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PHOTOGENERATION OF SINGLET OXYGEN BY PSORALENS

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Furocoumarins (psoralens), in conjunction with near UV irradiation (UV-A, 315-400 nm), are widely used for the treatment of psoriasis and other skin diseases (PUVA-therapy) [1]. The therapeutic effect is connected with the photochemical activity of furocoumarin. Besides their therapeutic effects, they also give side-effects — erythema, changes in the mechano-electrical properties of the skin, etc. [7, 9]. The role of singlet oxygen ($^1O_2$) in the photobiological action of furocoumarins has recently been discussed in the literature. Experiments have shown that these compounds can generate $^1O_2$ during irradiation in solutions [9]. However, the basic information has been obtained by the use of indirect chemical methods of detection of $^1O_2$, which permit a different interpretation. Much more reliable results can be obtained by means of photosensitized luminescence of oxygen, which has recently been found in solutions of many sensitizers [2-6, 8]. Preliminary data obtained by the writers previously showed that such luminescence is also observed in solutions of 8-methoxypsoralen [10].

The aim of the present investigation was to determine the excitation spectra of luminescence of $^1O_2$ and quantum yields of $^1O_2$ generation in solutions of three furocoumarins: psoralen, 8-methoxypsoralen (8-MOP), and angelicin. The structural formulas and absorption spectra of these compounds are given in Fig. 1.

EXPERIMENTAL METHOD

Luminescence of oxygen was measured on instruments with photomultipliers described previously [3, 8]. The furocoumarins were generously provided by Professor G. Rodiguerro (University of Padua, Italy). CCl_4 (analytical grade), obtained from VEB Laborchemie, Apold, East Germany) was used as the solvent. Since this solvent, on excitation in UV rays, gave relatively intensive luminescence in the region of oxygen emission, it was purified by distillation immediately before the measurements. Distillation weakened luminescence by about one order of magnitude.

EXPERIMENTAL RESULTS

Excitation Spectra of Luminescence of $^1O_2$. Illumination of the furocoumarins in CCl_4 was shown to lead to the appearance of luminescence with a maximum at 1272 nm, corresponding to the $^1\Delta_g$-state of oxygen. The emission spectrum is given, for example, in [3, 4]. Excitation spectra of this luminescence, measured in the region of long-wave absorption bands of furocoumarins, are illustrated in Fig. 2. The accuracy of measurements of the spectra in the region of short-wave absorption bands was found to be insufficient because of superposition with luminescence of the solvent, and the short-wave maxima of luminescence excitation are therefore not shown in Fig. 2. It will be clear from Fig. 2 that in solution of psoralen the

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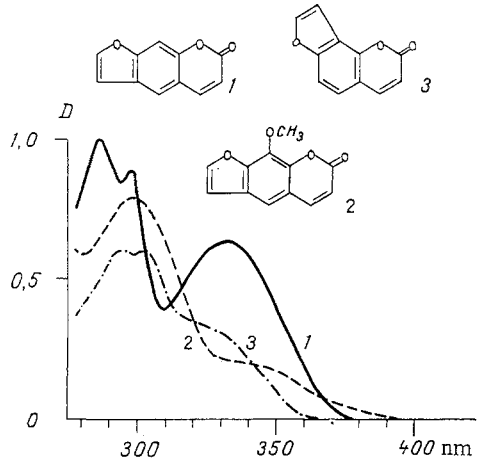

Fig. 1. Structural formulas and absorption spectra of furocoumarins in CCl_4. 1) Psoralen; 2) 8-MOP; 3) angelicin. Abscissa, wavelength (in nm); ordinate, optical density.

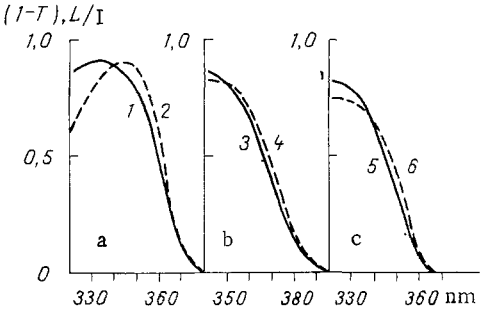

Fig. 2. Excitation spectra of luminescence of $^1O_2$ (2, 4, 6) and absorption spectra (1, 3, 5) of solutions of furocoumarins in CCl_4. a) Psoralen (10^{-4} M); b) 8-MOP (2×10^{-4} M); c) angelicin (2×10^{-4} M). Abscissa, wavelength (in nm); ordinate: (1 − T) — coefficient of absorption of solutions; L and I) intensity of luminescence and of exciting light respectively (in relative units).

long-wave excitation maximum was located at 345 nm and was shifted by 10 nm toward longer wavelengths relative to the absorption maximum. Similar differences from the absorption spectra were found previously in action spectra of photobiological reactions of psoralen [12]. In solutions of angelicin and 8-MOP the absorption spectra and excitation spectra almost coincided and this effect was not found. However, it must be pointed out that because of the lower intensity of luminescence of oxygen in solutions of angelicin and 8-MOP the accuracy of measurement of the excitation spectra was rather less than in solutions of psoralen. The character of the absorption spectra of these compounds (absence of any marked maximum; Fig. 1) also makes reliable detection among the absorption and excitation bands more difficult. Ultimately analysis of the excitation spectra leads to the conclusion that furocourmarins can generate $^1O_2$ during illumination. It can be postulated on the basis of differences described above between absorption and excitation spectra in solutions of psoralen that the relative efficiency of $^1O_2$ generation by the lower excited levels of the furocoumarins is higher than by levels with greater energy.

Quantum Yields of $^1O_2$ Generation (γ_g). Values of γ_g were estimated by comparing excitation spectra of oxygen luminescence in mixed solutions of furocoumarins and tetraphenylporphin with the absorption spectra of the mixtures [8]. It was found that in the region of long-wave excitation maxima, γ_g for furocoumarins was 100-350 times less than that of tetraphenylporphin, for which $\gamma_g = 0.7$ [3]. For comparison, γ_g was measured in a solution of the aromatic hydrocarbon chrysene, which has absorption maxima in the same region of the spectrum as furocoumarins. Absolute values of γ_g are given in Table 1. They show that the efficiency of $^1O_2$ generation by furocoumarins is very low.

TABLE 1. Values of γ_g and K_q for Furocoumarins in CCl_4

Photosensitizers and quenchers	γ_g	K_q, $M^{-1} \cdot sec^{-1}$	K_q/n_H, $M^{-1} \cdot sec^{-1}$
Psoralen	0,0055	$5,6 \cdot 10^3$	$9 \cdot 10^2$
Angelicin	0,0026	$6,5 \cdot 10^3$	$11 \cdot 10^2$
8-MOP	0,002	$9,4 \cdot 10^3$	$12 \cdot 10^2$
Chrysene	0,2	—	—
Pentane	—	$5,9 \cdot 10^3$	$4,9 \cdot 10^2$
Dodecane	—	$11,0 \cdot 10^3$	$4,2 \cdot 10^2$

Legend. n_H) Number of hydrogen atoms in molecules of quenchers.

Quenching of $^1O_2$. It was shown previously that molecules of photosensitizers — chlorophylls, porphyrins, retinals — in the basic state quench $^1O_2$ during collisions [3-6]. This may lead to a decrease in efficiency of $^1O_2$ generation in systems with a high local concentration of sensitizers. It was therefore interesting to determine the velocity constants of quenching of $^1O_2$ by furocoumarins (K_q). K_q was measured by analysis of quenching of oxygen luminescence sensitized by tetraphenylporphin (region of excitation 652 nm). The constants were calculated by the Stern–Volmer equation $L_0/L = 1 + K_q C_q \tau_0$, where L_0 and L denote the intensity of luminescence in the absence and in the presence of the quencher respectively, C_q the concentration of the quencher, and τ_0 the life of the luminescence without quenchers. In CCl_4 τ_0 = 26 msec. The value of K_q are given in Table 1. They show that furocoumarins are very weak quenchers of $^1O_2$ and their activity is close to that of alkanes, which quench $^1O_2$ by a physical mechanism, probably on account of the transfer of energy from $^1O_2$ to high-frequency overtones of oscillations of hydrogen atoms [4]. However, the ratio of K_q to the number of hydrogen atoms in furocoumarins is 2-3 times higher than for alkanes. It can accordingly be postulated that another mechanism of quenching, such as complex formation with the charge carrier (CCC) between quencher and $^1O_2$, also is possible. The writers showed previously in the case of porphyrins that quenching through CCC formation is accompanied by destruction of the quenches [5]. It was shown recently that furocoumarins are in fact destroyed during photosensitized $^1O_2$ generation [11].

To sum up, it can be concluded that furocoumarins are weak quenchers and ineffective generators of $^1O_2$. This suggests that although singlet oxygen can take part in the photobiological reactions of the furocoumarins, the quantum yield of these reactions must be low. Attention is also drawn to the fact that, according to our measurements angelicin generates $^1O_2$ with an efficiency rather higher than γ_g for 8-MOP (Table 1). This result differs from data in the literature [9], according to which angelicin cannot generate $^1O_2$ at all. It must be pointed out, however, that photoexcitation of angelicin in the investigation [9] was carried out by light at 350 nm, i.e., in the region where the absorptive capacity of this compound is very low (Fig. 1). It is therefore possible that during excitation in this way the sensitivity of the chemical methods of detection $^1O_2$ used in [9] was insufficient to record the effect.

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