

Reactions of Methylated Quercetin Derivatives with Singlet Molecular Oxygen

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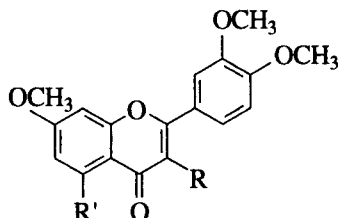
Abstract—The rate constants of the reactions of $^1\text{O}_2$ with 3,5,7,3',4'-pentamethoxy-, 3-hydroxy-5,7,3',4'-tetramethoxy-, and 5-hydroxy-3,7,3',4'-tetramethoxyflavones in benzene were measured. The methylation of quercetin was found to result in an increase in the rate due to the enhancement of the electron-donating activity of the molecules.

INTRODUCTION

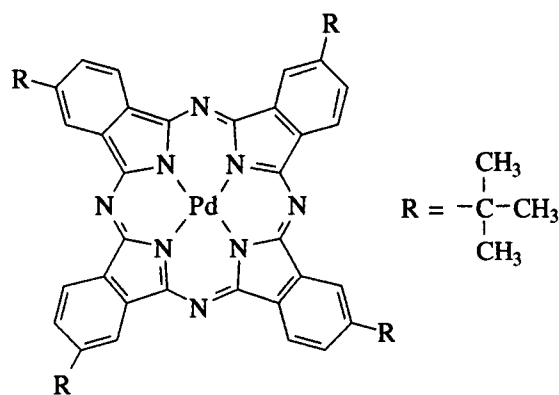
This paper continues the series devoted to the catalytic and photochemical properties of a biologically important class of compounds, flavonoids [1–3]. It reports the study of the reactions of methylated quercetin derivatives with singlet molecular oxygen ($^1\text{O}_2$, $^1\Delta_g$).

EXPERIMENTAL

3,5,7,3',4'-Pentamethoxyflavone (PMOF), 3-hydroxy-5,7,3',4'-tetramethoxyflavone (3-OH-TMOF), 5-hydroxy-3,7,3',4'-tetramethoxyflavone (5-OH-TMOF), anthracene (analytical grade, Reakhim), tetracene, and Pd-tetra(4-*tert*-butyl)phthalocyanine were used. Flavones were prepared by the methylation of quercetin (Fluck) or rutin (Shchelkovo Plant of Vitamins, Moscow oblast, Russia) using dimethyl sulfate in acetone and isolated according to the known procedure [4]. Purity of the compounds was estimated from UV spectra recorded in ethanol (Table 1). Spectral parameters correspond to the published data [4, 5]. The data from Table 1 were a source for calculating the concentrations of flavones in solutions from the Lambert–Bouguer–Beer law. Pd-tetra(4-*tert*-butyl)phthalocyanine (PdT(4-*t*-b)Phc) was synthesized by the Linsted method [6] from the corresponding 4-*tert*-butylphthalodinitrile and PdCl_2 in the presence of sodium acetate. The product was dissolved in chloroform and purified by column chromatography on silica gel (using chloroform as an eluent) and then concentrated and precipitated with methanol. The metallophthalocyanine synthesized was characterized by long-wave absorption in benzene at 662 nm ($\epsilon = 1.8 \times 10^5 \text{ mol}^{-1} \text{ l cm}^{-1}$).



PMOF: $\text{R} = \text{R}' = \text{OCH}_3$; 3-OH-TMOF: $\text{R} = \text{OH}$; $\text{R}' = \text{OCH}_3$; 5-OH-TMOF: $\text{R} = \text{OCH}_3$; $\text{R}' = \text{OH}$



PdT(4-*t*-b)Phc

The overall rate constant of deactivation of $^1\text{O}_2$ was determined by quenching the $^1\text{O}_2$ luminescence; measurements were carried out on an LIF-200 pulse laser fluorimeter [7]. Figure 1 shows the schematic for a setup. An IGT-50 laser served as a source of the excitation light. This laser worked on molecular nitrogen and generated light pulses with a wavelength of 337.1 nm. The time of pulse damping depended on nitrogen purity. It was 1.5 ns for the gas obtained by the evaporation of liquid nitrogen produced on a ZIF-1002 setup. The frequency of pulses was maintained at a level of 25 Hz. Light pulses passed through an OSP-200 optical system where the beam split into two beams. One of them was directed to the sample, and the other

Table 1. Parameters of the long-wave region of UV spectra of flavone solutions

Compound	λ_{max} , nm (ϵ , $\text{mol}^{-1} \text{ l cm}^{-1}$)	
	in ethanol	in benzene
PMOF	340 (21 900)	330 (19 600)
3-OH-TMOF	366 (20 300)	356 (22 000)
5-OH-TMOF	352 (21 900)	351 (19 200)

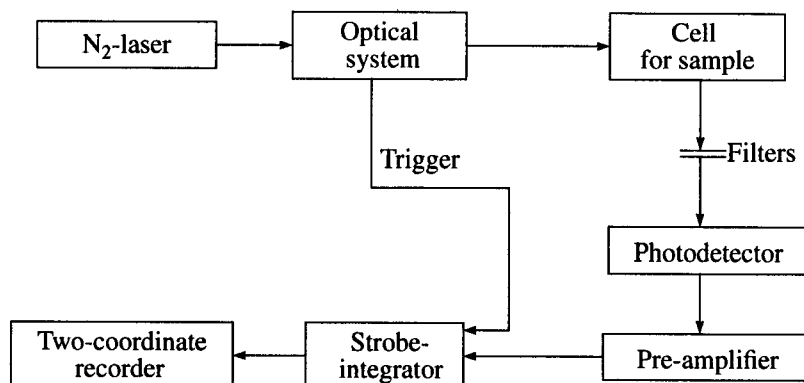


Fig. 1. Schematic of the setup for measuring the time-resolved photosensitized luminescence of singlet molecular oxygen.

was used in the system of the switching-on (trigger) of a BCI-280 strobe-integrator. The light absorbed by a sensitizer (PMOF or anthracene) caused the formation of $^1\text{O}_2$, whose deactivation was accompanied by luminescence in the near-IR spectral region (the main emission band at 1270 nm). The luminescence was selected by an IKS-7 glass light filter with a short-wave transmission cut off at 950 nm and detected at an angle of 90° to the excitation light. A FD-10G germanium photodiode served as a receiver. A signal was enhanced by an operation amplifier and fed to the inlet of the strobe-integrator from which it came to an XY Endim 620.02 two-coordinate recorder. The drift of the system (determined by the photoreceiver and a preamplifier) was estimated from the time of increasing the signal from 10 to 90% of the maximum value upon irradiating the photoreceiver with short photopulses. This increasing period was equal to 1.6 μs .

The rate constant of the chemical process was determined in a comparative study of the Pd-T(4-t-b)Phc-photosensitized oxidation of flavones and tetracene. The latter was an acceptor of $^1\text{O}_2$ with a known value of the reaction rate constant. The concentrations of tetracene, flavones, and sensitizer were 1.3×10^{-4} , 4×10^{-5} , and 2×10^{-5} mol/l, respectively. Samples were irradiated in a quartz cell 0.5-cm thick through a KS-15 glass light filter with a short-wave transmission cut off at 640 nm, which made it possible to selectively excite the sensitizer. An OBS-1 illuminator with a KGM-9-70 lamp (70 W) was used as a light source. The oxidation was monitored by a decrease in the extinction at the absorption maxima of $^1\text{O}_2$ acceptors. Table 1 lists the maxima of absorption bands for flavones. The oxidation of tetracene was measured at 476 nm.

UV spectra were recorded on a Specord-M400 spectrophotometer. Kinetic measurements were performed on an SF-46 single-beam spectrophotometer.

Spectroscopic studies were carried out in benzene- h_6 (for-UV-spectroscopy grade, Chemapol) and benzene- d_6 (Izotop) at 290 K.

RESULTS AND DISCUSSION

Flavones can be classified into two groups [2, 3, 8] depending on their ability to sensitize the formation of $^1\text{O}_2$ in the light. For example, PMOF is a typical sensitizer [2]. The photoexcitation of molecules of this compound in solutions saturated with the air oxygen results in the formation of $^1\text{O}_2$ in the $^1\Delta_g$ state, whose deactivation is accompanied by luminescence in the near-IR spectral region.

The time dependence of the luminescence intensity is exponential and approximated in the coordinates of $\ln I$ vs. t by a straight line. The slope is a measure of the rate constant (k) for damping. The value of k was $(3.6 \pm 0.4) \times 10^4 \text{ s}^{-1}$ in benzene- h_6 . It remained constant at PMOF concentrations of $\leq 3 \times 10^{-4}$ mol/l. The k value is an apparent value determined by three processes: monomolecular radiative (k_r) and two bimolecular processes due to the interaction with the solvent (k_{solv}) and PMOF (k_q):

$$k = k_r + k_{\text{solv}}[\text{Solv}] + k_q[\text{PMOF}]. \quad (1)$$

The radiative rate constant in organic solvents is small [9, 10]. The independence of k on the concentration shows that the interaction with PMOF can also be neglected under these conditions. Therefore, the interaction with the solvent is the main cause for $^1\text{O}_2$ deactivation. Based on these data, we may assume that the quenching rate constant of $^1\text{O}_2$ with flavone does not exceed a ceiling determined from the condition $k_q[\text{PMOF}] \ll 3.6 \times 10^4 \text{ s}^{-1}$, which suggests that $k_q \ll 10^8 \text{ mol}^{-1} \text{ l s}^{-1}$.

The constraint associated with the use of benzene- h_6 is removed when the process is performed in a deuterated solvent [7] because C-D bond vibrations are less significant in the deactivation of $^1\text{O}_2$ than C-H bond vibrations [11]. The k value linearly increases in benzene- d_6 with an increase in the PMOF concentration (Fig. 2) as described by the Stern-Volmer equation

$$k = k^0 + k_q[\text{PMOF}], \quad (2)$$

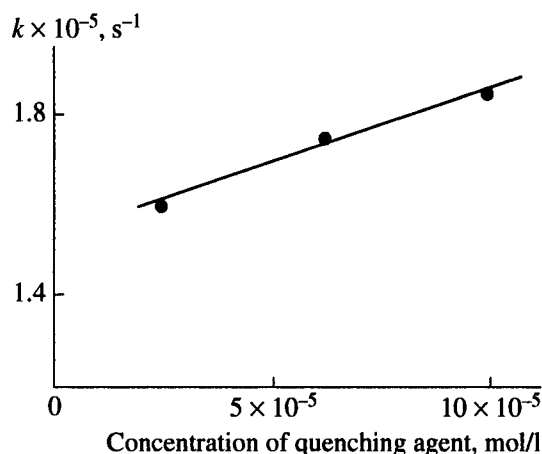


Fig. 2. Rate constant of quenching of $^1\text{O}_2$ luminescence in benzene- d_6 as a function of the concentration of 3,5,7,3',4'-pentamethoxyflavone.

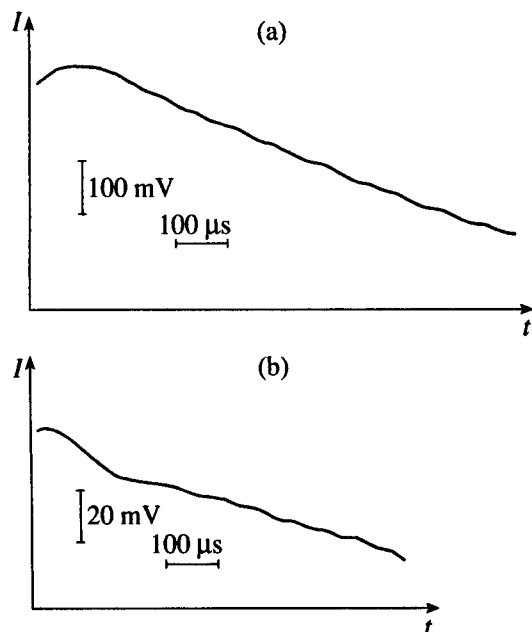


Fig. 3. Decay kinetics of the anthracene-photosensitized ($C = 3.4 \times 10^{-4}$ mol/l) luminescence of $^1\text{O}_2$ in the (a) absence and (b) presence of 3-OH-TMOF (1.0×10^{-4} mol/l) in benzene- d_6 .

where k^0 is the rate constant of luminescence decay at $[\text{PMOF}] \rightarrow 0$. The slope is equal to $k_q = (3.5 \pm 0.5) \times 10^6 \text{ mol}^{-1} \text{ l s}^{-1}$.

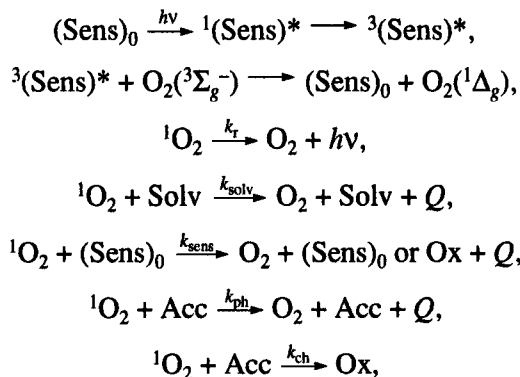
Unlike PMOF, 3-OH- and 5-OH-TMOF do not sensitize the formation of $^1\text{O}_2$ [3, 7]. Therefore, the k_q value for these compounds was determined from the data on the quenching of the anthracene-sensitized luminescence of $^1\text{O}_2$. Due to a strong overlap in the UV spectra of the sensitizer and flavones in the excitation region, the measurement of k over a wide range of flavone concentrations was difficult. Nevertheless, we succeeded in achieving the effect of luminescence quenching in the

presence of flavones at 1×10^{-4} mol/l. Figure 3 shows the experimental results for 3-OH-TMOF. The k value found for a solution containing anthracene (3×10^{-4} mol/l) is $(1.4 \pm 0.1) \times 10^3 \text{ s}^{-1}$. In the presence of 3-OH- and 5-OH-TMOF, $k = (1.7 \pm 0.2) \times 10^3$ and $(1.6 \pm 0.2) \times 10^3 \text{ s}^{-1}$, respectively. Assuming the linearity of the concentration dependence of k , the k_q value was estimated from equation (2). Table 2 presents the results. As can be seen from the data, the k_q constants are close for the compounds under study.

According to [8], the overall rate constant of $^1\text{O}_2$ deactivation by flavones can be described by the sum of the rate constants of physical (k_{ph}) and chemical (k_{ch}) processes:

$$k_q = k_{ph} + k_{ch}. \quad (3)$$

They can be separated in an experiment to find the k_{ch} value. For this purpose, we used the method of comparing the rates of the sensitized photooxidation of flavones and tetracene in benzene- h_6 , taking into account the fact that for tetracene, $k_{ch} = 6 \times 10^6 \text{ mol}^{-1} \text{ l s}^{-1}$ [12]. We suggested that the photosensitized oxidation in solutions involves $^1\text{O}_2$ and includes the following elementary steps [1, 3]:



where $(\text{Sens})_0$, ${}^1(\text{Sens})^*$, and ${}^3(\text{Sens})^*$ designate the sensitizer in the ground and excited states, respectively (in this case, this is Pd-T(4-t-b)Phc); Acc is the acceptor (flavone or tetracene); Ox stands for the oxidation products; Q is the energy of electron excitation dissipated as heat; and k_t , k_{solv} , k_{sens} , k_{ph} , and k_{ch} are the rate constants of the corresponding processes.

The equation of the steady-state oxidation rate takes the form

$$\frac{d[\text{Acc}]}{dt} = \frac{k_{ch, \text{Acc}} \gamma_{\Delta} \tau_{\Delta} I_0 (1 - 10^{-A}) [\text{Acc}]}{1 + \{k_{\text{sens}}[\text{Sens}] + (k_{ph} + k_{ch})[\text{Acc}]\} \tau_{\Delta}}. \quad (4)$$

Here $\tau_{\Delta} = 1/(k_t + k_{\text{solv}}[\text{Solv}])$ is the lifetime of $^1\text{O}_2$ in the solvent, γ_{Δ} is the quantum yield of $^1\text{O}_2$, I_0 is the intensity of the excitation light, and A is the extinction of the solution at the excitation wavelength. The k_{sens} and $(k_{ph} + k_{ch})$ values for tetracene and τ_{Δ} obtained from the results of direct experiments are equal to $(2.6 \pm 0.2) \times 10^7 \text{ mol}^{-1} \text{ l s}^{-1}$, $(1.2 \pm 0.1) \times 10^7 \text{ mol}^{-1} \text{ l s}^{-1}$, and $(29 \pm 2) \times 10^{-6} \text{ s}$, respectively. These data suggest that the denom-

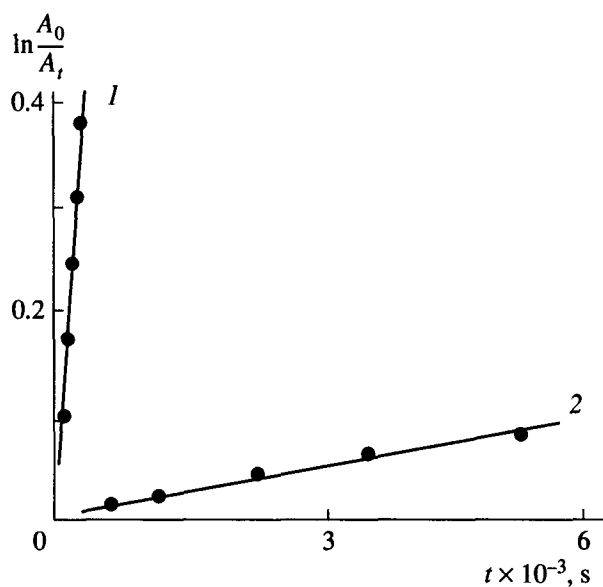


Fig. 4. Kinetic dependences for the photosensitized oxidation of (1) tetracene and (2) PMOF in benzene- h_6 .

inator is close to 1 in the regions of the studied concentrations of the sensitizer of ($\leq 1 \times 10^{-5}$ mol/l) and acceptors of ($\leq 1 \times 10^{-4}$ mol/l). Taking into account this approximation, equation (4) becomes

$$-\frac{d[\text{Acc}]}{dt} = k_{\text{app}}[\text{Acc}], \quad (5)$$

where $k_{\text{app}} = k_{\text{ch, Acc}} \gamma_{\Delta} \tau_{\Delta} I_0 (1 - 10^{-4})$ is the apparent rate constant of the process. Analysis of changes in the extinction of flavone solutions with time confirms that the reaction follows the first-order rate law. Therefore, the slope of the straight lines in the $\ln A_0/A_t$ - t coordinates, which equals k_{app} , can be a criterion for the reactivity of acceptors. Here, subscripts 0 and t correspond to the initial and current values of the extinction of solutions, respectively. Figure 4 shows kinetic dependences for the processes involving TMOF and tetracene. Evidently, the value of the k_{app} constant is unin-

formative if the intensity of the excitation or absorbed light is unknown. The apparent rate constants for flavones ($k_{\text{app, F}}$) and tetracene ($k_{\text{app, T}}$) found under different conditions, when the equality of the extinction of the sensitizer in the excitation region is fulfilled, are related to the true constants as follows:

$$k_{\text{ch, F}} = k_{\text{ch, T}} \frac{k_{\text{app, F}}}{k_{\text{app, T}}}. \quad (6)$$

Table 2 presents the experimental values of the $k_{\text{app, F}}/k_{\text{app, T}}$ ratio along with the $k_{\text{app, F}}$ values. Comparison of the k_{ch} and k_{q} values shows that the physical channel of $^1\text{O}_2$ quenching is most important. The obtained values indicate that the difference in the molecular structure of the compounds under study slightly affects their reactivity. At the same time, methylated derivatives are more active than quercetin, probably because of an increase in their electron-donating ability.

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Table 2. Rate constants of $^1\text{O}_2$ deactivation by flavone

Compound	$k_{\text{q}} \times 10^{-6}$, mol $^{-1}$ l s $^{-1}$	$k_{\text{app, F}}/k_{\text{app, T}}$	$k_{\text{ch, F}} \times 10^{-4}$, mol $^{-1}$ l s $^{-1}$
PMOF	3.5	0.010	6
3-OH-TMOF	2.5	0.010	6
5-OH-TMOF	2.0	0.008	5
Quercetin [8]	0.31	—	0.9