatty acids in human diets. *J. Food Sci.* **2006**, *71*, R66−R71.
(20) DiLabio, G. A.; Pratt, D. A.; LoFaro, A. D.; Wright, J. S. Theoretical study of X−H bond energetics (X = C, N, O, S): Application to substituent effects, gas phase acidities, and redox potentials. *J. Phys. Chem. A* **1999**, *103*, 1653−1661.
(21) Penzkofer, A. Photoluminescence behavior of riboflavin and lumiflavin in liquid solutions and solid films. *Chem. Phys.* **2012**, *400*, 142−153.
(22) Li, H.; Melo, T. B.; Naqvi., K. R. Triplets, radical cations and neutral semiquinone radicals of lumiflavin and riboflavin: An overhaul of previous pump-probe data and new multichannel absolute absorption spectra. *J. Photochem. Photobiol., B* **2012**, *106*, 34−39.
(23) Schoneich, C.; Asmus, K. D.; Dillinger, U.; Vonbruchhausen, F. Thiyl radical attack on poly-unsaturated fatty-acids—A possible route to lipid-peroxidation. *Biochem. Biophys. Res. Commun.* **1989**, *161*, 113−120.
(24) Warren, J. J.; Mayer, J. M. Predicting organic hydrogen atom transfer rate constants using the Marcus cross relation. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 5282−5287.
(25) Huvaere, K.; Skibsted., L. H. Light-induced oxidation of tryptophan and histidine. Reactivity of aromatic N-heterocycles toward triplet-excited flavins. *J. Am. Chem. Soc.* **2009**, *131*, 8049−8060.
(26) Agapito, F.; Nunes, P. M.; Cabral, B. J. C.; Santos, R. M. B.; Simoes, J. A. M. Energetics of the allyl group. *J. Org. Chem.* **2007**, *72*, 8770−8779.
(27) Lengyel, J.; Rimarcik, J.; Vaganek, A.; Fedor, J.; Lukes, V.; Klein, E. Oxidation of sterols: Energetics of C−H and O−H bond cleavage. *Food Chem.* **2012**, *133*, 1435−1440.
(28) Baltes, H.; Steckhan, E.; Schafer, H. J. Anodic-oxidation of organic-compounds. 21. Anodic-oxidation of conjugated dienes. *Chem. Ber./Recl.* **1978**, *111*, 1294−1314.
(29) Davies, M. J.; Truscott, R. J. W. Photo-oxidation of proteins and its role in cataractogenesis. *J. Photochem. Photobiol., B* **2001**, *63*, 114−125.