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Chemiluminescence of indole and its derivatives

R F Vasil'ev, A V Trofimov, Yu B Tsaplev

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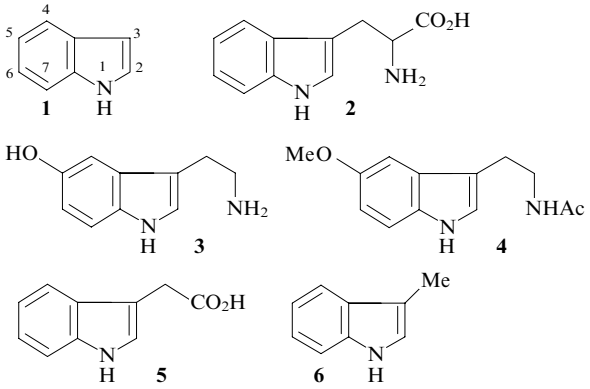
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Abstract. The results of studies on chemiluminescence of indole and its derivatives are critically analyzed. It is shown that chemical transformations of indoles lead, depending on the structure and experimental conditions, to various electronically excited products and emission of light. Many reactions considered are used as a basis for highly sensitive methods for detection of indoles in biology, medicine, ecology and forensics. The bibliography includes 100 references.

I. Introduction

In the early 1960s it was found that luciferins of some glowing living organisms (the ostracod *Cypridina*, the marine worm *Balanoglossus*) contain an indole fragment in the molecule. The strong smell of indole served as the tracer for isolation of marine worm luciferin. This prompted the idea that indole plays an important role in bioluminescence; therefore, the chemiluminescence properties of indoles have become subjects of active research.

Currently it is known for certain that chemiexcitation of the above-mentioned luciferins is not related to transformations of the indole fragment but the interest in chemiluminescence (CL) of indoles arising during oxidation in both biological and non-biological systems is retained. This is not surprising because some derivatives of indole (**1**) are very important, including the essential amino acid tryptophan (**2**); the neuromediator serotonin (**3**); the hormones melatonin (**4**) and indol-3-ylacetic acid (**5**); a great number of alkaloids and biologically active compounds (vinblastine, reserpine, ajmaline, strychnine, brucine, *etc*.), and exotic compounds such as skatole (**6**) having a strong fecal odor (or jasmine odor on high dilution).



The problem of indole determination in mixtures is encountered by specialists in various fields, including biologists, medical scientists, ecologists, forensic medicine experts and criminal experts, food industry workers, and others. Chemiluminescence-based detection methods, which are fast and highly sensitive, are best suited for this purpose. The review considers the more than 50-year history of investigations of indole CL the results of which underlie the analytical applications.

II. Early studies

1. Chemiluminescence of indoles in the presence of a base

Chemiluminescence in indole reactions was first reported at the Symposium on Chemiluminescence in 1965.[1,2] In one of the cited works,[1] CL of 22 compounds was studied under conditions designated as A1 and B1. Conditions A1: aqueous aerated solution of a sample (2.5 mmol litre^{-1}) in the presence of NaOH (0.5 mol litre^{-1}) and $K_2S_2O_8$ (0.08 mol

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Table 1. The maximum CL intensity for some indole compounds depending on the conditions.

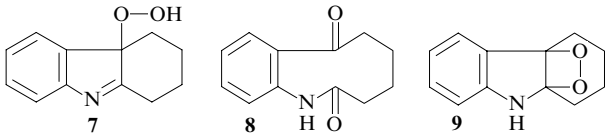
Compound	A1	B1	A9	B9
	$H_2O-NaOH-S_2O_8^{2-}$	DMSO–KOH	$EtOH-H_2O-KOH-H_2O_2-K_3Fe(CN)_6$	$DMSO-H_2O-KOH$
3-Methylindole	0.6	95.0	0.5	13.0
2-Methylindole	0.3	20.0	0.5	0.05
1,2-Dimethylindole	0.4	2.9	–	–
Indol-3-ylacetic acid	0.07	0.9	1.5	0.4
Indole-5-carboxylic acid	0.4	0	–	–
L-Tryptophan	0.2	0	–	0
2,3-Dimethylindole	0	29.0	–	31.0
2,5-Dimethylindole	0	5.7	–	–
Oxindole	0	1.1	–	–
2-(Indol-3-yl)propionic acid	0	1.1	–	–
3-Acetylindole	0	0.6	–	–
Indole-3-carbaldehyde	0	0.3	–	–
Indol-3-ylacetaldehyde	–	–	–	13.0
3-(2-Diisopropylaminovinyl)-indole	–	–	590.0	–

Note. All values are referred to the CL intensity of indole.

litre^{-1}); conditions B1: aerated solution of a sample (0.05 mol litre^{-1}) in dimethyl sulfoxide (DMSO) in the presence of KOH.† Chemiluminescence was found for compound **1** and six derivatives under conditions A1 and for 11 compounds under conditions B1 (Table 1). No CL was found for 2-phenylindole, indole-3-carboxylic acid, indol-3-ylacetonitrile, 3-chloroindole, 5-nitroindole, 3-acetyl-2-methylindole, 2,3-dichloroindole, 1-methyl-2,3-diphenylindole, 1-ethyl-2,3-diphenylindole, isatin or gramine.

Berger *et al.*[3] cited data from the report[4] on the CL of indole (**1**), indole-5-carboxylic acid and 3-methylindole, from which it follows that Philbrook *et al.*[1] observed CL of these compounds not only under conditions B1 but also in dimethylformamide (DMF) in the presence of 0.67 mol litre^{-1} of Bu^{t}OK and that the highest intensity of CL of 3-methylindole in DMF was almost 4 times higher than in DMSO. The observation of CL in DMF is important in relation to the problem of reactive oxygen species involved in these reactions. In 1981 it was shown[5] that in aerated solution of DMSO‡ (but not DMF), dissolved oxygen is converted under the action of alkali into superoxide radical anion the concentration of which can reach 0.4 mmol litre^{-1}. As the H_2O content in DMSO increases, the concentration of the superoxide radical anion decreases.[6]

McCapra *et al.*[2] were initially interested in peroxides, in particular, tetrahydrocarbazole hydroperoxide (**7**), whose melting or heating in xylene is accompanied by blue light flash. This gives lactam as a major product. In compound **7**, the oxygen atoms are arranged asymmetrically relative to the C(2)–C(3) bond, while in compound **8**, they are arranged symmetrically. As regards the reaction mechanism, a discussion between Witkop's and Beer's groups was started in the early 1950s. Witkop proposed a decomposition mechanism of peroxide **7** *via* a four-centre transition state, dioxetane **9**. One could hardly foresee at that time the significance dioxetane structures would acquire for chemiluminescence studies 15–20 years later.§



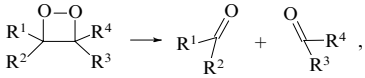
McCapra *et al.*[2] observed CL on treatment of hydroperoxide **7** or **10** in DMSO or the *N*-methyltryptamine in the presence of O_2 with Bu^{t}OK. In 1966 McCapra and Chang reported bright CL on treatment of solutions of 2,3-dimethylindolenyl 3-hydroperoxide (**11**) and hydroperoxide **10** in DMSO with Bu^{t}OK.[7,8] It was found that the peaks in the CL spectra of these compounds (518 and 495 nm) coincide with the fluorescence maxima of the anions of amidoacetophenones, which are formed as the major reaction products. The transformations of the studied hydroperoxides are shown in Scheme 1.

The above-cited studies[1,2,7,8] stimulated the interest in the CL of indoles. In 1967–1968, a group of Japanese chemists published a series of four studies[9–12] devoted to indole CL; later one more related publication appeared.[13] Sugiyama *et al.*[9] performed the search for CL among 42 compounds under conditions A9 and B9. According to conditions A9, 1 ml of a sample solution in an alkaline (0.1 mol litre^{-1} KOH) solution of ethanol was mixed with 1 ml of an aqueous solution containing H_2O_2 (1.5%) and $K_3Fe(CN)_6$ (1.5%); according to conditions B9, 1 ml of a

† The solubility of KOH in anhydrous DMSO does not exceed 0.003 mol litre^{-1} [see M Vasilescu, T Constantinescu, M Voicescu, H Lemmetyinen, E Vuorimaa *J. Fluoresc.* **13** 315 (2003)].

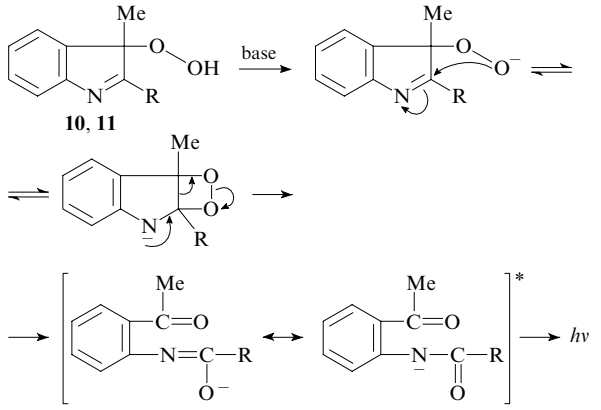
‡ It was shown that available DMSO samples (without special purification) contains Fe(II) and Cu(II) ions in a concentration of up to 3 mmol litre^{-1} [see X Qiao, S Chen, L Tan, H Zheng, Y Ding, Z Ping *Magn. Reson. Chem.* **39** 207 (2001)].

§ If the energy released in the transformation



is estimated from the bond energies, this value will exceed 300 kJ mol^{-1}. This energy can ensure electronic excitation of the products. Therefore, now dioxetanes are often called high-energy molecules or light storage molecules.

Scheme 1

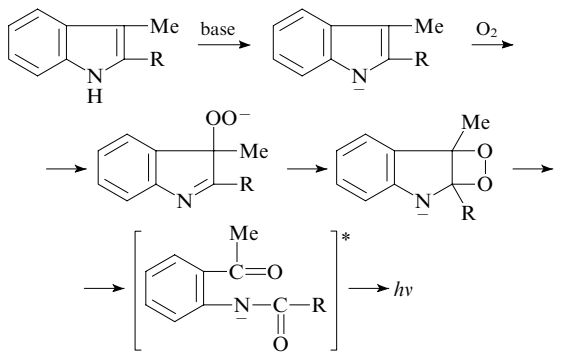


R = 4-MeOC$_6$H$_4$ (**10**), Me (**11**).

sample solution (1 mmol litre^{-1}) in DMSO was mixed with 1 ml of an aqueous solution of KOH (5 mol litre^{-1}). Some data from this work are presented in Table 1. Comparison of the results obtained under conditions B1 and B9 has shown that they are similar for 2,3-dimethylindole but differ considerably for 3-methylindole and 2-methylindole. This seems strange because conditions B1 and B9 formally differ only in the sample and water concentrations in DMSO.

Peroxide CL was also reported[9] for compounds **11** and 2,3-diethylindolenyl 3-hydroperoxide. Chemiluminescence of 2,3-dimethylindole and hydroperoxide **11** was studied[10,11] in more detail under conditions similar to B1. It was shown that CL of 2,3-dimethylindole required oxygen, while CL of compound **11** did not. *o*-Acetamidoacetophenone formed as the final product in both the oxidation of 2,3-dimethylindole and the alkaline decomposition of hydroperoxide **11**. The maxima of the CL spectra of 2,3-dimethylindole and compound **11** occur at 520 nm, which corresponds to the maximum of the fluorescence spectrum of *o*-acetamidoacetophenone. This provided solid grounds for assuming that *o*-acetamidoacetophenone is the CL emitter, while compound **11** is an intermediate of autooxidation of 2,3-dimethylindole. A number of indole derivatives have been studied,[11] namely, 2,3-dimethylindole, 2,3,5-trimethylindole, and 5-methoxy-2,3-dimethylindole. The positions of peaks in the CL spectra of these compounds are 520, 532, and 548 nm, respectively. The CL spectral peaks coincided with the positions of CL of the corresponding 3-hydroperoxides and the fluorescence maxima of amidoacetophenones. The transformations resulting in CL are presented in Scheme 2. This pattern differs from the authors' one in that the latter indicated additionally the

Scheme 2



isomeric forms of the excited anions with the substituents $-CCH_2O^-$ and $-COC^-H_2$.

The effect of 5- and 6-substituents in the series of 2,3-dimethylindoles (8 compounds) and hydroperoxides **11** (4 compounds) on the chemiluminescence properties of compounds have been studied.[12] Linear correlations of the logarithm of light yield with the Hammett function (σ) were found; the proportionality factors (ρ) were -1.24 for the indole series and -2.04 for the peroxide series. When constructing the dependences for 6- and 5-substituents, σ values corresponding to *para*- and *meta*-substituents were taken. Interpretation of the found correlations is hampered by the fact that the light yield in a chemiluminescence reaction is determined by the product of three terms: the chemical yield along the light reaction pathway, the chemiexcitation yield, and luminescence yield; each term depends on the starting compound structure.

In 1968 a group of chemists from the USA published the results of CL studies of more than 50 indole derivatives in the DMSO–Bu^{t}OK system.[14] Analysis of the vast experimental knowledge led the authors to conclusion that 3-alkylindoles are the most effective chemiluminophores in this system as regards the light yield, in particular, 5-benzyloxy-3-methylindole, 5-methoxy-3-methylindole, 3-methylindole, 3,5-dimethylindole and 3-ethylindole were most effective (this sequence corresponds to a decrease in this parameter). The CL spectra of 3-methylindole (corrected for self-absorption of the solutions, $\lambda_{max} \approx 500$ nm, uncorrected, 490 nm) and 2,3-dimethylindole ($\lambda_{max} \approx 530$ and 516 nm, respectively) coincide with the fluorescence spectra of *o*-formamidoacetophenone and *o*-acetamidoacetophenone anions, respectively (Fig. 1).

The acylamide anions were identified as primarily excited products of indole autooxidation and CL emitters. It was found that the anionic form of the corresponding derivative is the starting oxidation substrate. The authors also presented the transformation scheme for 3-methylindole; this is similar to Scheme 2. The most important points of this scheme are the formation of peroxide intermediate and its conversion to unstable 1,2-dioxetane. The quantum yield of CL of 3-methylindole is $(1.5 \pm 0.6) \times 10^{-3}$ Einstein mol^{-1}; hence, for the chemical yield of *o*-formamidoaceto-

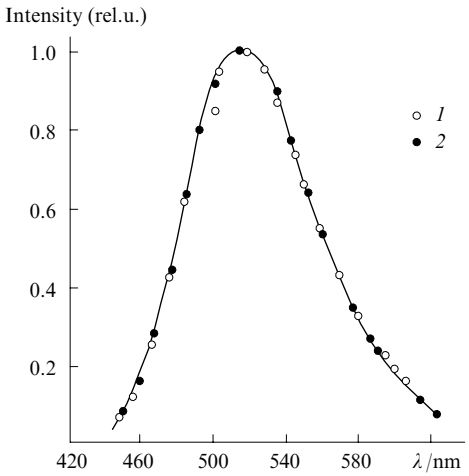


Figure 1. Chemiluminescence spectrum of 2,3-dimethylindole (*1*) and fluorescence spectrum of *o*-acetamidoacetophenone (*2*).

phenone equal to 0.4 and its luminescence quantum yield under the reaction conditions equal to 0.2, the excitation yield of the *o*-formamidoacetophenone anion in this reaction is ~ 0.02.

Note that the spectral data on the CL of 2,3-dimethylindole and hydroperoxide **11** reported by various authors do not coincide. This is due to the difficulty of experimental measurement of the spectra of weakly glowing subjects whose glow intensity varies with time. One more point is that the CL spectra either coincide or not with the fluorescence spectra of the putative emitters.

In the general case, the chemi- and photoluminescence spectra do not necessarily coincide. For example, lack of coincidence of the spectra was noted [15–17] in a comparison of the CL that accompanies decomposition of some substituted 1,2-dioxetanes with the photoluminescence of carbonyl compounds formed upon dioxetane decomposition, *i.e.*, CL emitters. However, in two of the cited studies,[15,16] different effects of the medium for photo- and chemiexcitation were found, whereas in the third work,[17] the difference (tens of nanometres) between CL and fluorescence spectra was attributed to structural features of the reactants. The presence of bulky substituents such as an anthrylphenol fragment results in different stereochemistry of the electronically excited state of the CL emitter, *viz.*, anthrylphenol-substituted ketoester, either generated upon the chemical reaction or populated by the photon absorption. These observations can be interpreted qualitatively in the following way.

Photoexcitation is a vertical Franck–Condon radiative transition with fixed nuclei of the molecular system. The transition takes place between spectroscopic minima on multidimensional potential energy surfaces (PES) of the ground and excited states of, in this case, the ketoester.

Chemiexcitation is a much more complex process. It takes place upon horizontal transition, namely configurational rearrangement of atomic nuclei, *i.e.*, migration of the imaginary point along the reaction coordinate on the ground state PES from the reactant (dioxetane) potential well to the product (ketoester) potential well. Half-way the system gets into the region where the ground and excited state PES are proximate (quasi-intersection area), it 'inertially' crosses to the excited state PES (of course, far from the minimum) and continues to move along towards the spectroscopic minimum. Also, the molecule can pause in another local well (corresponding to a product conformer) and from there, undergo a radiative transition to the ground surface (Fig. 2). Therefore, the spectrum of CL differs from the spectrum emitted upon photoexcitation.

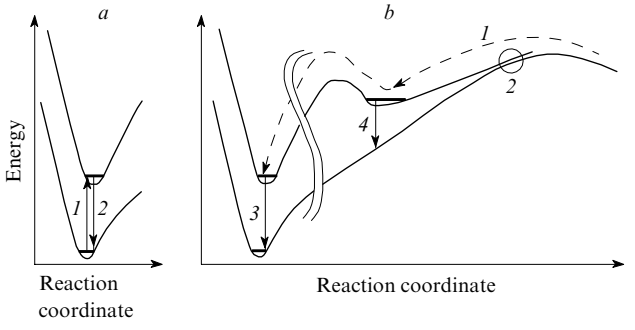


Figure 2. Scheme of transitions between potential energy surfaces. (*a*) Photoluminescence: (*1*) photon absorption (vertical radiative transition from the spectroscopic minimum), (*2*) fluorescence (vertical radiative transition from the spectroscopic minimum of the excited surface). (*b*) Chemiluminescence: (*1*) chemiexcitation (horizontal nonradiative transition to the excited surface), (*2*) quasi-intersection region of the potential energy surfaces, (*3*) vertical radiative transition from the spectroscopic minimum (the CL spectrum coincides with the fluorescence spectrum), (*4*) vertical radiative transition from the local minimum (the CL spectrum does not coincide with the fluorescence spectrum).

2. Chemiluminescence of indoles in the absence of a base

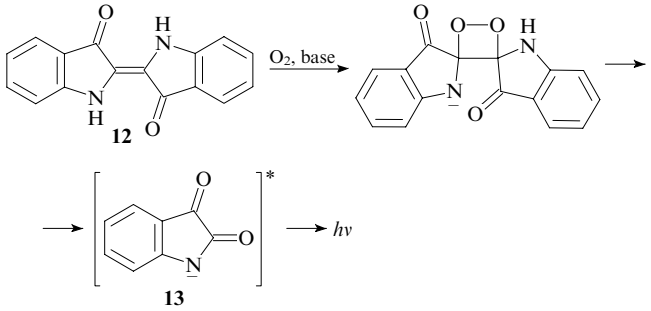
The above-noted publication [13] occupies a special position, and this is due to the fact that in all previous works, the indole CL was observed in highly alkaline medium, while this work reports CL of 3-methylindole, indole and 3-phenylindole in the reaction with peroxybenzoic acid in chloroform in the absence of a base. The CL intensity under these conditions was very low, and its spectrum was not measured.

Chemiluminescence of tryptophan (**2**) in sulfuric acid (~ 0.2 mol litre^{-1}) was found on oxidation with Ce(IV) ions [18] or peroxynitric acid (ONO_2H),[19] which was prepared in the reaction vessel by mixing solutions of H_2O_2 (0.25 mol litre^{-1}) and KNO_2 (0.1 mol litre^{-1}) directly during the experiment. Under similar conditions, tryptophan CL sensitized by trivalent terbium was observed upon oxidation with peroxynitric acid.[20] The CL intensity in the presence of Tb(III) (0.02 mol litre^{-1}) increases more than 5-fold. The spectra of non-sensitized CL recorded in two studies [19,20] do not coincide [peaks at 550 (Ref. 19) and 525 nm (Ref. 20)].

III. Chemiluminescence of indoles without pyrrole ring opening

Chemiluminescnce of indigo (**12**) was observed on air oxidation in the presence of strong alkali in DMSO and DMF.[21] The CL quantum yield reached 5.3×10^{-4} Einstein mol^{-1}. The isatin (indole-2,3-dione) anion (**13**) was the stable reaction product and a possible emitter. The authors suggested the intermediate formation of dioxetane at the $2-2'$ double bond of indigo (Scheme 3).

Scheme 3



The chemiluminescence reaction has a complex kinetics and has not yet been studied. The most intriguing points are the following: narrow temperature range (9–12 °C) in which CL is observed; unexpected sharp repeated CL build-up 3–4 min after the start of the reaction (Fig. 3), and giant enhancement of the CL upon the second addition of a solution of NaOH to the reaction medium.

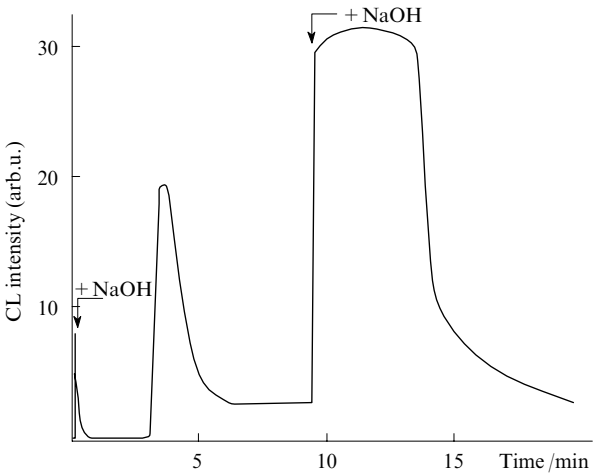


Figure 3. Chemiluminescence kinetics in aerated solution of indigo (**12**) in DMF.
The arrows mark the instants of addition of a NaOH solution.

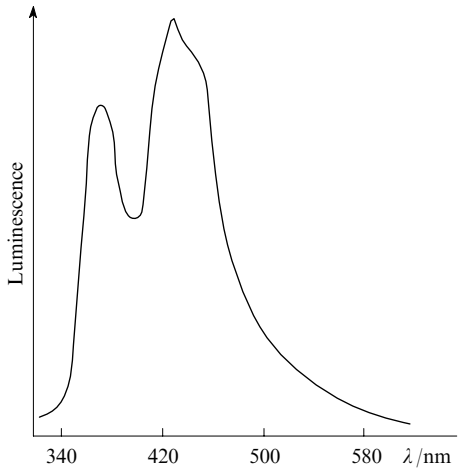
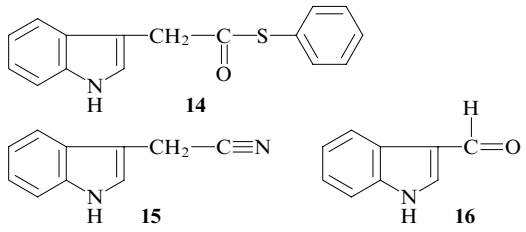


Figure 4. Photoluminescence spectrum of aldehyde **16** (1.72 mmol litre^{-1}) in deaerated DMSO in the presence of Bu^{t}OK (15 mmol litre^{-1}); λ_{excit} = 300 nm.

IV. Dioxetanones, dioxetanimines and record chemiluminescence of dioxetanes

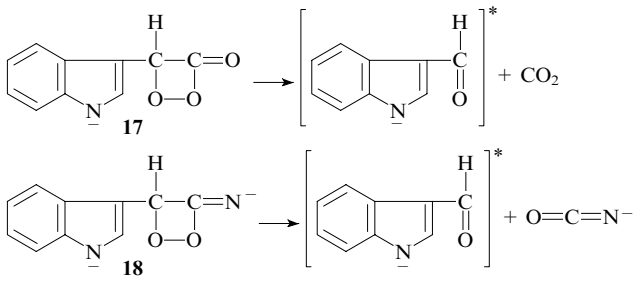
1. Dioxetanones and dioxetanimines

In 1976 biochemists from Brazil [22] detected CL for *S*-phenyl indol-3-ylthioacetate **14** and indol-3-ylacetonitrile (**15**) in an aerated DMSO solution (at a sample concentration of 0.6 mmol litre^{-1}) in the presence of Bu^{t}OK (4 mmol litre^{-1}). The maximum of the CL spectrum occurs at 435 nm in both cases; this corresponds to the luminescence maximum of aldehyde **16**, which was also detected among the reaction products.



The authors suggested that dioxetanone **17** and dioxetanimine **18** shown in Scheme 4 are intermediates preceding the formation of the excited aldehyde (we took the liberty to draw the indole fragment as an anion, which corresponds to both the reaction conditions and the conditions of recording the luminescence spectrum of aldehyde **16** presented in Ref. 22).

Scheme 4

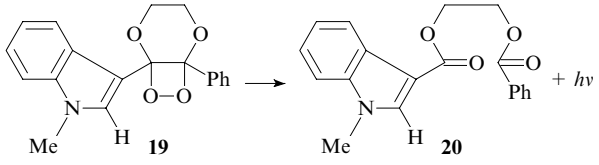


In the authors' opinion, the observed CL is emitted by a chemiexcited singlet molecule of aldehyde **16**. In the study cited,[22] the photoluminescence spectrum of compound **16** in a highly alkaline deaerated DMSO is reported (Fig. 4). The two-band structure of the spectrum (recall that the fluorescence of indole in ethanol has one band at 330 nm) and the absence of oxygen (phosphorescence quencher) in the solution suggest that the long-wavelength emission of compound **16** is phosphorescence. Thus, triplet aldehyde **16** is the major chemiexcited product. This is also in line with the results of other studies,[23–26] namely, with the fact that on dioxetanimine decomposition, the yield of chemiexcited triplet molecules is several orders of magnitude higher than the yield of singlet molecules.

An interesting feature of CL of compound **14** is that under certain conditions (the initial concentration >0.7 mmol litre^{-1}, temperature of the reaction medium <44 °C), the spectrum exhibits an additional band at 525 nm apart from the band at 435 nm. The authors demonstrated that the exciplex formed by the excited molecule **16** and molecule **14** is responsible for the emission in the green spectral band (This also implies that excited molecule **16** exists in the triplet state, because the exciplex formation by singlet excited molecule **16** is unlikely.)

2. Record chemiluminescence of dioxetanes

Compound **19** known among indole dioxetanes is converted to diester **20** on heating from -46 °C to room temperature.[27]



Characteristics of the CL of dioxetane **19** are unique, at least, among organic chemilumonophores. The maximum of the CL spectrum in non-polar solvents (*e.g.*, hexane) occurs in the UV region (320 nm), *i.e.*, upon chemiexcitation, a large portion of energy (>377 kJ mol^{-1}) transforms from chemical energy to electronic excitation. The chemiexcita-

tion during the rearrangement of compound **19** to diester **20** is highly efficient, its yield exceeding 0.5 (the CL yield is 0.036). In polar solvents ($CHCl_3$, tetrahydrofuran, CH_2Cl_2, acetone), a peak at 400–415 nm appears in the CL spectrum. This is assigned to light emission by the 'intramolecular exciplex' excited by charge transfer between the indole and benzoate chromophores. However, an attempt to synthesize a fluorescing molecule with the geometry of the hypothetical exciplex failed.

Indole dioxetanes relatively stable at room temperature were prepared.[28,29] The increase in the stability was attained by acylation of nitrogen of the indole fragment.

V. Indoles and reactive species

1. Indoles and singlet oxygen

One route to dioxetanes is addition of singlet oxygen to the C=C bond.[30] This reaction was used to obtain indole-containing dioxetanes at the C(2)–C(3) bond for the subsequent chemiluminescence determination.

Kawatani *et al.*[31] obtained singlet oxygen by oxidation of sodium molybdate with hydrogen peroxide

$$2\,H_2O_2 \xrightarrow{MoO_4^{2-}} {}^1O_2 + 2\,H_2O.$$

In this process molybdate functions as a catalyst of hydrogen peroxide disproportionation, the generation rate of singlet oxygen is 4×10^{-6} mol litre^{-1} s^{-1} at an H_2O_2 concentration of more than 0.07 mol litre^{-1} and MoO_4^{2-} concentration of more than 1 mmol litre^{-1} (Ref. 32). The data on light yield during the first 200 s (Σ_{200}) under reaction conditions where 100-μl portions of H_2O_2 (0.1 mol litre^{-1}), indole derivative (1 mmol litre^{-1}) and MoO_4^{2-} (0.02 mol litre^{-1}) solutions were mixed successively are summarized in Table 2.

Table 2. Light yield in the reactions of indole and its derivatives.

Compound	Σ_{200} (arb. u.)	Relative to indole
Melatonin (*N*-acetyl-5-methoxytryptamine)	1.2×10^8	364.0
3-Methylindole	7.5×10^6	23.0
Indol-3-ylacetic acid	6.1×10^6	19.0
Serotonin (5-hydroxytryptamine)	1.5×10^6	5.0
5-Hydroxyindol-3-ylacetic acid	1.4×10^6	4.0
5-Hydroxyindole	8.9×10^5	3.0
5-Hydroxytryptophan	6.5×10^5	2.0
Tryptamine	3.4×10^5	1.0
Indole	3.3×10^5	1.0
Tryptophan	3.6×10^4	0.1
Indole-3-carbaldehyde	1.2×10^4	0.04
Ethanol (control run)	2.0×10^3	–

Note. Reaction conditions: H_2O_2 and sodium molybdate are used as aqueous solutions, indoles are dissolved in EtOH, pH of the mixture ~10.

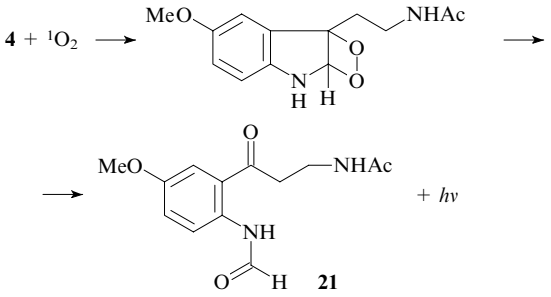
By using a flow-type reaction cell and by increasing the temperature of the reaction mixture, the authors attained a detection limit for compound **4** of about 2×10^{-9} mol litre^{-1} (sample volume 100 μl); the CL signal obeyed a linear dependence on the melatonin (**4**) concentration in the range of 10^{-8}–10^{-3} mol litre^{-1}.

Lu *et al.*[33] obtained singlet oxygen by the reaction between nitriles and hydrogen peroxide[34]

$$R-C\equiv N + H_2O_2 \longrightarrow {}^1O_2 + RCONH_2 + H_2O.$$

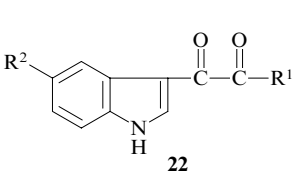
The authors[33] proposed the addition of $^1O_2$ to the C(2)–C(3) double bond and the subsequent reactions according to Scheme 5:¶

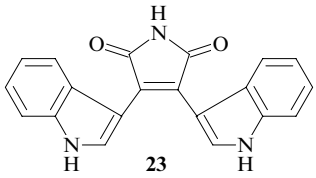
Scheme 5



This is a hypothetical scheme. The participation of singlet oxygen was substantiated[33] using inhibitor analysis (CL quenching by 1,4-diazabicyclo[2.2.2]octane and dimethylfuran). The formation of N^1-acetyl-N^2-formyl-5-methoxykynuramine (**21**) on melatonin oxidation with singlet oxygen was proved in another study.[35]

Under conditions similar to those used to observe CL in Ref. 33, the CL of 3-glyoxyl derivatives of indole (**22**)[36] and 2,3-bis(indol-3-yl)maleimide (**23**)[37,38] was found.

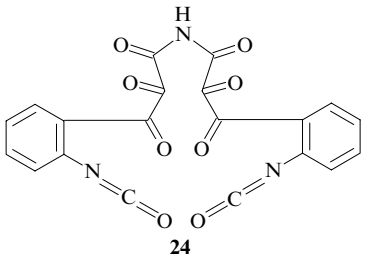




R^1 = Cl, OH, OMe, NMe_2;
R^2 = H, OH, OPh.

For 3-indoleglyoxylyl chloride, two reaction pathways resulting in light emission were proposed (Scheme 6).

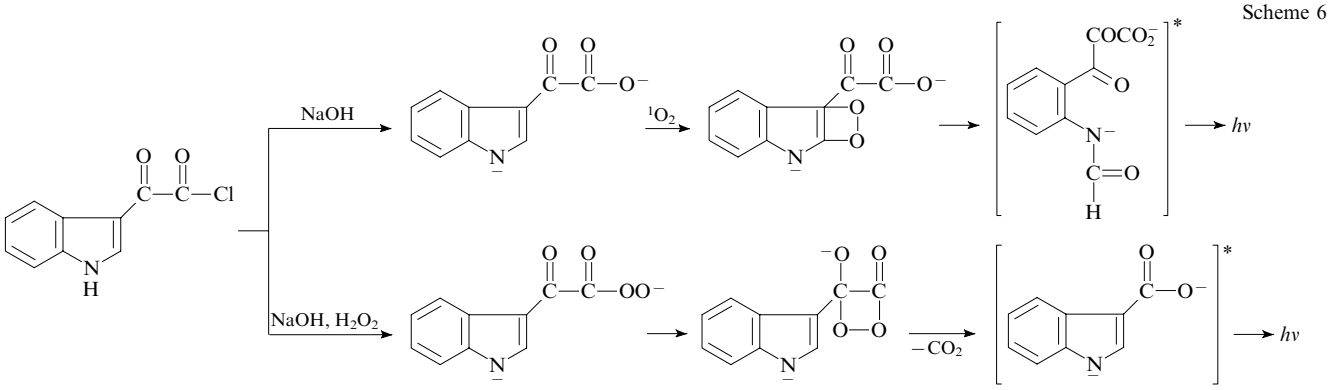
The CL spectral maximum of compound **23** is located near 430 nm; the CL emitter is octacarbonyl **24**, which is formed upon rearrangement of a complex molecule incorporating three (!) dioxetane fragments. This molecule is formed upon addition of three molecules of singlet oxygen to the pyrrole and two indole fragments [to the C(2)=C(3) double bonds].



Note that in the studies cited,[31,32,36–38] CL was observed in alkaline media, and the researchers observed considerable attenuation of CL at pH < 9. This might be

¶ Scheme 5 is reproduced with corrected mistakes in the structural formulas.

Scheme 6



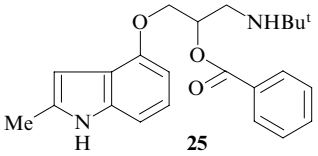
the reason for the fact that in another study[39] where $^1O_2$ was generated by the reaction between hydrogen peroxide and hypochlorite in acidic medium, only energy transfer was detected from singlet oxygen dimol to the indole fluorophore, tryptophan.

The chemiluminescence of indoles in the presence of hydrogen peroxide and hypochlorite in alkaline medium was employed for hypochlorite[40,41] and melatonin[42] determination (with a detection limit of 5×10^{-8} mol litre^{-1}) in tap water.

Below we discuss the works on the indole CL where the participation of singlet oxygen is highly probable but not adequately substantiated.

Chen *et al.*[43] observed CL by incubating aqueous solutions of indoles **1**, **2**, **5** in acidic medium in the presence of hydrogen peroxide and Fe^{3+} ions followed by addition of a solution of NaOH. Decomposition of H_2O_2 to O_2 and H_2O catalyzed by Fe^{3+} ions in low concentration is known to follow a radical chain mechanism giving intermediately $HO_2^{\bullet}$, $HO^{\bullet}$ and Fe^{2+} (see Ref. 44). The formation of singlet oxygen under these conditions is quite likely. Low CL was observed[45] in the $H_2O_2-Fe^{3+}$ system in the visible spectral region (apparently, this is luminescence of the singlet oxygen dimol).

The CL of bopindolol (**25**), a pharmaceutical agent used to treat cardiac arrhythmia and hypertension, has been studied.[46]



25

The chemiluminescence of bopindolol occurs in a carbonate buffer (pH 9.3) upon oxidation during H_2O_2 decomposition by Co(II) complex with ethylenediaminetetraacetic acid. The maximum of the CL spectrum occurs at ~500 nm; the spectrum has an additional peak for singlet oxygen dimol luminescence at 640 nm.

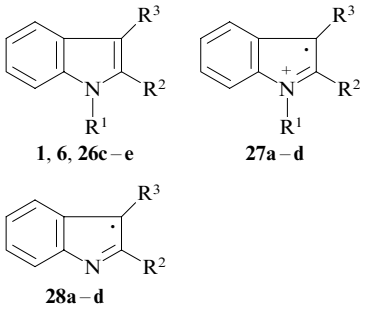
2. Indoles and carbonate radical anion

An important role of the $CO_3^{-\bullet}$ radical anion in the oxidation and CL of melatonin (probably, not only melatonin) was revealed.[47] In the presence of this radical, the CL intensity increases severalfold. In the reaction medium, $CO_3^{-\bullet}$ appears upon oxidation of the HCO_3^- anion added to the system. In aerated alkaline solutions, especially in alkaline DMSO, the HCO_3^- anions arise upon absorption of CO_2 from air.

3. Indoles and superoxide radical anions

The question of the ways of participation of superoxide radical anion in the indole CL was answered in a number of works.[48–50]

Shen *et al.*[48] studied radiolysis of the products of one-electron oxidation of indoles **1**, **6**, **26c–e**. It was found that one-electron oxidation of compounds **1**, **6**, **26c**,**d** in neutral solutions gives radicals **28a–d** as primary products, while radical cations **27a–d** are formed only in acidic solutions.



1, **6**, **26c–e** **27a–d** **28a–d**

	R^1	R^2	R^3
a	H	H	H
b	H	H	Me
c	H	Me	Me
d	H	Me	Ala
e	Me	H	H

When the radical cations (as shown for indole **27e**) react with $O_2^{-\bullet}$, electron transfer takes place

$$\mathbf{27e} + O_2^{-\bullet} \longrightarrow \mathbf{26e} + O_2 .$$

The authors noted that this reaction could yield singlet oxygen.

The reaction of radicals **28** with $O_2^{-\bullet}$ affords the corresponding 3-hydroperoxide anion, which initiates the light-generation (see Scheme 1).

Okajima and Ohsaka[49,50] generated the superoxide radical anion by electroreduction of dissolved oxygen in acetonitrile in the presence of tetraethylammonium perchlorate (supporting electrolyte) on pyrolytic graphite electrode. Synchronously, cyclic voltammogram and the CL signal were recorded. It was found that voltammograms measured in the presence and in the absence of compounds unable to act as proton donors are almost identical. In the presence of indoles, which are proton donors, the anodic current decreases, while the cathodic current increases; in addition, one more anodic current peak appears (without a pair in the cathodic region). The chemiluminescence is recorded at the instant of 'passage' of the additional anodic peak.

The authors explained the results by the following set of processes:

(i) proton transfer from the indole derivative (IndH) to the superoxide radical anion to give the hydroperoxide radical and the low-mobility anion of the indole compound

$$O_2^{\bullet-} + IndH \longrightarrow HO_2^{\bullet} + Ind^-,$$

(ii) single-electron electrooxidation of the Ind^- anion in the region of additional anodic current peak to give the $Ind^{\bullet}$ radical

$$Ind^- - e \longrightarrow Ind^{\bullet},$$

(iii) addition of the $HO_2^{\bullet}$ radical (or $O_2^{\bullet-}$) to the carbon atom in position 3 of the indole fragment of the $Ind^{\bullet}$ radical,

(iv) the subsequent steps are the same as in Scheme 1.

Thus, it can be concluded that the superoxide radical anion is necessary for CL of indoles provided that they have undergone preliminary single-electron oxidation.

Electrochemiluminescence of tryptophan and indole in the presence of hydrogen peroxide was used[51] for their determination with a detection limit of 10^{-7} mol litre^{-1}.

4. Indoles as non-specific participants of the chemiluminescence process

In some chemiluminescence reactions, indoles take part as reducing agents and ligands in coordination compounds. These reactions were used to develop chemiluminescence-based detection of a number of biologically active indole compounds in high performance liquid chromatography and in flow injection analysis.[52–60] They are highly sensitive providing the following detection limits (nmol litre^{-1}): for compound **3**, 2;[54] for tryptamine, 5;[55] for compound **4**, 20;[56] for compound **5**, 0.6,[57] 115;[58] and for compound **2**, 10,[55] 25,[59] and 2.5.[60]

Highly sensitive methods of chemiluminescence detection of indoles as dansyl derivatives (for *N*-methyltryptamine, 5-methyltryptamine, α-methyltryptamine, bufotenin and psilocin) and as 6-aminomethylphthalhydrazide derivatives (for 5-hydroxyindol-3-ylacetic acid and α-methylserotonin) have been developed.[61,62]

VI. Ultraweak luminescence of biological objects

The ultraweak luminescence (UWL) of biological objects[63] has long been attributed to peroxide oxidation of lipids.[64] In recent years, weighty grounds appeared for assuming that the oxidative degradation of amino acids and proteins can also make a considerable contribution to the UWL.

The CL of bovine serum albumin (BSA) on oxidation with peroxynitrite in the phosphate buffer at pH 8 was detected.[65,66] A study of the effects of dibromoanthracene (DBA) added as a test for triplets and diphenylanthracene (DPA) added as a test for singlets (20 μmol litre^{-1}) showed that DPA additive induces a marked increase in the CL intensity. This attests to a singlet nature of the emitter in BSA chemiluminescence. In oxidation of pure amino acids with peroxynitrite, it was found that only tryptophan oxidation is accompanied by pronounced CL.[66] The emission maximum for both BSA and tryptophan (**2**) is located at 475–575 nm. It was concluded that compound **2** is the substrate of oxidative CL of proteins.

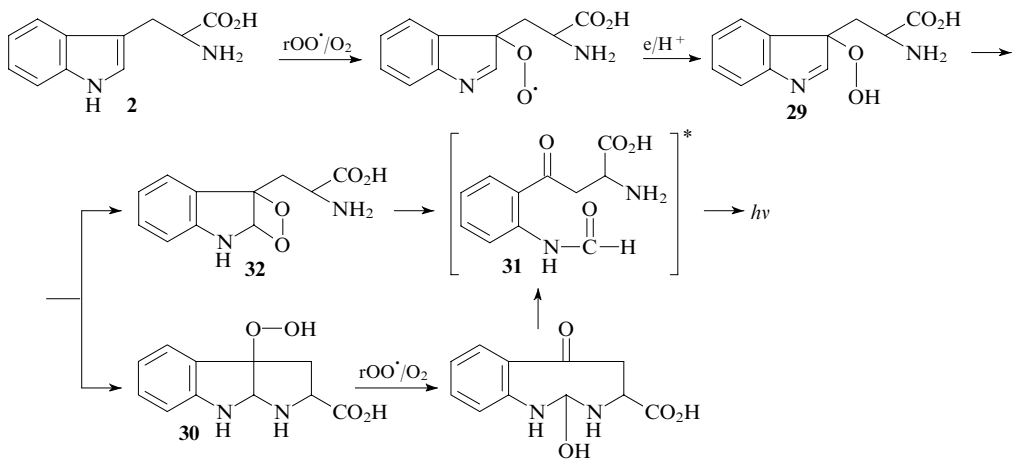
Chemiluminescence that accompanies the initiated oxidation of proteins and amino acids was studied.[67,68] 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was used as water-soluble radical initiator. Of all amino acids studied, only tyrosine and tryptophan show pronounced oxidative CL. Comparison of the chemiluminescence behavior of these amino acid and some proteins in the presence of AAPH elicited the existence of general trends for light generation during initiated free radical oxidation. Aspée and Lissi[67,68] proposed Scheme 7 for the mechanism of CL of tryptophan, a key participant of the 'protein' UWL processes. The Scheme is largely based on the knowledge on oxidative degradation of this amino acid initiated by other methods under other conditions. Tryptophan hydroperoxide (**29**) is a key intermediate product formed in the reaction of compound **2** with single-electron oxidants in the presence of oxygen.[48,67–71] Intermediate **30** was found in the photooxidation products of compound **2**.[72]

Scheme 7 shows the light reaction pathways (the dark reaction pathways are ignored) and the key peroxide intermediates that can be formed in the oxidative processes involving different reactive oxygen species. This Scheme was also used to describe the tryptophan CL during oxidation with singlet oxygen.[73]

In recent publications dealing with singlet oxygen oxidation of compound **2** (both free[74] and as a part of proteins and peptides[75]), the attention is concentrated on the goal to identify and structurally characterize the products and intermediates. However, Ronsein *et al.*[74] admitted that, despite the progress attained to date, it is still much to be done to establish the detailed mechanism of CL arising during oxidation of compound **2**.

The chemiluminescence properties of hydroperoxide **30** were studied.[74] This compound is formed as a mixture of

Scheme 7



cis- and *trans*-isomers in the oxidation of compound **2**. Their thermal transformation is accompanied by luminescence whose spectrum shows two bands with peaks at 436 and 510 nm. The shorter-wavelength band (436 nm) corresponds to the fluorescence spectrum of formylkynurenine (**31**), while the origin of the longer-wavelength is still not entirely clear. In the opinion of the researchers cited,[74] the nature of the latter emission band may be different ranging from the formation of exciplexes to a peculiar effect of the intramolecular hydrogen bond (between the N−H and carbonyl groups) in molecule **31** on chemiexcitation. It is also noteworthy that the CL of hydroperoxide **30** was enhanced upon introduction of alkali; this was attributed to catalytic acceleration of the decomposition of dioxetane intermediate **32**, which can also apparently be formed from hydroperoxide **30**.

Note that works considering the interaction of reactive oxygen species with tryptophan and the CL that accompanies these processes continue the studies on stimulated CL of tryptophan, glycyltryptophan and proteins carried out by I I Sapezhinsky and his followers.[76] The acquaintance with his works at a new phase of investigations is both a tribute of respect to the forerunners and considerable time saving, since, as noted in N Turro's interview to the journal *Spectrum*: 'Three months in the laboratory can save a couple of hours in the library.'†

VII. Chemiluminescence of indoles in enzymatic processes

The studies of CL that accompanies oxidation of indoles in enzymatic systems started in the late 1960s.[77] The main investigation objects in the 1970–1990 included, among indoles, indol-3-ylacetic acid (**5**) and, among enzymes, animal or plant peroxidases.

The knowledge on the classification and the current state of the studies of plant peroxidases have been presented in a number of publications.[78–83] In the context of this review, we would like to note that peroxidase, in particular horseradish peroxidase (HRP) isolated from the horseradish roots is a haem-containing enzyme (molecular mass ~40 000), which accomplishes its peroxidase function in the presence of hydrogen peroxide, while in the absence of H_2O_2 (or RO_2H) and in the presence of O_2, it functions as an oxidase. The HRP-catalyzed oxidation of compound **5** is a rather complex process. During this process, the initial enzyme transforms into a number of catalytically active and passive forms, which differ in the oxidative state of the haem iron [Fe(II), Fe(III) and Fe(IV)] and its environment. Compound **5** is oxidized along a number of pathways, differing by both the intermediates and products. Unlike animal peroxidases, HRP has a specific centre of binding of compound **5**.

The peroxidase cycle operates with various oxidizable substrates; it can be represented by the sequence of three transformations:

$$E + H_2O_2 \longrightarrow EI + H_2O,$$

$$EI + SH \longrightarrow EII + S^{\bullet},$$

$$EII + SH + H^+ \longrightarrow E + H_2O + S^{\bullet},$$

where E, EI, EII are native enzyme and its forms: compound I and compound II, and SH and $S^{\bullet}$ are the substrate and the product of its single-electron oxidation, respectively.

The aerobic oxidation of HRP is highly specific to the substrate. It starts with the formation of the enzyme−**5**−O_2 ternary complex in which the radical cation $\mathbf{5}^{+\bullet}$ and the superoxide radical anion are formed. The radical cation $\mathbf{5}^{+\bullet}$ is then decarboxylated to give the skatolyl radical (Ind-3-$CH_2^{\bullet}$), which is oxidized with oxygen to peroxyl radical. In neutral medium, radical cation $\mathbf{5}^{+\bullet}$ is rapidly deprotonated to give neutral radical the decarboxylation of which is more than 100 times slower.

Switching from the peroxidase oxidation to aerobic (oxidase) oxidation depends on the pH and on the concentration ratio of HRP to **5**.

In the radical pool formed in the **5**–HRP–H_2O_2–O_2 system, a series of radical (including chain) reactions take place in the case of both peroxidase and oxidase action of enzyme, some of these being accompanied by chemiexcitation and light emission.[84]

Of particular interest are reactions that accompany the aerobic oxidation as highly specific to indole **5**. First, the attention of researchers was attracted by the CL of compound **5** in the IR range, related to the appearance of excited oxygen molecules in the $^1\Delta_g$ state. The formation of singlet oxygen in the peroxidase-catalyzed aerobic oxidation of compound **5** was first assumed[85,86] and then established reliably[87] based on light emission in the IR range (~1270 nm), which is the 'visiting card' of singlet oxygen. The mechanism of formation of singlet oxygen assumed in the mentioned works[85,86] was based on energy transfer from primarily excited indole-3-carbaldehyde triplet to oxygen, while in other studies,[87,88] this was the Russell mechanism[89] of disproportionation of Ind-3-$CH_2OO^{\bullet}$ radicals to give six-centre tetroxide, which decomposed to oxygen, an alcohol and a carbonyl compound. Note that CL of compound **5** in the IR range is observed only for pH < 5 and disappears in the neutral medium.[87] The lifetime of singlet oxygen in D_2O is more than 10 times longer than in H_2O; therefore, the IR CL that accompanies oxidation of **5** is much more intense in heavy water, where it was found in experiments described by Kanofsky.[87]

Apart from the IR emission band (1270 nm), singlet oxygen also had an emission band of singlet oxygen dimols in the red region (635 nm).[85,86]

In a number of works,[90–92] the CL spectra were recorded in the range of 350–570 nm upon aerobic oxidation of compound **5** in neutral medium. The CL spectra in the HRP–**5**–O_2 system are shown in Fig. 5. The conditions of recording spectra *1* and *2* are closely similar: the HRP concentration is 0.4 μmol litre^{-1} (*1*) and 1.1 μmol litre^{-1} (*2*), the concentrations of compound **5** are 0.2 and 0.5 mmol litre^{-1}, pH is 7.0 and 7.4, temperature is 25 and 37 °C, respectively. However, the spectra differ considerably, although the principal maximum is at 410–430 nm in all three cases. It was assumed[91] that several emitters are involved. This assumption could be verified by a published method,[93] which consists of recording the CL kinetics in several, relatively narrow spectral ranges corresponding to the emission maxima of the assumed emitters. If the intensity ratios recorded in different spectral channels are time-dependent, then participation in the CL of several emitters arising in different chemical pathways can be assumed. Conversely, a constant ratio would be evidence in favour of only one emitter.

† *Spectrum* **17** (1) 3 (2004).

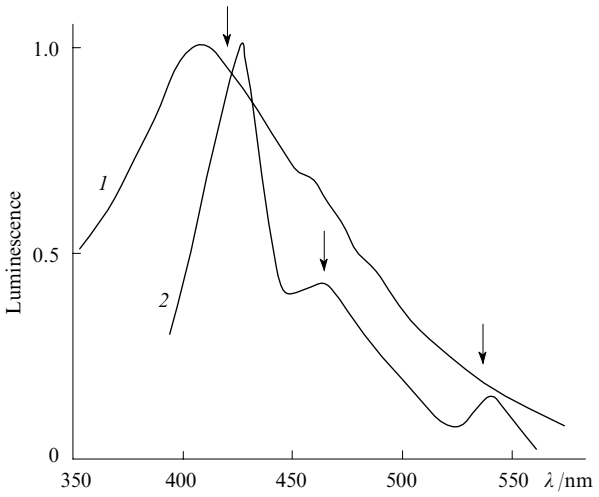
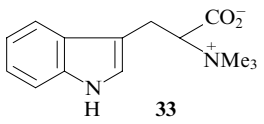


Figure 5. Chemiluminescence spectra in the HRP–**5**–O_2 system at neutral pH from Refs 90 (*1*) and 92 (*2*).
The arrows mark the positions of the spectral peaks from Ref. 91.

To estimate the proportion of light emitted by singlet oxygen dimols in the total flux of the emitted light, the CL kinetic curves were recorded [92] in the presence and in the absence of a red light filter mounted between the reaction vessel and the photodetector. It was found that at pH 7.4, emission in the red region provides a minor portion of the emitted light, and the profiles of the kinetic curves are different. At pH 5, the CL kinetics considerably differs from that observed at pH 7.4: the CL intensity considerably decreases, the proportion of the red light increases, and the kinetic profiles become similar. It was shown [90] that CL in the HRP–**5**–O_2 system occurs in the pH range from 5 to 9 and the light yield is maximum at pH 7. The replacement of H_2O by D_2O in the pH optimum is accompanied by a considerable decrease in the light yield. These data, together with the spectral kinetic results [92] indicate that CL observed at 350–570 nm at neutral pH is not a consequence of generation of singlet oxygen by the mechanism proposed by Khan and Kasha.[94]

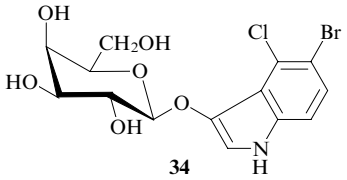
It was also noted [90] that the addition of a free radical inhibitor (2,5-di-*tert*-butylhydroquinone) in 19 μmol litre^{-1} concentration gives rise to an induction period in the kinetic curve of CL, which considerably increases as pH grows from 5 to 7. The effect of phenolic antioxidants on CL in the HRP–**5**–O_2 system was studied in detail.[95] Among the inhibitors of aerobic oxidation of compound **5**, special mention should be made of hypaphorine (**33**), tryptophan betaine. According to a publication,[80] compounds **33** and **5** compete for the peroxidase binding site specific to compound **5**. The use of hypaphorine in CL studies is of interest, first of all, as a tool of targeted interference.



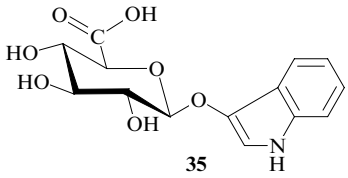
33

The wide use of HRP in analysis stimulated the interest in the search for new chemiluminogenic substrates, in particular, among indoles.

It was found [96] that 5-bromo-4-chloroindol-3-yl-β-D-galactopyranoside (**34**) is an effective chemiluminogenic substrate in the HRP–H_2O_2–O_2 system at pH 7–9. Probably, compound **34** is oxidized in the peroxidase cycle (no CL is observed without H_2O_2 or upon the addition of catalase to the reaction mixture, and superoxide dismutase does not affect CL). It is noteworthy that glycoside **35**, which exhibits oxidative CL under the action of Fenton's reagent, does not show CL in the HRP–H_2O_2–O_2 system.[97, 98]



34



35

Ximenes *et al.*[99] performed a search for chemiluminogenic substrates among 22 indoles in the HRP–H_2O_2–O_2 system at pH 9.3 and a temperature of 37 °C. Data on the chemiluminescence activity of these compounds are summarized in Table 3. It should be emphasized that, as in the above-mentioned study,[96] this concerns enzymatic oxidation in the peroxidase cycle (CL is absent after thermal inactivation of the enzyme and in the absence of H_2O_2). Analysis of the data presented in Table 3, especially in

Table 3. Light yield relative to indole for HRP-catalyzed oxidation of indole derivatives.

Compound	Light yield
2-Methylindole	1672.0
2,5-Dimethylindole	221.0
3-Methylindole	5.12
2,3-Dimethylindole	2.27
Melatonin	1.41
6-Chloromelatonin	1.06
Indole	1.00
6-Methoxyindole	0.98
4-Methoxyindole	0.96
2-Iodomelatonin	0.67
5-Methylindole	0.47
5-Methoxyindol-3-ylacetic acid	0.31
N-Methylindole	0.31
2-Methyl-5-methoxyindol-3-ylacetic acid	0.20
7-Methoxyindole	0.20
Indol-3-ylacetic acid	0.10
5-Methoxyindole	0.08
2-Phenylindole	0.04
6-Hydroxymelatonin	0
5-Hydroxytryptamine (serotonin)	0
5-Methoxytryptamine	0
Tryptophan	0

Note. Oxidation conditions: [HRP] = 0.2 μmol litre^{-1}, [H_2O_2] = 5 mmol litre^{-1}, pH 9.3, indole concentration 1 mmol litre^{-1}.

comparison with Tables 1 and 2, showes the following. First, this is the effect of position of the methoxy group in the benzene fragment (methoxylation into positions 4 and 6 does not affect, while that into positions 5 and 7 decreases the light yield 5 – 12-fold). Second, the effect of the position of the methyl group among methylindoles, the resulting sequence differing from that observed for non-enzymatic systems. Third, the enormous (more than 1600-fold) difference between the light yields for 2-methylindole compared to indole.

A study of 2-methylindole CL demonstrated [99] that an increase in the pH from 4.8 to 11.6 entails an increase in the light yield by a factor of more than 1400 and a decrease in the oxygen consumption rate by a factor of 470. This means that CL arises upon the peroxidase action of HRP; however, the oxidase cycle also functions and is also important for CL, as indicated by CL quenching by superoxide dismutase.

It may seem that the sequence of steps along the light reaction pathway is beyond doubt: the substrate undergoes single-electron oxidation in the peroxidase cycle, 2-methylindole being converted to the radical; the oxidase cycle is the supplier of $O_2^{-\bullet}$ radicals; the indole radical derivative is coupled with $O_2^{-\bullet}$ to give the 3-hydroperoxide anion and the subsequent events follow Scheme 1. However, in solution at pH 9, the dioxetane anion should undergo fast hydrolysis to give neutral dioxetane, and decomposition of neutral dioxetane gives rise to *o*-acetamidobenzaldehyde for which emission from the singlet level is at about 430 nm. However, the CL maximum of 2-methylindole is located at about 510 nm, which is apparently emitted by the acetamidobenzaldehyde anion (the luminescence maximum of the *o*-acetamidoacetophenone anion is at about 520 nm, see Fig. 1). Presumably, 2-methylindole and $O_2^{-\bullet}$ radicals interact if the pyrrole fragment of the molecule is located in the hydrophobic pocket of the enzyme, which protects the dioxetane anion from hydroysis. This assumption is also prompted by the enormous effect of the derivative structure on the CL (see Table 3).

Using investigation of the CL in the **5** – HRP – O_2 – H_2O_2 system as an example, we demonstrated that this phenomenon is intricate. Although the CL of indoles in other enzymatic systems is also known, we do not dare to plunge the reader more into this topic, at least, in this review.

VIII. Conclusion

The studies that were started as a result of incidental coincidence provided lots of non-trivial findings. Compounds that are only slightly inferior to luciferins in the chemiexcitation yield were obtained. The mechanisms of chemiluminescent transformations were established, although not universal but still covering a broad range of reactions involving various reagents. Many of the discovered reactions provided the grounds for highly sensitive methods of analysis, while some other bring about new questions.

Some of the publications dealing with the CL of indoles, specifically, the works dealing with light-induced CL of tryptophan and indol-3-ylacetic acid remained beyond the scope of this review, as they were considered in our previous work.[100]

When summing up, one cannot help considering whether it is accidental that the indole fragment can be found both in luciferins and hormones that regulate the circadian rhythm and in other metabolites. Perhaps, the expressions 'shine with happiness' and 'light with pleasure' are not without grounds and have a 'bioluminescence' sense.

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References

1. G E Philbrook, J B Ayers, F Garst, J R Totter *Photochem. Photobiol.* **4** 869 (1965)
2. F McCapra, D G Richardson, Y C Chang *Photochem. Photobiol.* **4** 1111 (1965)
3. A W Berger, J N Driscoll, J A Pirog *Photochem. Photobiol.* **4** 1123 (1965)
4. G E Philbrook, J R Totter *Techn. Rep. AD60279* (Athens: University of Georgia, 1964)
5. K Hyland, C Auclair *Biochem. Biophys. Res. Commun.* **102** 531 (1981)
6. R F Haseloff *Anal. Chim. Acta* **218** 179 (1989)
7. F McCapra, Y C Chang *J. Chem. Soc., Chem. Commun.* 522 (1966)
8. F McCapra *Quart. Rev. Chem. Soc.* **20** 485 (1966)
9. N Sugiyama, M Akutagawa, T Gasha, Y Saiga, H Yamamoto *Bull. Chem. Soc. Jpn.* **40** 347 (1967)
10. N Sugiyama, M Akutagawa *Bull. Chem. Soc. Jpn.* **40** 240 (1967)
11. N Sugiyama, M Akutagawa, H Yamamoto *Bull. Chem. Soc. Jpn.* **41** 936 (1968)
12. N Sugiyama, H Yamamoto, Y Omote, M Akutagawa *Bull. Chem. Soc. Jpn.* **41** 1917 (1968)
13. Y Omote, H Yamamoto, K Funasaki, M Akutagawa, N Sugiyama *Bull. Chem. Soc. Jpn.* **42** 3014 (1969)
14. A W Berger, J N Driscoll, J S Driscoll, J A Pirog, H Linschitz *Photochem. Photobiol.* **7** 415 (1968)
15. W Adam, I Bronstein, A V Trofimov *J. Phys. Chem. A* **102** 5406 (1998)
16. W Adam, M Matsumoto, A V Trofimov *J. Org. Chem.* **65** 2078 (2000)
17. M Matsumoto, N Watanabe, N Hoshiya, H K Ijuin *Chem. Rec.* **8** 213 (2008)
18. A A Alwarthan *Anal. Chim. Acta* **317** 233 (1995)
19. Y D Liang, J F Song *J. Pharm. Biomed. Anal.* **38** 100 (2005)
20. M Kaczmarek, S Lis *J. Alloys Compd.* **451** 186 (2008)
21. J Nikokavouras, G Vassilopoulos *Monatsh. Chem.* **112** 1239 (1981)
22. N Duran, K Zinner, R C De Baptista, C C C Vidigal, G Cilento *Photochem. Photobiol.* **24** 383 (1976)
23. Y Sawaki, Y Ogata *J. Am. Chem. Soc.* **99** 6313 (1977)
24. Y Ito, T Matsuura, H Kondo *J. Am. Chem. Soc.* **101** 7105 (1979)
25. Y Ito, H Yokoya, K Kyono, S Yamamura, Y Yamada, T Matsuura *J. Chem. Soc., Chem. Commun.* 898 (1980)
26. D T Breslin, M A Fox *J. Am. Chem. Soc.* **115** 11716 (1993)
27. H Nakamura, T Goto *Photochem. Photobiol.* **30** 27 (1979)
28. W Adam, M Ahrweiler, K Peters, B Schmiedeskamp *J. Org. Chem.* **59** 2733 (1994)
29. W Adam, D Reinhardt *J. Chem. Soc., Perkin Trans. 2* 1503 (1994)
30. W Adam, A V Trofimov, in *The Chemistry of Peroxides* (Ed. Z Rappoport) (Chichester: Wiley, 2006) p. 1171
31. T Kawatani, J-M Lin, M Yamada *Analyst* **125** 2075 (2000)
32. K Boehme, H D Brauer *Inorg. Chem.* **31** 3468 (1992)
33. J Lu, C Lau, M K Lee, M Kai *Anal. Chim. Acta* **455** 193 (2002)
34. E McKeown, W A Waters *Nature (London)* **203** 1063 (1964)
35. E A de Almeida, G R Martinez, C F Klitzke, M H de Medeiros, P Di Mascio *J. Pineal Res.* **35** 131 (2003)
36. M Nakazono, Y Sho, K Zaitsu *Anal. Sci.* **19** 123 (2003)
37. M Nakazono, A Uesaki, K Zaitsu *Talanta* **70** 128 (2006)
38. M Nakazono, S Nanbu, A Uesaki, R Kuwano, M Kashiwabara, K Zaitsu *Org. Lett.* **9** 3583 (2007)

39. P F de Violet, B Veyret, P Vincendeau, A Caristan *Photochem. Photobiol.* **39** 707 (1984)
40. M C Cheregi, J V G Mateo, J M Calatayud, A F Danet *Rev. Chim. (Bucharest)* **50** 325 (1999)
41. P Fletcher, K N Andrew, A C Calokerinos, S Forbes, P J Worsfold *Luminescence* **16** 1 (2001)
42. S-L Wei, L-X Zhao, X-L Cheng, J-M Lin *Chin. J. Chem.* **25** 535 (2007)
43. G-N Chen, X-Q Xu, J-P Duan, M-M He, F Zhang *Analyst* **120** 1699 (1995)
44. W G Barb, J H Baxendale, P George, K R Hargrave *Trans. Faraday Soc.* **47** 462 (1951)
45. V S Lebedev, I V Tsybanova, A P Rudenko *Zh. Fiz. Khim.* **49** 776 (1976) [a]
46. H Y Aboul-Enein, I Kruk, K Lichszteld *Biospectroscopy* **4** 229 (1998)
47. R Hardeland, B Poeggeler, R Niebergall, V Zelosko *J. Pineal Res.* **34** 17 (2003)
48. X Shen, J Lind, T E Erikson, G Merényi *J. Chem. Soc., Perkin Trans. 2* 597 (1990)
49. T Okajima, T Ohsaka *Electrochim. Acta* **47** 1561 (2002)
50. T Okajima, T Ohsaka *J. Electroanal. Chem.* **523** 34 (2002)
51. G N Chen, R E Lin, Z F Zhao, J P Duan, L Zhang *Anal. Chim. Acta* **341** 251 (1997)
52. T Nakagama, M Yamada, S Suzuki *Anal. Chim. Acta* **217** 371 (1989)
53. Y T Lee, C W Whang *J. Chromatogr., A* **771** 379 (1997)
54. N W Barnett, B J Hindson, S W Lewis *Anal. Chim. Acta* **362** 131 (1998)
55. A J Brown, C E Lenehan, P S Francis, D E Dunstan, N W Barnett *Talanta* **71** 1951 (2007)
56. G N Chen, F X Huang, X P Wu, F Z Zheng, J P Duan *Anal. Bioanal. Chem.* **376** 873 (2003)
57. A I P Neves, J R Albert-Garcta, J M Calatayud *Talanta* **71** 318 (2007)
58. Z Xi, Z Zhang, Y Sun, Z Shi, W Tian *Talanta* **79** 216 (2009)
59. H Chen, L Li, M Zhou, Y J Ma *Chin. Chem. Lett.* **19** 203 (2008)
60. Z Lin, X Chen, Z Cai, P Li, X Chen, X Wang *Talanta* **75** 544 (2008)
61. J Cepas, M Silva, D Pérez-Bendito *Analyst* **121** 49 (1996)
62. T Yakabe, J Ishida, H Yoshida, H Nohta, M Yamaguchi *Anal. Sci.* **16** 545 (2000)
63. B N Tarusov, A I Polivoda, A I Zhuravlev *Biofizika* 490 (1961) [b]
64. Yu A Vladimirov *Soros. Obr. Zh.* 25 (1999)
65. B P Watts Jr, M L Barnard, J F Turrens *Arch. Biochem. Biophys.* **317** 324 (1995)
66. E Pollet, J A Martínez, B Metha, B P Watts Jr, J F Turrens *Arch. Biochem. Biophys.*. **349** 74 (1998)
67. A Aspée, E A Lissi *Luminescence* **15** 273 (2000)
68. A Aspée, E A Lissi *J. Protein Chem.* **20** 479 (2001)
69. T J Simat, H Steinhart *J. Agric. Food Chem.* **46** 490 (1998)
70. M Nakagawa, S Kato, S Kataoka, T Hino *J. Am. Chem. Soc.* **101** 3136 (1979)
71. S Gebicki, J M Gebicki *Biochem. J.* **289** 743 (1993)
72. I Sato, T Matsuura, M Nakagawa, T Hino *Acc. Chem. Res.* **10** 346 (1977)
73. E Alarćon, C Henríquez, A Aspée, E A Lissi *Photochem. Photobiol.* **83** 475 (2007)
74. G E Ronsein, M C B Oliveira, S Miyamoto, M H G Medeiros, P Di Mascio *Chem. Res. Toxicol.* **21** 1271 (2008)
75. M Gracanin, C L Hawkins, D I Pattison, M J Davies *Free Radical Biol. Med.* **47** 92 (2009)
76. I I Sapezhinsky *Biopolimery. Kinetika Radiatsionnykh i Fotokhimicheskikh Prevrashchenii* (Biopolymers. The Kinetics of Radiation and Photochemical Inversions) (Moscow: Nauka, 1988)
77. H Degn *Biochim. Biophys. Acta* **180** 271 (1969)
78. I G Gazaryan, D M Khushpul'yan, V I Tishkov *Usp. Biol. Khim.* **46** 303 (2006) [c]
79. P A Savitsky, I G Gazaryan, V I Tishkov, L M Lagrimini, T Ruzgas, L Gorton *Biochem. J.* **340** 579 (1999)
80. T Kawano, N Kawano, H Hosoya, F Lapeyrie *Biochem. Biophys. Res. Commun.* **288** 546 (2001)
81. S N Krylov, H B Dunford *Biophys. Chem.* **58** 325 (1996)
82. S N Krylov, H B Dunford *J. Phys. Chem.* **100** 913 (1996)
83. D Metodiewa, M P de Melo, J A Escobar, G Cilento, H B Dunford *Arch. Biochem. Biophys.* **296** 27 (1992)
84. W Adam, G Cilento *Free Radical Biol. Med.* **19** 103 (1995)
85. M P de Mello, S M de Toledo, M Haun, G Cilento, N Duran *Biochemistry* **19** 5270 (1980)
86. N Duran, A Campa, L C Leite, G Cilento, E Cadenas *Photobiochem. Photobiophys.* **11** 281 (1986)
87. J R Kanofsky *J. Biol. Chem.* **263** 14171 (1988)
88. J R Kanofsky *Methods Enzym.* **319** 59 (2000)
89. G A Russell *J. Am. Chem. Soc.* **79** 3871 (1957)
90. S Kobayashi, K Sugioka, H Nakano, M Nakano, S Tero-Kubota *Biochemistry* **23** 4589 (1984)
91. S N Krylov, V V Lazarev, L B Rubin *Dokl. Akad. Nauk SSSR* **310** 1000 (1990) [d]
92. M P de Melo, J A Escobar, D Metodiewa, H B Dunford, G Cilento *Arch. Biochem. Biophys.* **296** 34 (1992)
93. I Neeman, D Joseph, W H Biggley, H H Seliger *Lipids* **20** 729 (1985)
94. A U Khan, M Kasha *J. Am. Chem. Soc.* **88** 1574 (1966)
95. S N Krylov, S M Krylova, I G Chebotarev, A B Chebotareva *Phytochemistry* **36** 263 (1994)
96. H Arakawa, A Tsuji, M Maeda *J. Biolum. Chemilum.* **13** 349 (1998)
97. S Agatsuma, T Nagoshi, M Kobayashi, M Usa, H Watanabe, H Sekino, H Inaba *Clin. Chem.* **38** 48 (1992)
98. S Agatsuma, H Sekino, T Nagoshi, H Watanabe *Clin. Chem.* **40** 1580 (1994)
99. V F Ximenes, A Campa, L H Catalani *Arch. Biochem. Biophys.* **387** 173 (2001)
100. R F Vasil'ev, Yu B Tsaplev *Usp. Khim.* **75** 1103 (2006) [*Russ. Chem. Rev.* **75** 989 (2006)]

[a] — *Russ. J. Phys. Chem. (Engl. Transl.)*
[b] — *Biophysics (Engl. Transl.)*
[c] — *Biol. Chem. Rev. (Engl. Transl.)*
[d] — *Dokl. Biochem. Biophys. (Engl. Transl.)*