Reactions of singlet oxygen with biomolecules
1. $^1O_2$ quenching by glycyrrhetic acid, isoliquiritigenin, and their glycosides — glycyrrhizic acid and licurazide

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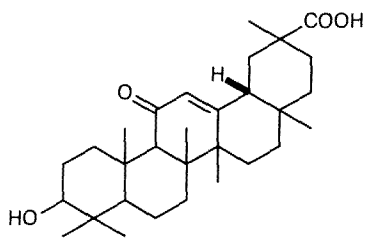
Rate constants of singlet oxygen quenching by glycyrrhetic acid, glycyrrhizic acid, isoliquiritigenin, licurazide, D-glucose, and L-arabinose were determined. An increase in the quenching rate constants by more than an order of magnitude is observed on going from aglycone to the corresponding glycoside.

Key words: singlet oxygen; biologically active substances, rate constants of singlet oxygen quenching.

Glycosides and flavonoids are contained in food products and enter the compositions of many medicinals. As all unsaturated compounds, they undergo photooxidation in the presence of oxygen and sensitizers.[1] Literature data on reactions of $^1O_2$ with flavonoids and glycosides are quite scarce.

In this work, the reactions of singlet oxygen with glycyrrhetic acid (**1**), isoliquiritigenin (**2**), and their glycosides, glycyrrhizic acid (**3**) and licurazide (**4**), were studied.

The rate constants of the reactions of singlet oxygen with D-glucose (**5**) and L-arabinose (**6**) were measured



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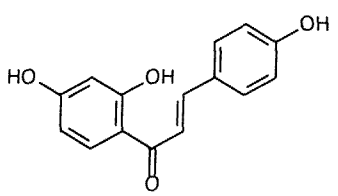

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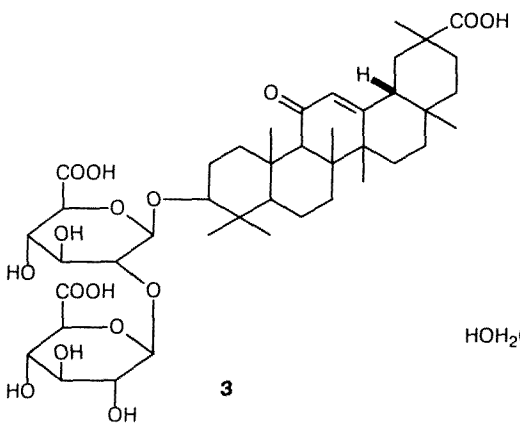

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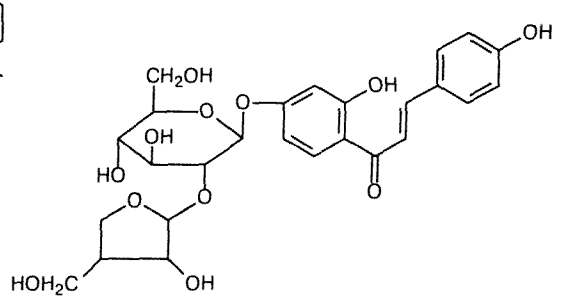

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for comparison with those of $^1O_2$ quenching by compounds **3**, **4**, and sugars.

Experimental

The reactivities of compounds **1**—**6** towards $^1O_2$ were studied by quenching of singlet oxygen luminescence in the IR spectral range, and triphenylphosphite ozonide was the source of $^1O_2$.[2,3] Glycyrrhetic acid (**1**), 2,4,4^1-trihydroxychalcone(isoliquiritigenin) (**2**), glycyrrhizic acid (**3**), and 2,4^1-dihydroxy-4-[2-*O*-(β-D-apio-D-furanosyl)-β-D-glucopyranosyloxy]chalcone (licurazide) (**4**) were obtained from licorice (*Glycyrrhiza glabra*) roots by extraction followed by preparative chromatography.[4] Overall rate constants of $^1O_2$ quenching were measured at −15±1 °C. Concentrations of compounds **1**—**6** were varied from 10^{-5} to 10^{-2} mol L^{-1}. Rate constants of chemical and physical quenching were determined separately by the stoichiometry of the consumption of triphenylphosphite ozonide and a substrate.[5] Concentrations of licurazide **4** were determined spectrophotometrically at λ = 368 nm.

Results and Discussion

The quenching of singlet oxygen by compounds **1**—**6** obeys the Stern—Volmer equation (Fig. 1): $I_0/I = 1 + k\tau[A]$, where I_0 and I are the intensities of $^1O_2$ luminescence in the absence and presence of a substrate, respectively; k is the overall rate constant of $^1O_2$ quenching ($k = k_r + k_q$, k_r and k_q are the rate constants of chemical and physical $^1O_2$ quenching, respectively); τ is the lifetime of $^1O_2$ in a solvent; and [A] is the initial substrate concentration in the reaction mixture.

The $k\tau$ value was found from the dependence of I_0/I on [A], and the overall quenching rate constant k was calculated for the known τ. In methylene chloride solutions $\tau = 0.91 \cdot 10^{-4}$ s (see Refs. 6 and 7).

The values of the overall rate constant of $^1O_2$ quenching by compounds **1**—**6** range from $1.1 \cdot 10^7$ to $1.9 \cdot 10^9$ L mol^{-1} s^{-1} (Table 1). It is of interest that the k value

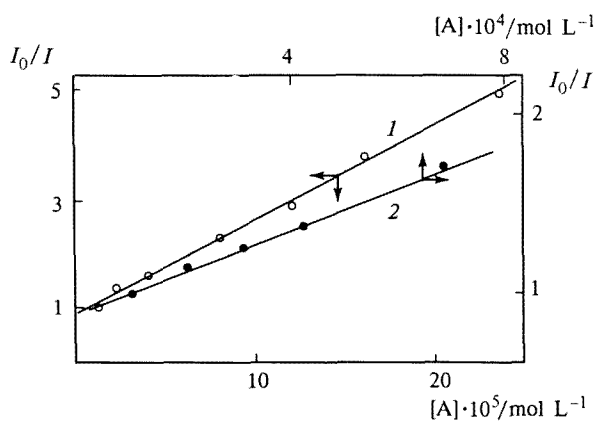

Fig. 1. Quenching of singlet oxygen luminescence by glycyrrhizic (*1*) and glycyrrhetic (*2*) acids in CH_2Cl_2 at −15 °C.

Table 1. Rate constants of $^1O_2$ quenching (k) at −15 °C in CH_2Cl_2

Compound	$k\tau$ /L mol^{-1}	$k \cdot 10^{-8}$ /L mol^{-1} s^{-1}	r*
Glycyrrhetic acid (**1**)	$9.9 \cdot 10^2$	0.11	0.983
Isoliquiritigenin (**2**)	$1.71 \cdot 10^4$	1.87	0.987
Glycyrrhizic acid (**3**)	$1.70 \cdot 10^4$	3.20	0.998
Licurazide (**4**)	$1.74 \cdot 10^5$	19.00	0.988
D-Glucose (**5**)	$1.18 \cdot 10^4$	1.37	0.838
L-Arabinose (**6**)	$5.00 \cdot 10^4$	6.04	0.983

* The correlation coefficient of the dependence of I_0/I on [A].

for glycoside is higher by an order of magnitude than that for the corresponding aglycone (*cf.* pairs glycyrrhizic acid—glycyrrhetic acid and licurazide—isoquiritigenin). In the case of sugars (see Table 1, compounds **5** and **6**), the k values are close to the rate constants of $^1O_2$ quenching by glycosides. These data allow one to assume that the carbohydrate residue protects aglycone from the singlet oxygenation ($^1O_2$ quenching by carbohydrate molecules mainly occurs *via* the mechanism of physical quenching[8]).

Rate constants of chemical and physical quenching were separately determined for flavonoid **4**. The main routes of consumption of $^1O_2$ and substrate A are reactions (1)—(3) in the triphenylphosphite ozonide (source of $^1O_2$)—acceptor of $^1O_2$—solvent system.

$$(PhO)_3PO_3 \xrightarrow{k_1} \begin{cases} \xrightarrow{\varphi_1} {}^1O_2 + (PhO)_3PO \\ \xrightarrow{1-\varphi_1} {}^3O_2 + (PhO)_3PO \end{cases} \quad (1)$$

$$^1O_2 \xrightarrow{k_2} \begin{cases} \xrightarrow{\varphi_2} {}^3O_2 + h\nu \\ \xrightarrow{1-\varphi_2} {}^3O_2 \end{cases} \quad (2)$$

$$^1O_2 + A \xrightarrow{k_3} \begin{cases} \xrightarrow{\varphi_3} AO_2 \\ \xrightarrow{1-\varphi_3} {}^3O_2 + A \end{cases} \quad (3)$$

Here k_1, k_2, and k_3 are the rate constants of the corresponding stages, φ_1 is the yield of singlet oxygen, φ_2 is the radiation quantum yield, and φ_3 is the efficiency of chemical quenching of $^1O_2$ by acceptor A ($k_r = \varphi_3 k_3$, $k_q = (1 - \varphi_3)k_3$).

According to the scheme presented,

$$\frac{-d[A]}{dt} = \varphi_3 k_3 [^1O_2][A]. \quad (4)$$

Table 2. Stoichiometry of consumptions of licurazide and triphenylphosphite ozonide at −30 °C in CH_2Cl_2

$[A]_0 \cdot 10^4$	$[A]_\infty \cdot 10^4$	$[(PhO)_3PO_3]_0 \cdot 10^3$
2.5	2.1	0.8
2.5	0.5	7.5
3.2	0.5	6.7
3.2	3.0	0.6
3.3	0.8	6.6
5.0	4.3	5.0
5.5	3.8	4.4

Note. All concentrations are given in mol L^{-1}; $[(PhO)_3PO_3]_\infty = 0$.

Since in the steady-state regime

$$[^1O_2] = \frac{\varphi_1 k_1 [(PhO)_3PO_3]}{k_2 + \{\varphi_3 k_3 + (1-\varphi_3)k_3\}[A]}, \quad (5)$$

$$\frac{d[A]}{dt} = -\frac{\varphi_3 k_3 [A]}{k_2 + \{\varphi_3 k_3 + (1-\varphi_3)k_3\}[A]} \cdot \varphi_1 k_1 [(PhO)_3PO_3],$$

it follows that

$$\frac{d[A]}{dt} = -\varphi_1 k_1 [(PhO)_3PO_3] \cdot \frac{\gamma[A]}{\beta + [A]},$$

$$d[A](\beta/[A]+1) = \{-\varphi_1 k_1 [(PhO)_3PO_3]\} \cdot \gamma dt, \quad (6)$$

where

$$\gamma = \frac{\varphi_3 k_3}{\varphi_3 k_3 + (1-\varphi_3)k_3}; \quad \beta = \frac{k_2}{\varphi_3 k_3 + (1-\varphi_3)k_3}.$$

Since ozonide decomposes according to a first-order law,

$$\varphi_1 k_1 [(PhO)_3PO_3] = \varphi_1 k_1 [(PhO)_3PO_3]_0 \cdot \exp(-k_1 t), \quad (7)$$

where $[(PhO)_3PO_3]_0$ and $[(PhO)_3PO_3]$ are the initial and current concentrations of triphenylphosphite ozonide, respectively.

Substituting Eq. (7) into Eq. (6) and integrating, we obtain

$$\varphi_1\gamma\{[(PhO)_3PO_3]_0 - [(PhO)_3PO_3]_\infty\} = \beta \ln([A]_0/[A_\infty]) + ([A]_0 - [A]_\infty). \quad (8)$$

Here $[A]_0$ and $[A]_\infty$ are the initial and final concentrations of the acceptor, respectively, and $[(PhO)_3PO_3]_\infty$ is the final concentration of ozonide. Under the experimental conditions $[(PhO)_3PO_3]_\infty = 0$. Therefore, from Eq. (8) it follows that

$$\frac{[A]_0 - [A]_\infty}{[(PhO)_3PO_3]_0} = \varphi_1\gamma - \frac{\beta \ln([A]_0/[A]_\infty)}{[(PhO)_3PO_3]_0}. \quad (9)$$

Equation (9) makes it possible to determine parameter γ, if the values of $[A]_0$, $[A]_\infty$, $[(PhO)_3PO_3]_0$, and φ_1 are known. The stoichiometry of consumptions of licurazide and ozonide in CH_2Cl_2 at 30 °C is presented in Table 2. Using the data in Table 2 and the value $\varphi_1 = 1$ taken from Ref. 5, we obtain $\gamma = 3.5 \cdot 10^{-2}$. Since, as shown above (see Table 1), the overall rate constant of $^1O_2$ quenching by licurazide $k = k_3 = k_r + k_q = 1.9 \cdot 10^9$ L mol^{-1} s^{-1}, the rate constants of chemical ($k_r = \varphi_3 k_3$) and physical ($k_q = (1-\varphi_3) \cdot k_3$) quenching are equal to $0.07 \cdot 10^9$ and $1.83 \cdot 10^9$ L mol^{-1} s^{-1}, respectively. Thus, physical quenching is the main channel of $^1O_2$ quenching by flavonoid **4**.

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