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Triplet Excited States and Singlet Oxygen Production by Analogs of Red Wine Pyranoanthocyanins

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Abstract

During the maturation of red wines, the anthocyanins of grapes are transformed into pyranoanthocyanins, which possess a pyranoflavylium cation as their basic chromophore. Photophysical properties of the singlet and triplet excited states of a series of synthetic pyranoflavylium cations were determined at room temperature in acetonitrile solution acidified with 0.10 mol dm⁻³ trifluoroacetic acid (TFA, to inhibit competitive excited state proton transfer) and at 77 K in a rigid TFA-acidified isopropanol glass. In solution, the triplet states of these pyranoflavylium cations are efficiently quenched by molecular oxygen, resulting in sensitized formation of singlet oxygen, as confirmed by direct detection of the triplet state decay by laser flash photolysis and of singlet oxygen monomol emission in the near infrared. The strong visible light absorption, the relatively small singlet-triplet energy differences, the excited state redox potentials and the reasonably long lifetimes of pyranoflavylium triplet states in the absence of molecular oxygen suggest that they might be useful as triplet sensitizers and/or as cationic redox initiators in polar aprotic solvents like acetonitrile.

Introduction

As red wines mature, the anthocyanins initially present are transformed into pyranoanthocyanins (Scheme 1) via reaction with yeast metabolic products, colorless copigment molecules or other additives present in the wine (1-6). The pyranoanthocyanin chromophore is largely responsible for the color of aged red wines (1,6) and presumably contributes to the taste (e.g., astringency) (7,8), anti-oxidant capacity (9,10) and health benefits (11) of moderate consumption of red wines.

Scheme 1. Transformation of red wine anthocyanins (such as malvidin O-glycoside) into pyranoanthocyanin derivatives.

Recently we reported (12) the preparation of a series of substituted pyranoflavylium cations that contain the same basic chromophoric unit as pyranoanthocyanins, facilitating systematic studies of substituent and structural effects on the ground and excited state properties of pyranoanthocyanin-type chromophores. Similar to anthocyanins, these synthetic pyranoflavylium cations are weak acids in the ground state (pK_a typically of about 3.5-4) (13). However, the color of pyranoflavylium cations is more stable than that of anthocyanins or their synthetic analogs, 7-hydroxyflavylium cations, due to the presence of the additional pyran ring, which prevents the chemistry that leads to the loss of color of anthocyanins at neutral pH (13-

15). Like anthocyanins and 7-hydroxyflavylium cations (16,17), pyranoflavylium cations become stronger acids in the first singlet excited state, but the photoacidity of pyranoflavylium cations is much less pronounced and the rate of proton transfer to water much slower by nearly two orders of magnitude than those of anthocyanins or 7-hydroxyflavylium cations (13,18). As a result, the excited state lifetimes of pyranoflavylium singlet states are typically much longer than those of analogous flavylium cations.

A particularly intriguing aspect of the photochemistry of anthocyanins and 7-hydroxyflavylium cations is the apparent absence of significant triplet state formation in solution of these compounds (16). To date, no clear evidence for triplet-state formation has been reported at room temperature for synthetic flavylium cations or any of the naturally-occurring anthocyanins such as cyanin or malvidin 3-O-glucoside in aqueous solution or organic solvents such as alcohols or acetonitrile. In aqueous solution, this could be attributed to the dominance of ultrafast proton transfer to water, making intersystem crossing non-competitive. Although the 7-methoxy-4-methylflavylium cation, a compound in which ultrafast proton transfer is blocked, does emit weak phosphorescence at 77 K in a methanol-ethanol glass (16), unequivocal evidence could not be found for triplet state formation at room temperature in dry acetonitrile solution.

In contrast, we report here that the pyranoflavylium cations **1-8** (Scheme 2) not only phosphoresce rather well at 77 K in an isopropanol glass, but also form triplet states in acidic acetonitrile solution that are readily detectable by nanosecond laser photolysis and quenched by molecular oxygen with concomitant formation of singlet oxygen. The strong visible light absorption, the relatively small excited singlet-triplet energy differences, the excited state redox potentials and the reasonably long triplet lifetimes of these pyranoflavylium triplet states in the absence of molecular oxygen

suggest that they might be useful as triplet sensitizers and/or as cationic redox initiators in polar aprotic solvents like acetonitrile.

Scheme 2. Structures of the pyranoflavylium cations.

Materials and Methods

The preparation and purification of the pyranoflavylium chloride salts has been described elsewhere (12). Trifluoroacetic acid (TFA, Sigma-Aldrich, 99%), isopropanol (Merck p.a.) and potassium hydroxide (Sigma-Aldrich, p.a.) were used as received. Acetonitrile (Merck p.a.) was dried by distillation from calcium hydride and methanol (Merck p.a.) by distillation from magnesium turnings.

Absorbance spectra were measured with Varian Cary 50 UV-vis Bio or Cary 100 spectrophotometers, a Hewlett Packard 8452A diode array spectrometer or a Shimadzu UV-1800 Spectrometer at room temperature (ca. 22 °C) in 1.00 cm path length cuvettes. Steady-state fluorescence spectra were determined with a Hitachi F-4500 fluorescence spectrometer. Solutions in dry acetonitrile containing 0.10 mol dm⁻³ TFA (in order to suppress excited state proton transfer) were contained in 1.00 cm path length fluorescence cuvettes at room temperature and spectra were corrected for the detector response; the corresponding fluorescence quantum yields at room temperature were determined using quinine sulfate (Sigma) in 0.10 mol dm⁻³ sulfuric acid (Vetec) as

standard ($\Phi_f = 0.55$) (19). Fluorescence decays were measured in acetonitrile solution containing 0.10 mol dm^{-3} TFA (to suppress ESPT) with picosecond resolution by the time-correlated single-photon counting technique as described in detail elsewhere (20). Basically, the laser excitation pulse (410 nm) and sample fluorescence emissions (498-535 nm) were collected at the magic angle (54.7°) using automatic alternate measurements of pulse and sample (1000 counts in the maximum channel per cycle) until a total of 3000-5000 counts had been collected at the maximum. The instrument response function (IRF), measured at 1.53 ps/channel, was 25 ps (full width at half-maximum, fwhm). Decay times were determined by fits of the decays employing the Sand program (21). The values agreed within experimental error with lifetimes determined by single photon counting with sub-nanosecond time resolution employing an Edinburgh Instruments FLS 980 lifetime spectrometer (Edinburgh EPLED-340 nm diode excitation). Fluorescence lifetimes were obtained from convolution fits of the decay curves employing the Edinburgh Instruments decay analysis software.

Steady-state emission (fluorescence and phosphorescence) spectra were determined at 77 K (liquid nitrogen temperature) with a Hitachi F-4500 fluorescence spectrometer equipped with phosphorescence accessory (quartz Dewar). Solutions of the pyranoflavylium cations in isopropanol with 0.10 mol dm^{-3} TFA, contained in stoppered 5 mm diameter NMR tubes (Sigma-Aldrich) were rapidly cooled to 77 K (liquid nitrogen) to obtain a rigid glass. Phosphorescence lifetimes were determined from single exponential fits of the temporal decay of the phosphorescence intensity.

Laser flash photolysis experiments for detection of the triplet states in fluid solution were performed on a previously described (22) system using a Quanta Ray Lab-130 4 Hz Nd:YAG laser at 355 nm from Spectra Physics as the excitation source. Spectra were measured by averaging 6 individual decays at each wavelength and

lifetime measurements at 680 nm were averaged for 7-10 decays. Absorbance values were averaged between set time windows and the midpoint of the time window is taken to be the time delay. The spectra were constructed point-by-point from two interleaved series of measurements offset by 10 nm starting at the highest wavelengths and using 20 nm wavelength increments. Static cells (7 mm × 7 mm) were used for all measurements. The samples were prepared in acetonitrile (Caledon, HPLC grade) containing 0.10 mol dm⁻³ TFA (Caledon, Reagent grade), both of which were used as received. The samples were purged with N₂ or O₂ for at least 20 min or used without purging for measurements in air-equilibrated solutions. The absorbance of the samples at 355 nm was adjusted to between 0.35 – 0.45. No appreciable changes were observed in the ground-state absorption spectra of the pyranoflavylium cations after the laser flash photolysis experiments, showing that these compounds are extremely photostable.

Singlet oxygen quantum yields [$\Phi_{\Delta}(\text{air})$] were determined using air-equilibrated solutions of the pyranoflavylium cations in dry acetonitrile containing 0.10 mol dm⁻³ TFA. A laboratory version of the TCMPC-1270-LED (SHB Analytics GmbH, Berlin, Germany) was used for direct detection of the singlet oxygen phosphorescence kinetics on samples (0.70 mL; absorbance of ca. 0.1 at 400 nm) contained in 1.00 cm diameter cylindrical quartz cuvettes. A 400 nm LED excitation module (APLED-BL1) was employed to excite the sample with a 160 ns light pulse. The singlet oxygen phosphorescence emission, centered at 1270 nm, is discriminated with singlet oxygen emission filter optics (OM+1270) and the light detected by a specially modified version of the Hamamatsu H10330-45 photomultiplier. The decays were obtained by time-resolved single photon counting with an excitation frequency of 3.125 kHz. The luminescence kinetics were fitted with the standard growth and decay model (23) for singlet oxygen kinetics in homogenous environments (Eq. 1, in which τ_T and τ_{Δ} are the

sensitizer triplet lifetime and the singlet oxygen lifetime, respectively, and A is the pre-exponential factor; in all samples, the much shorter sensitizer triplet lifetime τ_T determined the rise time). The ratio of photons absorbed by the sample and reference was corrected for the finite bandwidth of the excitation LED and for differences in the absorption profile of the sample and reference. Singlet oxygen quantum yields were then determined by comparing the pre-exponential factors of the samples to that of the standard tris(bpy)Ru(II)²⁺, for which $\Phi_{\Delta}(\text{air})$ is 0.57 in air-equilibrated acetonitrile (24). Samples were corrected for possible background luminescence by subtracting out the emission detected through 1200 nm background filter optics (OM+1200). The measurement error of the singlet oxygen quantum yields is estimated to be ± 0.01 in the absolute value of the quantum yield (not taking into account eventual systematic errors of the reference value). In all cases, the lifetime of the singlet oxygen decay was $\tau_{\Delta} = 80 \pm 3 \mu\text{s}$, in agreement with literature lifetimes in acetonitrile without added TFA (25).

$$C(t) = \frac{A}{1 - \frac{\tau_T}{\tau_{\Delta}}} \cdot \left(e^{-\frac{t}{\tau_{\Delta}}} - e^{-\frac{t}{\tau_T}} \right) \quad (1)$$

Results and Discussion

Figure 1 presents the absorption spectra at room temperature and the fluorescence and phosphorescence spectra of the pyranoflavylium cations at 77 K in an isopropanol glass. Table 1 lists the fluorescence quantum yields and lifetimes of the pyranoflavylium cations in acetonitrile containing 0.10 mol dm⁻³ trifluoroacetic acid (TFA) at room temperature, along with the singlet and triplet excited state energies and the triplet lifetimes determined from the emission at 77 K. Although the fluorescence quantum yields and excited state energies tend to be higher and the triplet lifetimes longer for the electron donating substituents than for the more electron-withdrawing

substituents (26), the trend is at best qualitative. The fluorescence rate constants are in the range of $3\text{--}12 \times 10^7 \text{ s}^{-1}$ (Table 1) consistent with a lowest π,π^* excited singlet state. The upper limit for the intersystem crossing rate constants calculated from $(1 - \Phi_f)/\tau_f$ are the order of $10^8\text{--}10^9 \text{ s}^{-1}$, i.e., not inordinately large for an aromatic chromophore or for intersystem crossing between π,π^* states given the rather small $S_1\text{--}T_1$ energy gap.

INSERT Figure 1 HERE

INSERT Table 1 HERE

Nanosecond laser flash photolysis (355 nm laser pulse) of solutions of the pyranoflavylium cations in acetonitrile containing 0.10 mol dm^{-3} TFA showed two main transient signals, i.e., ground state bleaching between about 400 and 500 nm and net absorption starting about 500–520 nm and extending beyond 700 nm (Figure 2 and Figures S1 and S2 of the Supplementary Information) and an isosbestic point at times longer than a few tenths of a microsecond (when interference due to fluorescence no longer affects the signal). The lifetime of the long-wavelength transient, monitored at 680 nm, is strongly dependent on the oxygen concentration, consistent with the identification of this transient as the absorption of the pyranoflavylium triplet state. In N_2 purged solutions, an additional transient could also be observed at about 380 nm. Although we have not yet identified this transient species, it appears to be absent or at least strongly quenched in both air-equilibrated and oxygen saturated solutions and does not interfere with the triplet state decay kinetics at 680 nm. In this context, the remarkable photostability of all of the compounds in both the presence and absence of oxygen is emphasized by the lack of appreciable spectral changes or signs of decomposition after the point-by-point determination of the transient spectra, which

required up to 250 laser shots per sample (Figure S3 of the Supplementary Information), as well as the lack of influence of any of the compounds on the lifetime of singlet oxygen.

INSERT Figure 2 HERE

Table 2 collects the data for the triplet state lifetimes in air-equilibrated solution (τ_{air}) and the values of the second-order rate constants, k_q , for oxygen quenching of the triplet states, together with the singlet oxygen quantum yields measured in air-equilibrated acetonitrile [$\Phi_{\Delta}(\text{air})$] and extrapolated to 100% quenching of the donor triplet state [$\Phi_{\Delta}(\text{max})$] by oxygen using Eq. 2 (24,27):

$$\Phi_{\Delta}(\text{max}) = \Phi_{\Delta}(\text{air})/(\tau_{\text{air}}k_q[\text{O}_2]) = \Phi_{\text{isc}}\phi_{\Delta}^{\circ} \quad (2)$$

where τ_{air} is the triplet lifetime in air equilibrated solutions, Φ_{isc} is the intersystem crossing yield of the donor or sensitizer and ϕ_{Δ}° the intrinsic quantum efficiency of singlet oxygen formation upon quenching the donor triplet state. The values of k_q were determined from the slopes of three-point (O_2 saturated, air-equilibrated and N_2 purged) Stern-Volmer plots (Figure S4 of the Supplementary Information) of the reciprocal of the triplet lifetime ($1/\tau$) vs. the nominal oxygen concentrations in air-equilibrated and O_2 saturated acetonitrile, viz., 1.9 mmol dm^{-3} and 9.1 mmol dm^{-3} , respectively (19). Relative to phenyl, both electron donating and electron withdrawing substituents decrease $\Phi_{\Delta}(\text{max})$ and increase k_q .

Assuming that $\Phi_{\text{isc}} \sim (1 - \Phi_{\text{f}})$, Eq. 2 can be arranged into the form:

$$(1 - \Phi_{\text{f}})/\Phi_{\Delta}(\text{max}) \sim \Phi_{\text{isc}}/\Phi_{\Delta}(\text{max}) = 1/\Phi_{\Delta}^{\circ} = k_q/k_{\Delta} = (k_{\Delta} + k_{\text{d}})/k_{\Delta} \quad (3)$$

where the overall quenching rate constant (k_q) is the sum of the bimolecular rate constants for the pathways that do (k_{Δ}) and do not (k_{d}) result in singlet oxygen

formation. A plot of $(1 - \Phi_f)/\Phi_{\Delta}(\text{max})$ vs. k_q (Figure 3) exhibits a particularly interesting behavior. For compounds **1**, **2**, **4** and **5** with the lowest quenching efficiencies, $(1 - \Phi_f)/\Phi_{\Delta}(\text{max})$ approaches a limiting value of ca. 4, or a maximum singlet oxygen yield of $\Phi_{\Delta}^0 \sim 0.25$. However, for compounds **3** and **6-8**, $(1 - \Phi_f)/\Phi_{\Delta}(\text{max})$ increases almost linearly with increasing k_q , indicating that k_d is bimodal, with one component that is relatively structure insensitive and another that is responsible for an increase in k_q . In general, k_q is found to correlate with the oxidation potential of the sensitizer, as expected for charge transfer quenching or electron transfer from the sensitizer to molecular oxygen (24,27-28). In the present case, however, the sensitizer is cationic and electron-withdrawing substituents (26) such as nitro or cyano, which should increase the oxidation potential, actually increase k_q much more than electron-donating substituents (26) such as methyl or methoxy. This is contrary to the trend for quenching of singlet oxygen by anthocyanins, where an increase in the number of electron-donating groups in the B-ring increases k_q (29-30). It is, however, consistent with a chemical quenching pathway rather than a pathway involving charge transfer.

INSERT Table 2 HERE

INSERT Figure 3 HERE

As pointed out in the Introduction, these synthetic pyranoflavylium cations contain the same basic chromophore as the pyranoanthocyanins that are formed in red wine during its maturation. Thus, could the natural pyranoanthocyanins in red wine also form triplet states upon exposure to ambient light and, if left open to air, potentially generate singlet oxygen? In this context, the photophysics of these synthetic pyranoflavylium cations is quite different in aqueous media from that in acidic acetonitrile. In aqueous media, very fast adiabatic excited state proton transfer (ESPT)

efficiently deactivates the singlet excited state of these pyranoflavylium cations (13,18), to the detriment of intersystem crossing. Moreover, compared to compounds **1-8**, the pyranoanthocyanins derived from malvidin (Scheme 1) have multiple additional electron-donating substituents that enhance the charge-transfer character of the lowest energy excited state and hence modify its photophysics. Thus, even in acetonitrile solution, the more electron-rich trimethoxyphenyl pyranoflavylium cation **9** exhibits a very short fluorescence lifetime (41 ps at 650 nm with 410 nm excitation), laser flash photolysis showed no transients attributable to triplet state formation and there was no detectable sensitized singlet oxygen formation. Red wines also contain a number of antioxidants and anthocyanins themselves have been shown to quench singlet oxygen (29-30). Taken together, these considerations argue against the extrapolation of the current photophysical results for compounds **1-8** to red wine pyranoanthocyanins or the postulation of sensitized singlet oxygen production as a significant contributor to the oxidation of wine (31).

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References

1. Brouillard, R., S. Chassaing, A. Fougerousse (2003) Why are grape/fresh wine anthocyanins so simple and why is it that red wine color lasts so long? *Phytochemistry* 64, 1179-1186.
2. Schwarz, M., T. C. Wabnitz, P. Winterhalter (2003) Pathway Leading to the Formation of Anthocyanin-Vinylphenol Adducts and Related Pigments in Red Wines. *J. Agric. Food Chem.* 51, 3682-3687.
3. Fulcrand, H., M. Dueñas, E. Salas, V. Cheynier (2006) Phenolic Reactions during Winemaking and Aging. *Am. J. Enol. Vitic.* 57, 289-297.
4. Marquez, A., M. P. Serratos, J. Merida (2013) Pyranoanthocyanin Derived Pigments in Wine: Structure and Formation during Winemaking. *J. Chem.* (Hindawi), Article ID 713028, 15 pp; DOI: 10.1155/2013/713028.
5. Oliveira, J., N. Mateus, V. Freitas (2014) Previous and recent advances in pyranoanthocyanins equilibria in aqueous solution. *Dyes Pigments* 100, 190-200.
6. Figueiredo-Gonzalez, M., B. Cancho-Grande, J. Simal-Gandara, N. Teixeira, N. Mateus, V. Freitas (2014) The phenolic chemistry and spectrochemistry of red sweet wine-making and oak-aging. *Food Chem.* 152, 522-530.
7. Quaglieri, C., G. Iachetti, M. Jourdes, P. Waffo-Teguo, P. L. Teissedr (2016) Are pyranoanthocyanins involved in sensory effect in red wines? *BIOWeb of Conferences* (39th World Congress of Vine and Wine) 7, 02007, 5 p.; DOI: 10.1051/bioconf/20160702007.

8. Garcia-Estevez, I., L. Cruz, J. Oliveira, N. Mateus, V. Freitas, S. Soares (2017) First evidences of interaction between pyranoanthocyanins and salivary proline-rich proteins. *Food Chem.* 228, 574-581.
9. Azevedo, J., J. Oliveira, L. Cruz, N. Teixeira, N. F. Bras, V. Freitas, N. Mateus (2014) Antioxidant features of red wine pyranoanthocyanins: Experimental and theoretical approaches. *J. Agr. Food Chem.* 62, 7002–7009.
10. Tománková, E., J. Balík, I. Soral, P. Bednár, B. Papoušková (2016) Colour and Antioxidant Properties of Malvidin-3-glucoside and Vitisin A. *Acta Alimentaria* 45, 85-92.
11. Oliveira, H., N. Wu, Q. Zhang, J. Wang, J. Oliveira, V. Freitas, N. Mateus, J. He, I. Fernandes (2016) Bioavailability studies and anticancer properties of malvidin based anthocyanins, pyranoanthocyanins and non-oxonium derivatives. *Food Funct.* 7, 2462- 2468.
12. Silva, C. P., R. M. Pioli, L. Liu, S. Zheng, M. Zhang, G. T. M. Silva, V. M. T. Carneiro, F. H. Quina (2018) An Improved Synthesis of Analogs of Red Wine Pyranoanthocyanin Pigments. *ACS Omega* 3, 954-960.
13. Freitas , A. A., C. P. Silva, G. T. M. Silva, A. L. Maçanita, F. H. Quina (2018) Ground and Excited State Acidity of Analogs of Red Wine Pyranoanthocyanins. *Photochem. Photobiol.* <https://doi.org/10.1111/php.12944>
14. Oliveira, J., N. Mateus, V. Freitas (2017) Wine-Inspired Chemistry: Anthocyanin Transformations for a Portfolio of Natural Colors. *Synlett.* 28, 898-906.

15. Souza, A., L. Cabrita, P. Araújo, N. Mateus, F. Pina, V. Freitas (2014) Color stability and spectroscopic properties of deoxyvitisins in aqueous solution. *New J. Chem.* 38, 539-544.
16. Quina, F. H., P. F. Moreira, C. Vautier-Giongo, D. Rettori, R. E. Rodrigues, A. A. Freitas, P. F. Silva, A. L. Maçanita (2009) Photochemistry of anthocyanins and their biological role in plant tissues. *Pure Appl. Chem.* 81, 1687-1694.
17. Silva, V. O., A. Freitas, A. L. Maçanita, F. H. Quina (2016) Chemistry and photochemistry of natural plant pigments: The anthocyanins. *J. Phys. Org. Chem.* 29, 594-599.
18. Freitas, A. A., C. Pacheco Silva, G. T. M. Silva, A. L. Maçanita, F. H. Quina (2017) From Vine to Wine: Photophysics of a Pyranoflavylum Analog of Red Wine Pyranoanthocyanins. *Pure Appl. Chem.* 89, 1761–1767.
19. Montalti, M., A. Credi, L. Prodi, M. T. Gandolfi (2006) *Handbook of Photochemistry*, 3rd Ed., CRC Press, Boca Raton, FL.
20. Freitas, A. A., F. H. Quina, A. L. Maçanita (2014) Femtosecond and Temperature-Dependent Picosecond Dynamics of Ultrafast Excited-State Proton Transfer in Water–Dioxane Mixtures. *J. Phys. Chem. A* 118, 10448–10455.
21. Striker, G.; V. Subramaniam, C. A. M. Seidel, A. J. Volkmer (1999) Photochromicity and Fluorescence Lifetimes of Green Fluorescent Protein. *J. Phys. Chem. B* 103, 8612-8617.
22. Liao, Y., C. Bohne (1996) Alcohol Effect on Equilibrium Constants and Dissociation Dynamics of Xanthone–Cyclodextrin Complexes. *J. Phys. Chem.* 100, 734–743.

23. Pfitzner, M., J. C. Schlothauer, E. Bastien, S. Hackbarth, L. Bezdetnaya, H.-P. Lassalle, B. Röder (2016) Prospects of *in vivo* singlet oxygen luminescence monitoring: Kinetics at different locations on living mice. *Photodiagnosis Photodyn. Ther.* 14, 204–210.
24. Abdel-Shafi, A. A., D. R. Worrall, A. Y. Ershov (2004) Photosensitized generation of singlet oxygen from ruthenium(II) and osmium(II) bipyridyl complexes. *Dalton Trans.* 2004, 30-36.
25. Bregnhøj, M., M. Westberg, F. Jensen, P. R. Ogilby (2016) Solvent-dependent singlet oxygen lifetimes: temperature effects implicate tunneling and charge-transfer interactions. *Phys.Chem.Chem.Phys.* 18, 22946-22961.
26. Hansch, C., A. Leo, R. W. Taft (1991) A Survey of Hammett Substituent Constants and Resonance and Field Parameters. *Chem. Rev.* 91, 165-195.
27. Fresnadillo, D. G., S. Lacombe (2016) Reference photosensitizers for the production of singlet oxygen. In: *Singlet Oxygen: Applications in Biosciences and Nanosciences*, Vol. 1. S. Nonell, C. Flors, Eds., Royal Society of Chemistry, Cambridge, UK; pp. 105-143.
28. Schmidt, R. (2006) Photosensitized generation of singlet oxygen. *Photochem. Photobiol.* 82, 1161-1177.
29. de Rosso, V. V., F. E. M. Vieyra, A. Z. Mercadante, C. D. Borsarelli (2008) Singlet oxygen quenching by anthocyanin's flavylium cations. *Free Radical Res.* 42, 885-891.
30. Nagaoka, S.-I., Y. Bandoh, U. Nagashima, K. Ohara (2017) Correlation among singlet-oxygen quenching, free-radical scavenging, and excited-state intramolecular-proton-transfer activities in hydroxyflavones, anthocyanidins and 1-hydroxyanthroquinones. *J. Phys. Chem. A.* 121, 8069-8079.

31. Oliveira, C. M., A. C. S. Ferreira, V. De Freitas, A. M. S. Silva (2011) Oxidation mechanisms occurring in wine. Food Res. Internat. 44, 1115-1126.

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Figure 1. Normalized absorption (black) in acetonitrile containing 0.10 mol dm^{-3} TFA at room temperature and total emission (red) and phosphorescence (blue) emission at 77 K in isopropanol containing 0.10 mol dm^{-3} TFA of (a)-(h) compounds **1-8**.

Figure 2. Transient absorption spectra (left) and corresponding kinetics (right) at 680 nm for Compound **1** in: (a, b) N₂ purged, (c, d) air-equilibrated and (e, f) O₂ saturated acetonitrile containing 0.10 mol dm⁻³ TFA.

Figure 3. Bimodal relationship between $(1 - \Phi_f)/\Phi_{\Delta}(\max)$ and the rate constant for bimolecular quenching of the pyranoflavylium triplet state by molecular oxygen (Eq. 3).

Table 1. Quantum yield (Φ_f), lifetime (τ_f) and rate constant (k_f) for fluorescence of the pyranoflavylium cations in acetonitrile containing 0.10 mol dm⁻³ TFA, energies of the singlet (E_S) and triplet (E_T) excited states and the phosphorescence lifetime at 77 K in isopropanol containing 0.10 mol dm⁻³ TFA.

Compound	Substituent	$\Phi_f^{a,b}$	τ_f , ns ^{a,b}	$10^{-7}k_f$, s ⁻¹	E_S , eV ^c	E_T , eV ^c	ΔE_{ST} , eV	τ_T , s ^{a,c}
1	p-MeO-phenyl	0.43 (0.41) ^d	3.45	12	2.51	2.21	0.30	1.42
2	p-CH ₃ -phenyl	0.17	4.05	4.2	2.51	2.23	0.28	0.64
3	2-Naphthyl	0.13	2.92	4.5	2.48	2.15	0.33	0.93
4	Phenyl	0.16	3.55	4.5	2.51	2.24	0.27	0.95
5	p-F-phenyl	0.12	3.60	3.3	2.52	2.23	0.29	1.25
6	m-Pyridyl	0.023	0.62 (66%) 2.36 (34%)	(3.7)	2.46	2.22	0.24	0.83
7	p-CN-phenyl	0.048	1.56	3.1	2.45	2.18	0.27	0.49
8	p-NO ₂ -phenyl	0.030	1.1	2.7	2.41	2.17	0.24	0.64

^a $\pm 10\%$;

^b at 20 °C in air-equilibrated acetonitrile containing 0.10 mol dm⁻³ TFA.

^c at 77 K in an isopropanol glass containing 0.10 mol dm⁻³ TFA.

^d Ref. 18

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Table 2. Pyranoflavylium cation triplet state properties in air-equilibrated acetonitrile containing 0.10 mol dm⁻³ TFA; triplet lifetime (τ_{air}), bimolecular rate constants for quenching of the triplet state by molecular oxygen (k_q) and the singlet oxygen formation quantum yields (Φ_{Δ}) in air-equilibrated solution and extrapolated to 100% quenching of the donor triplet state.

Compound	Substituent	τ_{air} , ns ^a	$10^{-9}k_q^b$ dm ³ mol ⁻¹ s ⁻¹	$\Phi_{\Delta}(\text{air})^c$	$\Phi_{\Delta}(\text{max})^d$
1	p-MeO-phenyl	570	0.85	0.12	0.13
2	p-CH ₃ -phenyl	620	0.70	0.16	0.19
3	2-Naphthyl	440	1.16	0.05	0.05
4	Phenyl	560	0.57	0.13	0.21
5	p-F-phenyl	560	0.69	0.13	0.18
6	m-Pyridyl	330	1.33	0.03	0.04
7	p-CN-phenyl	350	1.02	0.06	0.09
8	p-NO ₂ -phenyl	330	1.09	0.04	0.06

^a In air-equilibrated acetonitrile containing 0.10 mol dm⁻³ TFA; [O₂] = 0.0019 mol dm⁻³. From decay of the transient signal at 680 nm, lifetimes $\pm 5\%$.

^b From the slope of three-point plots of $1/\tau$ vs. [O₂]; ± 5 -10% (Figure S3 of the Supplementary Information).

^c Quantum yield of singlet oxygen in air-equilibrated acetonitrile containing 0.10 mol dm⁻³ TFA; ± 0.01 .

^d Quantum yield of singlet oxygen extrapolated to 100% donor triplet quenching, Eq. 2.