Back electron transfer in electron-exchange chemiluminescence of oxyaryl-substituted spiroadamantyl dioxetane, an analog of firefly bioluminescence

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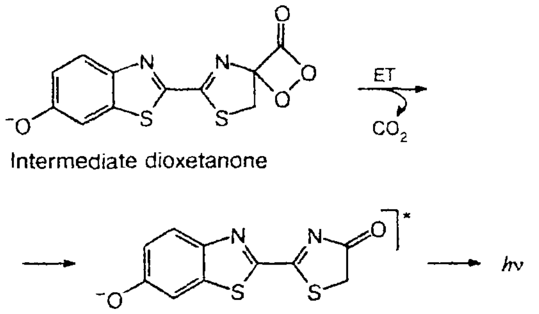
The viscosity dependence of the excitation yield of the singlet oxybenzoate anion, an emitter of electron-exchange chemiluminescence of oxyaryl-substituted spiroadamantyl dioxetane, a chemical analog of firefly bioluminescence, was studied. At 299 K the excitation yield increases from 8 to 23% as the content of diphenylmethane in its mixture with benzene increases to 97%. This effect was quantitatively interpreted in terms of a probabilistic model of the solvent-cage effect, assuming that the chemiexcitation results from the back electron transfer between the products of chemically initiated decomposition of the starting reagent.

Key words: chemiluminescence, firefly bioluminescence, dioxetanes, excited states, electron transfer, cage effect.

An interest in the chemistry of dioxetanes and dioxetanones (α-peroxylactones), four-membered cyclic peroxides, is mainly provoked by their chemiluminescence (CL) properties[1,2]: the chemical energy of the dioxetane cycle is efficiently converted to light. A striking example is represented by firefly bioluminescence, which, as assumed, is originated from the decomposition of intermediate dioxetanone, the product of the chemical reaction between luciferin and the enzyme luciferase.[3,4] The decomposition of dioxetanone proceeds due to electron transfer (ET) to the dioxetanone group (the overall reaction is shown in Scheme 1). This phenomenon was named chemically initiated electron-exchange luminescence (CIEEL).[5] It is the most efficient of the known CL processes[6]: the CL yield is ~0.9 quanta per reaction act. Such an efficient conversion of chemical energy to light is of interest from both scientific and practical points of view. This phenomenon is especially attractive for molecular biology and immunoanalysis.[6–8] Since luciferin-luciferase systems are unavailable for practical purposes, many efforts were made to search for efficient synthetic dioxetane systems.[6,9,10] The mechanism of this phenomenon should be known for understanding of the nature of chemical generation of excited states and development of efficient bioanalytical CL methods. However, unlike CL during thermal decomposition of dioxetanes,[1,2,11,12] the CIEEL mechanism is poorly studied.

Scheme 1



The CIEEL phenomenon was observed for the first time for diphenoyl peroxide[13] and almost simultaneously in reactions of other cyclic peroxides[14,15] with easily oxidizable aromatic luminophores. The generation of CIEEL has also been found convincingly in reactions of Ru^{II} complexes with dioxetanes[1] and dimethyldioxirane.[16,17] Note, however, that an alternative mechanism of chemical generation of the excited

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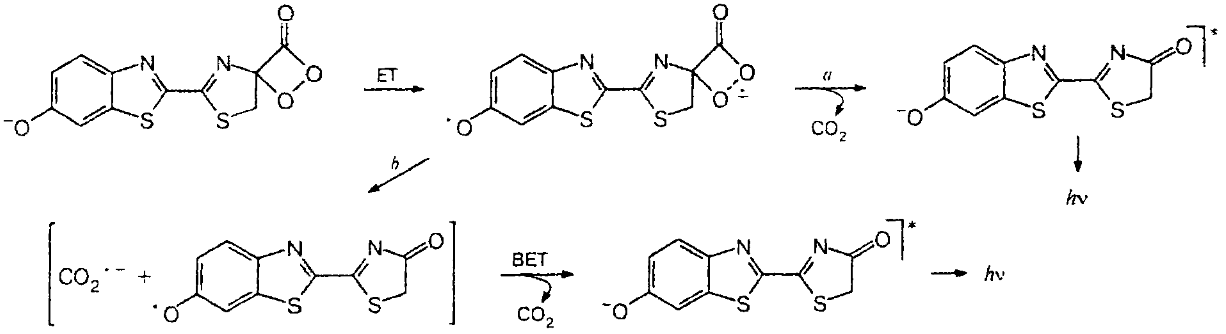
Scheme 2

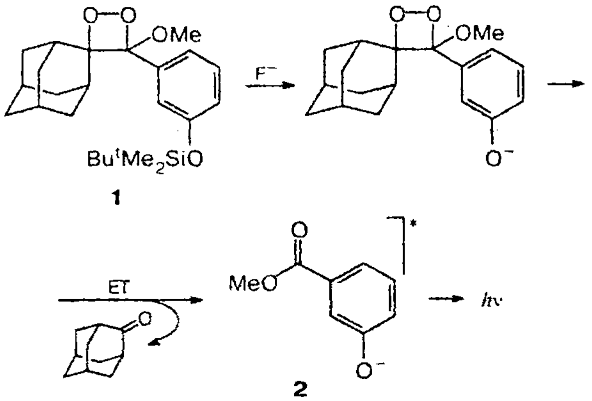


state through a charge-transfer complex[18,19] has been proposed for the explanation of low chemiexcitation yields in the reactions of diphenoyl peroxide and anthracene endoperoxide.

Electron-exchange luminescence can be initiated not only by intramolecular but also by intermolecular ET. The intramolecular ET has been postulated for firefly bioluminescence[3] (see Scheme 1).

What is the mechanism of this process? It can be assumed that after ET to the dioxetanone group the excited emitter is immediately generated in the act of decarboxylation of the dioxetanone fragment (Scheme 2, pathway *a*). An alternative CIEEL mechanism is also possible: the Schuster scheme[5,13] with back electron transfer (BET) from the CO$_2^{\cdot -}$ radical anion to an intermediate radical, the precursor of the photoemitter (Scheme 2, pathway *b*). Dioxetanone intermediates resulting in firefly bioluminescence are knowingly unstable and cannot be convenient objects for the study of the CIEEL mechanism. In this work, oxyaryl-substituted adamantyldioxetane, 3-(2′-spiroadamantane)-4-methoxy-4-(3″-*tert*-butyldimethylsilyloxy)phenyl-1,2-dioxetane (**1**), was used as the model (Scheme 3).

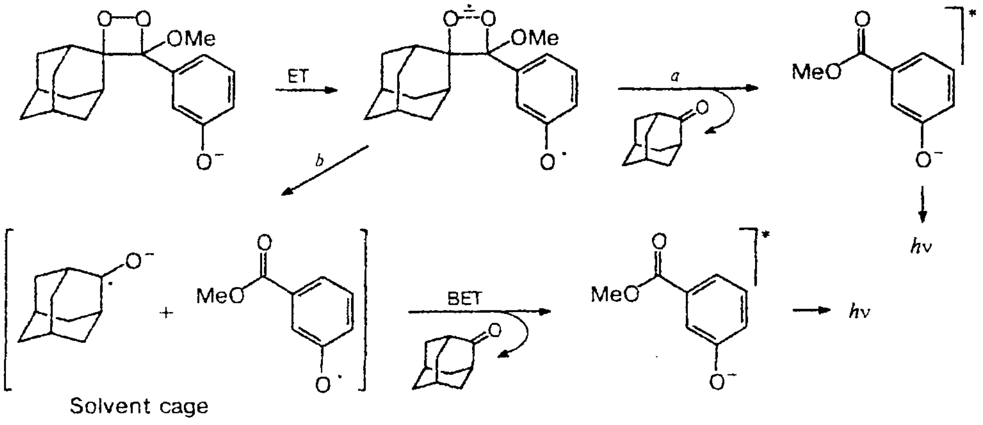
Scheme 3



The oxygen atom in the aryl fragment of dioxetane **1** is protected by the electron-withdrawing SiMe$_2$Bu^t group, which prevents ET to the O—O bond. This dioxetane is thermally very stable due to the adamantyl fragment.[11c,20] To initiate the dioxetane decomposition and CIEEL generation, the protective SiMe$_2$Bu^t group is removed by the action of F$^-$ ions (see Scheme 3), after which the aryl fragment becomes an intramolecular electron donor. The ET from O$^-$ to the dioxetane fragment results in the cleavage of the O—O bond and, thus, in dioxetane decomposition accompanied by CIEEL excitation. We have recently reported the kinetic[21,22] and spectral[23] characteristics of CIEEL of spiroadamantyldioxetanes and their thermal CL.[24] Thermolysis does not result in the elimination of the protective group, but the O—O and C—C bonds are broken and C=O groups are formed; the excited states of both decomposition products of dioxetane are generated: S$_1$($n\pi^*$) and T$_1$($n\pi^*$) of adamantanone, T$_1$($\pi\pi^*$) of ester.[24] Unlike chemiexcitation during thermolysis, in the case of CIEEL, only one of the decomposition products, methyl-*m*-oxybenzoate anion (**2**), is excited. The CIEEL is the fluorescence from the S$_1$($\pi\pi^*$) state of **2** (the band at 460 nm with a half-width of ~100 nm). The AM1 calculations show[21] that the energy of the T$_1$($\pi\pi^*$) state of **2** is equal to ~47 kcal mol^{-1}, *i.e.*, ~34 kcal mol^{-1} lower than that of S$_1$. Therefore, phosphorescence (even if its existence is proved) cannot contribute to the emission of CIEEL.

As can be seen in Scheme 4, the same alternatives as in the case of the firefly (see Scheme 2) can be proposed for the CIEEL mechanism of oxyaryl-substituted dioxetane. Thus, oxyaryl-substituted spiroadamantyl-dioxetane can be considered indeed as a chemical model of the firefly. How can the two possible CIEEL mechanisms shown in Schemes 2 and 4 be distinguished? Along with the increase in the thermal stability of dioxetane, the adamantyl substituent has an additional advantage: the adamantanone radical anion (see Scheme 4), unlike CO$_2^{\cdot -}$ (see Scheme 2), is a bulky fragment. Thus, the channel with back electron transfer shown in Scheme 4 (pathway *b*) should be prone to the cage effect; and if

Scheme 4



CIEEL is excited in fact during BET, the excitation yield should be sensitive to the solvent viscosity. In this work, we quantitatively studied the viscosity dependence of the excitation yield (Φ_{S1}) of the singlet state of the CIEEL emitter, methyloxybenzoate 2.

Experimental

Materials. The starting reactant, 3-(2′-spiroadamantane)-4-methoxy-4-(3″-*tert*-butyldimethylsilyloxy)phenyl-1,2-dioxetane (**1**), was synthesized by the previously described procedure.[21] A 1 *M* solution of $NBu^n{}_4F$ in THF (Aldrich) was used as the source of F^- ions. Methyl-*m*-oxybenzoate ions (**2**) were prepared in a DMSO solution by the reaction of $NBu^n{}_4F$ with 3-(butyldimethylsilyloxy)benzoate.* Solvents, benzene and diphenylmethane (Fluka), were distilled in a nitrogen atmosphere under a reduced pressure in the presence of ethylenediaminetetraacetic acid (50 g L^{-1}).

Measurements of chemiluminescence. Chemiluminescence (CIEEL) in mixtures of benzene and diphenylmethane at 26 °C was detected on a photometric setup.[25] The decomposition of dioxetane **1** was initiated by the removal of the protective $SiMe_2Bu^t$ group in the reaction with F^- ions (see Scheme 3). Small amounts (1.6 vol.%) of a solution of $NBu^n{}_4F$ (the source of F^- ions) in THF—DMSO (1 : 1) were introduced into a solution of dioxetane **1** in a C_6H_6—Ph_2CH_2 mixture. The yields of CIEEL (Φ^{CIEEL}) and chemiexcitation of product **2** (Φ_{S1}) can be found from relationship (1) for the intensity of CIEEL (I^{CIEEL}):

$$I^{CIEEL} = \Phi^{CIEEL} v = \Phi_{S1}\Phi^{fl} v, \qquad (1)$$

where Φ^{fl} is the quantum yield of fluorescence of **2**, and v is the reaction rate. The CIEEL yield was determined as follows. The number of photons $N_{h\nu}$ emitted due to the complete decomposition of **1** was measured as the area under the curve $I^{CIEEL}(t)$:

$$N_{h\nu} = \int_0^{\infty} I^{CIEEL} dt = \Phi^{CIEEL}\int_0^{\infty} v\, dt = \Phi^{CIEEL}[1]. \qquad (2)$$

According to Eq. (2), $\Phi^{CIEEL} = N_{h\nu}/[1]$. To find the chemiexcitation yield $\Phi_{S1} = \Phi^{CIEEL}/\Phi^{fl}$, Φ^{fl} should be known. The Φ^{fl} values were measured in C_6H_6—Ph_2CH_2 mixtures with the same composition as those used for the determination of Φ^{CIEEL}, using a Perkin Elmer LS 50 spectrofluorimeter relative to the fluorescence of quinine bisulfate (QBS, [QBS] = $1.19 \cdot 10^{-6}$ mol L^{-1}) in 1 *N* H_2SO_4 ($\Phi^{fl}{}_{QBS} = 0.55$ [26]).

Viscosity effect. The cage effect in the CIEEL process (see Scheme 4) was determined from the influence of the viscosity on the excitation yield Φ_{S1} of the singlet state of the CIEEL emitter. The viscosity of mixtures of the fluid (C_6H_6) and viscous (Ph_2CH_2) components (from 0 to 97 vol.% Ph_2CH_2 in mixtures) was measured by an Ostwald capillary viscosimeter.[27] The C_6H_6—Ph_2CH_2 system was chosen because the factor $1/n^2 - 1/\varepsilon$ was approximately equal for both components of the solution, being necessary to reduce to a minimum the change in the energy of solvent reorganization during variation of the composition of the C_6H_6—Ph_2CH_2 mixture, which could, according to the Marcus theory, substantially affect the electron transfer.

Results and Discussion

The Φ^{CIEEL}, Φ^{fl}, and Φ_{S1} values found at different viscosities of the solution (*i.e.*, at different [Ph_2CH_2]) are presented in Table 1.

If we assume that both possible channels of excitation, namely, the direct generation of the excited emitter (see Scheme 4, pathway *a*) and BET (see Scheme 4, pathway *b*), contribute to the CIEEL process, the excitation yield Φ_{S1} of the singlet state (S_1) of the CIEEL emitter is summated of two components:

$$\Phi_{S1} = \Phi_{S1}{}^0 + \Phi_{S1}(\eta). \qquad (3)$$

One of them, $\Phi_{S1}{}^0$, corresponds to the contribution of the direct channel (pathway *a*) and should be independent of the viscosity, whereas the component $\Phi_{S1}(\eta)$ corresponding to the channel with BET (pathway *b*) depends on the viscosity η.

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* The sample of 3-(butyldimethylsilyloxy)benzoate was kindly presented by Dr. M. Schulz (Würzburg University).

Table 1. Viscosity (η), estimated mean value ($\bar{m}$) of repeated collisions in the cage, CIEEL yield ($\Phi^{\rm CIEEL}$), and fluorescence ($\Phi^{\rm fl}$) and chemiexcitation ($\Phi_{\rm S1}$) yields of the methyl-*m*-oxybenzoate ion at different concentrations of diphenylmethane in benzene—diphenylmethane mixtures at 299 K*

$[\mathrm{Ph_2CH_2}]$ (vol.%)	η /cP	$\bar{m}$	$\Phi^{\rm CIEEL}$	$\Phi^{\rm fl}$	$\Phi_{\rm S1}$
0	0.62	60	0.022	0.24	0.09
10	0.71	70	0.024	0.24	0.10
20	0.82	80	0.024	0.22	0.11
40	1.09	110	0.029	0.22	0.13
60	1.44	145	0.040	0.24	0.17
80	1.91	190	0.048	0.23	0.21
97	2.42	240	0.052	0.22	0.24

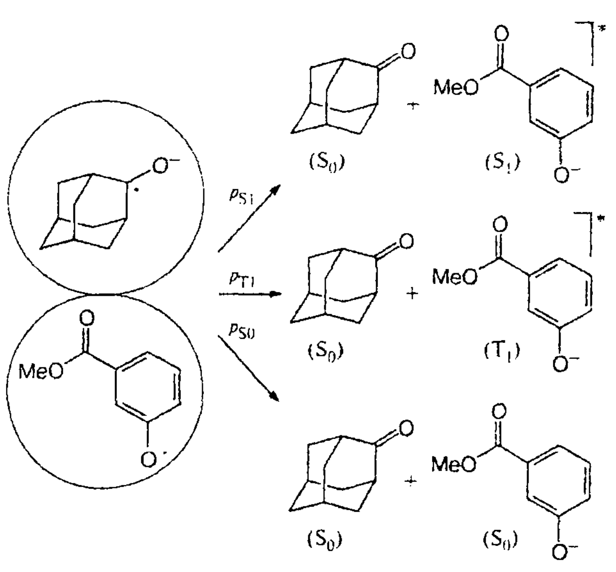
* Measurement errors of $\Phi^{\rm CIEEL}$ and $\Phi^{\rm fl}$ are 5—7%.

To find the analytical form of the dependence $\Phi_{\rm S1}(\eta)$, we used a probabilistic approach, which can be named the kinetic model of discrete tests. The essence of the approach is in the consideration of collisions in an encounter complex in the solvent cage. The collision is the contact at a distance between the centers of molecules of the partners approximately equal to the sum of their radii (the collision diameter) when some overlapping of the electron clouds sufficient for the interaction resulting in ET is observed. The charged partner is surrounded by a solvating molecules, and the reaction coordinate is a combination of coordinates of nuclei of the reagent and solvent molecules. The reorganization energy of the solvent necessary for the contact is tightly related to the activation energy, *i.e.*, it is borrowed from a thermostat. It can be expected that the characteristic collision time is of the order of the vibration period, *i.e.*, 10^{-13}—10^{-12} s. The encounter implies multiple collisions in the solvent cage within its whole lifetime. Since the probability of electron transfer is sharply decreased at distances longer than the collision diameter, in the first approximation it is reasonable to consider the electron transfer only at the moment of collision. Designate the probability of BET at the moment of collision of the partners in the radical ion pair in the solvent cage as $p_{\rm BET}$ (see Scheme 4, pathway *b*). Then the probability of a collision without the electron transfer is equal to $1 - p_{\rm BET}$. The probability that the partners do not react after m collisions is $(1 - p_{\rm BET})^m$. Thus, the BET probability after m collisions ($P_{\rm BET}$) is expressed by the following equation:

$$P_{\rm BET} = 1 - (1 - p_{\rm BET})^m. \qquad (4)$$

This approach to the cage effect has been suggested[28] for the description of the quenching of luminescence. However, the cage distribution over lifetimes was not taken into account, but was done later[29] (using the kinetic theory of Ya. I. Frenkel[30]) for the description of triplet-triplet energy transfer.

Scheme 5



To obtain an expression for the viscosity dependence of the excitation yield, we should consider three possible channels of BET in the solvent cage. In Scheme 5, $p_{\rm S0}$, $p_{\rm S1}$, and $p_{\rm T1}$ are the elementary probabilities of BET at the moment of collision of the partners resulting in the formation of the CIEEL emitter in the $\rm S_0$, $\rm S_1$, and $\rm T_1$ states, respectively. The probability of BET ($p_{\rm BET}$) to at least one of these states after one collision is equal to the sum of the elementary probabilities $p_{\rm S0}$, $p_{\rm S1}$, and $p_{\rm T1}$, *i.e.*, $p_{\rm BET} = p_{\rm S0} + p_{\rm S1} + p_{\rm T1}$. Thus, relationship (4) is transformed into:

$$P_{\rm BET} = 1 - (1 - p_{\rm S0} - p_{\rm S1} - p_{\rm T1})^m. \qquad (5)$$

The number of collisions m within time t is determined by the relationship

$$m = t/\tau_{\rm c}, \qquad (6)$$

in which $\tau_{\rm c}$ is the mean time between successive collisions of the partners in the solvent cage.

Taking into account the cage distribution over the lifetimes,[29,30] using Eqs. (5) and (6), we obtain the expression for the mean probability of BET per cage:

$$\bar{P}_{\rm BET} = \int_0^\infty [1 - (1 - p_{\rm S1} - p_{\rm T1} - p_{\rm S0})^{t/\tau_{\rm c}}]e^{-t/\tau}{\rm d}(t/\tau) = $$
$$= \{1 - [\bar{m}\ln(1 - p_{\rm S1} - p_{\rm T1} - p_{\rm S0})]^{-1}\}^{-1}, \qquad (7)$$

where $\bar{m} = \tau/\tau_{\rm c}$ is the mean number of collisions, τ is the mean lifetime of the cage, $1 - (1 - p_{\rm S1} - p_{\rm T1} - p_{\rm S0})^{t/\tau_{\rm c}}$ is the probability of BET after $m = t/\tau_{\rm c}$ collisions in the cage, and $e^{-t/\tau}{\rm d}(t/\tau)$ is the lifetime distribution of cages. The excitation yield of the $\rm S_1$ state of the CIEEL emitter is nothing but the probability $\bar{P}_{\rm BET}$ multiplied by the relative contribution $p_{\rm S1}/(p_{\rm S1} + p_{\rm T1} + p_{\rm S0})$ of the single ($\rm S_1$) channel to the overall BET process. Thus,

$$\Phi_{\rm S1} = [p_{\rm S1}/(p_{\rm S1} + p_{\rm T1} + p_{\rm S0})]\,\bar{P}_{\rm BET}. \qquad (8)$$

Similarly, the excitation yield of the triplet (T_1) and the yield of the ground state (S_0) of the emitter are the following:

$$\Phi_{T1} = [p_{T1}/(p_{S1} + p_{T1} + p_{S0})]\,\overline{P}_{BET}\,, \tag{9}$$

$$\Phi_{S0} = [p_{S0}/(p_{S1} + p_{T1} + p_{S0})]\,\overline{P}_{BET}\,. \tag{10}$$

From expressions (8)—(10), we obtain the identity

$$\Phi_{BET} = \Phi_{S0} + \Phi_{T1} + \Phi_{S1} = \overline{P}_{BET}\,. \tag{11}$$

Thus, the yield of the overall BET, Φ_{BET}, is equivalent to P_{BET}.

Since oxybenzoate ion **2** does not phosphoresce in the liquid phase at room temperature,[21] its triplet state is not manifested in the chemiluminescence experiment, and only the excitation yield Φ_{S1} of the singlet state of oxybenzoate is the measured value. We obtain the dependence of Φ_{S1} on η by Eqs. (7) and (8), if in Eq. (7) the mean number of repeated collisions of the partners $\overline{m}$ is related to the viscosity of the medium η. This relationship can easily be found expressing the viscosity in the Arrhenius form

$$\eta = A_\eta e^{E\eta/(RT)}, \tag{12}$$

$$k = \tau^{-1} = \tau_c^{-1} e^{-E\eta/(RT)}, \tag{13}$$

$$\overline{m} = \tau/\tau_c = \tau_c e^{E\eta/(RT)}/\tau_c = e^{E\eta/(RT)} = \eta/A_\eta, \tag{14}$$

where A_η is the pre-exponential factor of the viscosity, and E_η is the activation energy of the fluidity.

According to the theory of Ya. I. Frenkel,[30] the jump of the particles to a new equilibrium position after some vibrations is related to the overcoming of a potential barrier virtually equal to the E_η energy in the Arrhenius equation (12). The Frenkel settled lifetime is equivalent to the cage lifetime τ. Thus, the radical ion pairs caged in the solvent disappear with the rate constant $k = \tau^{-1}$ given by relationship (13), in which τ_c^{-1} is the collision frequency of the partners in the cage. The interrelation of the number of collisions $\overline{m}$ and the viscosity η in the form of Eq. (14) follows from expressions (12) and (13). From where, substituting $\overline{m}$ from Eq. (14) into expression (7) and inserting the thus obtained correlation into Eq. (8), we obtain the yield Φ_{S1} as a function of the viscosity η

$$\Phi_{S1}(\eta) = \frac{p_{S1}\eta}{p_{S1} + p_{T1} + p_{S0}} \cdot \frac{\ln(1 - p_{S1} - p_{T1} - p_{S0})}{\eta \ln(1 - p_{S1} - p_{T1} - p_{S0}) - A_\eta}\,. \tag{15}$$

Since the probabilities p_{S0}, p_{S1}, and p_{T1} of BET per collision are much lower than unity, then $\ln(1 - p_{S1} - p_{T1} - p_{S0}) \approx -(p_{S1} + p_{T1} + p_{S0})$ and Eq. (15) can be reduced to

$$\Phi_{S1}(\eta) = \frac{p_{S1}\eta}{(p_{S1} + p_{T1} + p_{S0})\eta + A_\eta}\,. \tag{16}$$

Expressions (15) and (16) are attributed to the chemiexcitation exclusively in the process with BET (see Scheme 4, pathway *b*). For the general case (correlation (3)), using Eq. (16), we obtain the dependence of the excitation yield on the viscosity

$$\Phi_{S1} = \Phi^0_{S1} + \frac{p_{S1}\eta}{(p_{S1} + p_{T1} + p_{S0})\eta + A_\eta}\,. \tag{17}$$

We calculate the pre-exponential factor of the viscosity A_η in expressions (16) and (17) from the measured by us temperature dependences of the viscosity for the mixtures of benzene and diphenylmethane. The Arrhenius law was found to be well fulfilled for η. The pre-exponential factor A_η is $(9.9\pm0.6)\cdot10^{-3}$ cP and independent of the viscosity, *i.e.*, of $[Ph_2CH_2]$, whereas the activation energy linearly increases with increasing $[Ph_2CH_2]$: $E_\eta = E_\eta{}^0 + \alpha[Ph_2CH_2]$, where $\alpha = (8.3\pm0.5)$ cal mol^{-1} (vol.%)$^{-1}$ and $E_\eta{}^0 = (2.46\pm0.03)$ kcal mol^{-1}. The fact that for the C_6H_6—Ph_2CH_2 mixtures A_η is independent of $[Ph_2CH_2]$ is convenient for the quantitative analysis of the experimental dependence of Φ_{S1} on η, because A_η enters into Eqs. (16) and (17) as the constant. Another positive feature is that the fluorescence yield of **2**, the CIEEL emitter ($\Phi^{fl} = 0.230\pm0.015$), in the C_6H_6—Ph_2CH_2 mixtures is also virtually independent of $[Ph_2CH_2]$ (see Table 1).

The increase in Φ_{S1} with increasing η (see Table 1) indicates the participation of BET in the generation of CIEEL. Let us determine the parameters of this process. When BET is the predominant mechanism of the CIEEL generation, Eq. (17) is reduced to expression (16). For checking this correlation, it is convenient to use its linear form

$$\Phi_{S1}{}^{-1}(\eta) = \frac{p_{S1} + p_{T1} + p_{S0}}{p_{S1}} + \frac{A_\eta}{p_{S1}}\eta^{-1}. \tag{18}$$

As can be seen in Fig. 1, the dependence of Φ_{S1} on η (see Table 1) satisfactorily obeys Eq. (18), which indicates the predominant contribution of BET to the CIEEL generation (see Scheme 4, pathway *b*). Using the above value of A_η and the slope of the straight line in Fig. 1 ($A_\eta/p_{S1} = 5.70\pm0.34$ cP), we obtain the probability of formation of the singlet-excited CIEEL emitter per collision in the cage: $p_{S1} = (1.7\pm0.1)\cdot10^{-3}$. Multiplying the intercept on the ordinate, $(p_{S0} + p_{S1} + p_{T1})/p_{S1} = 2.0\pm0.1$, by p_{S1}, we find the probability $p_{BET} = p_{S0} + p_{S1} + p_{T1} = (3.4\pm0.1)\cdot10^{-3}$ for the overall BET. From the obtained values of p_{S1} and p_{BET}, we can estimate the mean number of collisions in the solvent cage per

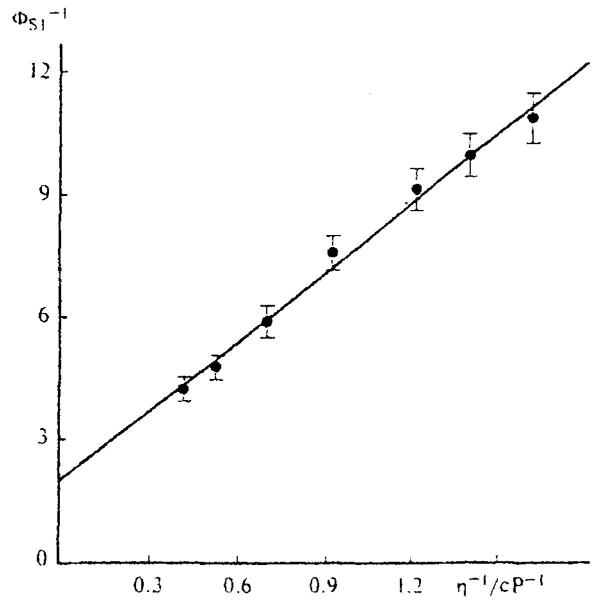


Fig. 1. Linear anamorphosis of the experimental dependence of the excitation yield Φ_{S1} of the singlet state of the CIEEL emitter on the viscosity η (see Table 1) in the coordinates of Eq. (18); R^2 = 0.993.

successful encounter resulting in BET with the excitation of the S_1 state, $p_{S1}^{-1} \approx 590$, and the mean number of collisions preceding the back electron transfer to at least one of the S_0, S_1, or T_1 states, $p_{BET}^{-1} \approx 295$. Thus, before the electron transfer, the partners undergo multiple collisions in the solvent cage.

Thus, the experimental dependence of the chemiexcitation yield on the viscosity is well described by the regularities derived under the assumption that BET is the only channel of CIEEL generation. From which we may conclude that the mechanism with BET predominates, indeed, in the studied case of CIEEL. Since the latter is the chemical model of *in vivo* chemiexcitation, it is probable that the excitation of firefly bioluminescence also proceeds mainly through a mechanism with back electron transfer.

The excitation yields in the electron-exchange decomposition of **1** are very high: they approach those *in vivo* (the highest value is ~0.9) and are much higher than those in many other CL reactions occurring through the mechanism of nuclei rearrangement. This can most likely be explained in terms of the modern ET theories[31] by a peculiar kinetic forbiddance of the population of the ground state of the product and an allowance of the pathway to the excited state. The electron transfer is preceded by the slow reorganization of the medium due to thermal fluctuations, resulting in change of the energy of an electron in the donor. The electron tunneling occurs when this energy becomes equal to the energy of one of the levels in the acceptor. The electron transfer occurs within the time during which nuclei may be considered as fixed. When the accepting level is excited, the chemical (*i.e.*, electronic) energy is more easily converted to electronic excitation energy than to heat. This favors efficient chemiexcitation. When the electron transfer reaction is energy deficient and the energy should be converted to heat, this hinders a particular elementary act, but the average yield is still high because the number of energy deficient acts is greater than that of energy sufficient acts.

In fact, although the probabilities of the populations of lumunescent and "dark" states per collision are low, they are values of the same order (10^{-3}). Owing to this, similar probabilities of the populations of these states per encounter are observed due to many repeated collisions, which results in sufficiently high absolute excitation yields.

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