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Thermochemiluminescence as a fast method to detect and characterize hydroperoxide moieties in novel 3-hydroperoxyisothiazole 1,1-dioxides

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ABSTRACT

Hydroperoxy compounds are important for selective oxidation of organic precursors in the chemical industry. Moreover, novel 3-hydroperoxyisothiazole 1,1-dioxides (*N*-phthalimidyl sultams) have been shown to be potent and specific inhibitors of acetylcholinesterase (AChE) an important enzyme in the neurotransmitter process. The inhibitory potential of these novel compounds is clearly linked to the hydroperoxide moiety. Hydroperoxides are known for their chemi- and thermochemiluminescent reactivity. In this study we tested thermochemiluminescence (TCL) as a fast method to detect and characterize the hydroperoxide moiety in structurally different synthetic organic hydroperoxides.

1. Introduction

Chemi- and thermochemiluminescent light emission (TCL) from synthetic organic hydroperoxides is a well-known phenomenon. Lophine (2,4,5-triphenylimidazole) was the first artificial chemiluminescent compound described in 1877 by Radziszewski.[1] 4-Hydroperoxy-2,4,5-triphenyl-4*H*-imidazole was determined as the intermediate responsible for the chemiluminescence (CL) of lophine by Sonnenberg and White,[2] White and Harding[3,4] and Kimura.[5] However, the luminescent reaction mechanism of lophine has only been gradually revealed and is still under investigation.[6] A far better understanding concerning the mechanisms of chemiluminescence has been achieved for cyclic peroxides of the 1,2-dioxetane type.[7,8] Thermal decomposition of these compounds mainly results in the formation of triplet-excited carbonyl products showing phosphorescence.[9–11] Instead, the yield of singlet excited carbonyl states is far lower.[9–11] However, under aerated conditions fluorescence originating from these states is responsible for the observed direct chemiluminescence. Chemiluminescence originating from carbonyl triplet states can only be observed in the absence of oxygen, because of triplet oxygen being a strong triplet quencher.

α-Hydroperoxy sultams, which are the product of isothiazolium salt oxidation, have great potential as novel chemoselective electrophilic oxidants.[12] The selective oxidation of organic compounds is crucial in the chemical industry for the production of necessary oxygen containing compounds.[12] Furthermore, isothiazole derivatives have been described as bioactive compounds in both the medical and agrochemical area.[13–15] The novel *N*-phthalimidyl sultams (**1**–**4**, Fig. 1) have been recognized as efficient inhibitors of acetylcholinesterase (AChE).[16] AChE is responsible for the hydrolytic destruction of acetylcholine in neurotransmission. The medicinal control of this specific enzyme is important due to 'nonclassical' activities in disease development, e.g., Alzheimer's disease. The inhibitory potential of these novel compounds (**1**–**4**, Fig. 1) is clearly associated with the hydroperoxide moiety. Substitution of the hydroperoxide group to 3-hydroxy or 3-oxo function strongly diminished inhibitory properties and AChE activity remained >85%.[16]

In this study we tested if thermochemiluminescence (TCL) is suitable to detect and characterize the hydroperoxide moiety in structurally different synthetic organic hydroperoxides.

2. Results and discussion

The presence of a hydroperoxide moiety in the tested synthetic organic compounds (Fig. 1) was a prerequisite for

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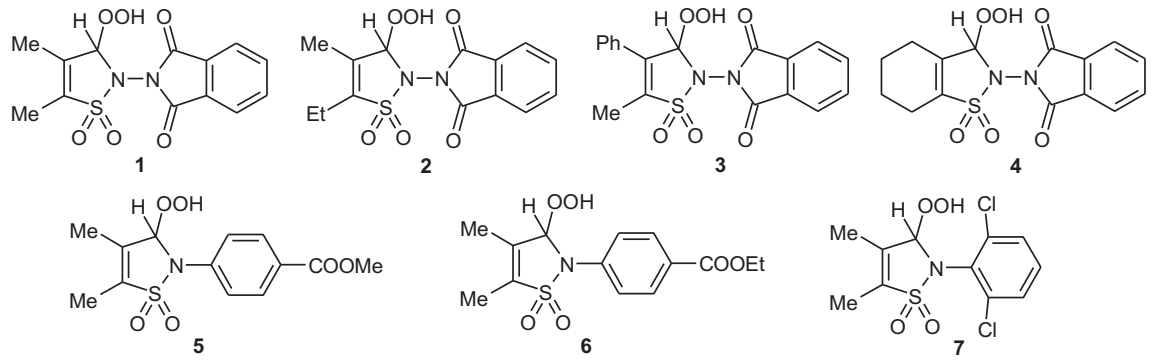

Fig. 1. Chemical structures of the studied organic hydroperoxides.

thermochemiluminescent light emission. The isothiazolium salts used for synthesis or structurally similar compounds carrying a hydroxy- or keto- instead of the hydroperoxide group did not show any thermochemiluminescent activity (data not shown).

The shape and the quantum yield of thermochemiluminescence (TCL) from the synthetic organic hydroperoxides depended strongly on their chemical structure, the type of metal surface and the composition of atmosphere during the heating process (Fig. 2).

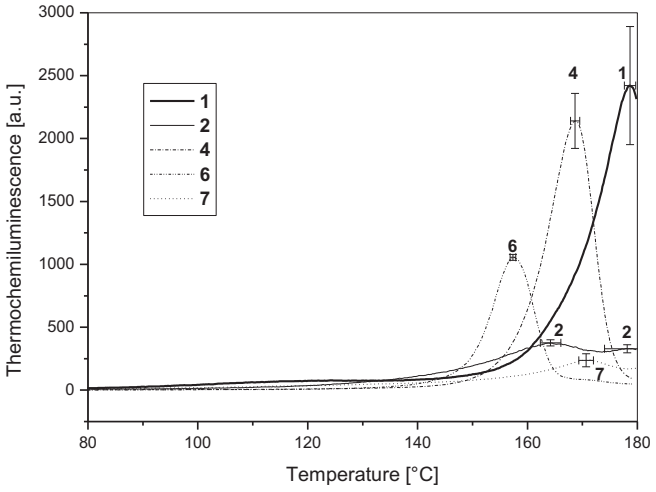

Fig 2. TL glow curves of different micro-crystalline organic hydroperoxides in the temperature range between 80 and 180 °C (*measured in air*). Each hydroperoxide of 0.5 μmol dissolved in acetonitrile was applied to round gold plates of 0.65 cm^2 resulting in a micro-crystalline cover after evaporation of the solvent. Curve of compound **2** was multiplied by a factor of 3 for better visibility.

In air, hydroperoxides **1**, **4** and **5** showed on gold the highest thermochemiluminescent amplitudes ranging from 2000 to 2500 units with peak temperatures T_m from 171.0 to 178.6 °C (Fig. 2, Table 1). Hydroperoxide **6** (Au, air) had an intermediate amplitude of about 1000 units, whereas hydroperoxides **2**, **3** and **7** displayed a rather low light emission between 160 and 670 units and peak temperatures T_m between 157.5 and 179.5 °C (Table 1). Different from the other hydroperoxides, compound **2** (Au, air) showed instead of one two distinct peaks (Fig. 2, Table 1).

Only in hydroperoxides **1** and **6** the amplitude was significantly affected by the composition of atmosphere. When air (Au) was replaced by N_2 the amplitudes increased by 70 and 40%, respectively (Table 1). In hydroperoxides **2** (peak 1) and **3**, N_2 atmosphere (Au) down-shifted T_m by 4–5 °C.

When samples were prepared on copper instead of gold most hydroperoxides showed a strong or even complete reduction in thermochemiluminescence by 30–100% (Table 1). Only hydroperoxide **7** was unaffected by the copper surface. Hydroperoxides with residual light emission (**1**, **3**, **6**, **7**) showed a down-shift of T_m in air by 2–34 °C (Table 1). Similar to gold the T_m of some hydroperoxides (**3**, **6**) was down-shifted on copper by 1–5 °C when N_2 atmosphere was applied instead of air (Table 1). Decomposition of hydroperoxides in the presence of transition metal ions or in contact with metal surfaces of the respective elements (Co^{2+}, Cu^{2+}, Fe^{2+}/Fe^{3+}) has already been described in other research studies.[17] Furthermore, the copper-induced down-shift of T_m (Table 1) indicates to a decrease in activation energy and a catalytic mechanism by transition metals in the decomposition process.[18,19]

IR spectra (Fig. 3) clearly showed that the hydroperoxide group is almost completely lost after heating as can be seen in the quenching of the rather broad band at 3270 cm^{-1}. This band is typical for a hydrogen bonded O–H stretching mode of the hydroperoxide group.[20,21] Since no –O–H groups and also no –N–H groups are present in the chemical structures of the studied hydroperoxides a contribution of O–H or N–H stretching modes can be excluded in this frequency band.[22] There is also an almost complete loss of the 871 cm^{-1} vibration, which is ascribed to one of the O–O stretching modes of hydroperoxides.[21]

Table 1
Results of TL glow curve measurements from different organic hydroperoxides characterized by their amplitude and peak temperature T_m (mean±SD). The influence of the sample holder material, copper (Cu) or gold (Au), and the atmosphere (air, N_2) in the sample compartment on the TL glow curve parameters is also shown. *, **, *** indicate significant effects on TL glow curve parameters when heating is performed under different atmospheres on the same sample holder material. $^{+}$, $^{++}$, $^{+++}$ indicate significant effects on TL glow curve parameters when heating is performed on different sample holder materials but under the same atmosphere. *,$^{+}$: $p<0.05$; **, $^{++}$: $p<0.01$; ***, $^{+++}$: $p<0.001$; n.d.=not detectable; n.m.=not measured

TCL parameters of 3-hydroperoxyisothiazole 1,1-dioxides

Hydroperoxide			Amplitude [a.u.]		T_m [°C]	
			Air	N_2	Air	N_2
1		Au	2583±470	4300±1212**	178.6±1.0	178.5±1.2
		Cu	154±33^{+}	127±16^{+++}	177.0±1.1^{+}	177.8±1.3
2	Peak 1	Au	165±24	183±32	164.7±1.9	160.8±1.9***
		Cu	n.d.	n.d.	n.d.	n.d.
	Peak 2	Au	156±32	n.d.	175.2±4.1	n.d.
		Cu	n.d.	n.d.	n.d.	n.d.
3		Au	668±163	555±128	179.5±0.4	175.0±2.1***
		Cu	129±22^{+++}	118±14^{+++}	145.6±3.0^{+++}	140.4±1.7^{+++}***
4		Au	2243±219	2448±166	168.9±0.8	168.4±0.6
		Cu	n.d.	n.d.	n.d.	n.d.
5		Au	1947±434	2255±280	171.0±0.5	170.6±0.1
		Cu	n.m.	n.m.	n.m.	n.m.
6		Au	1060±24	1488±123**	157.5±0.5	157.6±0.7
		Cu	341±67^{+++}	384±103^{+++}	153.4±0.5^{+++}	152.7±0.7^{+++}*
7		Au	245±52	293±43	171.0±1.3	169.5±1.7
		Cu	323±116	284±70	154.6±1.5^{+++}	154.6±2.7^{+++}

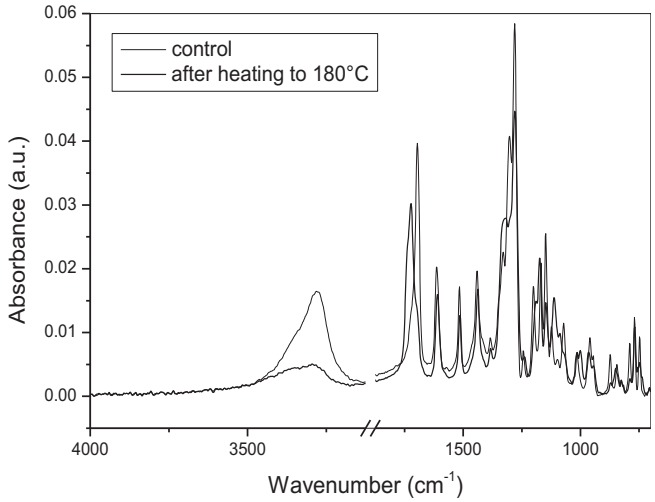

Fig. 3. FTIR spectra of micro-crystalline hydroperoxide **5** before and after heating to 180 °C.

The thermochemiluminescent light emission in air induced by the heat-induced decomposition of hydroperoxides is dominated by blue-green and green light (Fig. 4A, B) in the spectral range between 460 and 600 nm (**4**–**6**). However, due to scanning of the emission wavelength in parallel to the heating gradient, the spectra represent a convolution of both time-dependent parameters (c.f. Section 4.3.). Hence the peaks displayed in the spectra do not exactly represent true emission peak(s). This can be observed when the spectrum is scanned once from 450 to 600 nm (Fig. 4A) and the other time from 370 to 520 nm (Fig. 4B), yielding emission peaks at 550 and 485 nm, respectively. However, the intensity of the TCL emission in Fig. 4B is significantly lower as well as the band is not fully resolved at the long wavelengths part compared to that in Fig. 4A. TCL emission scans from 300 to 450 nm and 600 to 750 nm were also performed yielding only very noisy light emission well below 1000 cps of uncertain origin (Supplementary data; Fig. S-1). At least, from these experiments it can be deduced that the main light emission of TCL takes place in the range from 460 to 600 nm.

Beside the measurements of TCL emission spectra we also carried out steady state fluorescence emission and excitation scans of the reaction products of compound **4** by re-dissolving them in acetonitrile. Reaction products formed after heating were a yellowish amorphous solid on the gold plates and a white fume deposit on the protective glass filter (Supplementary data; Fig. S-2) shielding the photomultipliers in the TL apparatus. The latter deposit clearly indicated that due to vaporization/sublimation and/or reactions in the gas phase volatile molecular species were produced, which at least partially deposited at the cold glass filter surface above the sample holder. When the white fume deposit was re-dissolved in acetonitrile a clearly structured double band between 500 and 600 nm in the fluorescence emission scan could be resolved showing a peak at 525 nm and a shoulder about 555 nm (Fig. 4C). Excitation spectra between 220 and 450 nm at Em=525 and 555 nm showed rather similar shapes and intensities with highest excitation between 300 and 350 nm (Fig. 4D). For the thermolytic yellowish decomposition product from the gold plate we only found a rather unstructured large fluorescence emission band peaking about 400 nm including a broad shoulder between 480 and 600 nm (Ex=300 nm, Supplementary data; Fig. S-3). When diluting the latter deposit down to the linear range of fluorescence intensity the broad emission shoulder between 480 and 600 nm could not be any longer resolved. In the non-heated control sample the broad emission shoulder between 480 and 600 nm was missing. Here the broad emission band peaking about 400 nm, common for all samples, declined monotonously with a long tail to higher wavelengths (Supplementary data; Fig. S-4). In the excitation

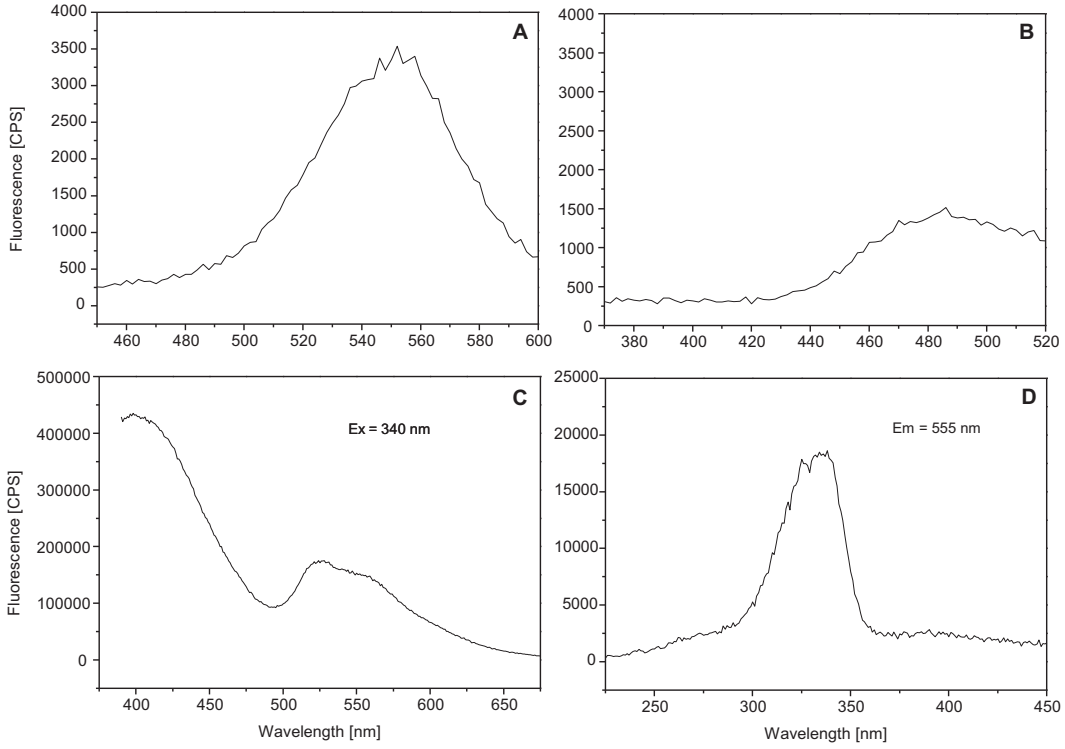

Fig. 4. A, B: TCL emission spectra of hydroperoxide **4** in *air atmosphere*. The displayed wavelength range was scanned in parallel with the heating range from 160 to 175 °C. C, D: fluorescence emission (C) and excitation (D) spectra of the fume deposit from **4** in acetonitrile after heating, excited at 300 nm (C) and detected at 555 nm (D), respectively. For details, see Section 4.3.

spectra of control samples no significant excitation with 300–350 nm was observed at Em=525 or 555 nm. This clearly indicates that the fluorescence emission spectrum resolved between 500 and 600 nm in the re-dissolved fume deposit represents reaction products not present in the untreated hydroperoxide **4**. Since the broad shoulder in the emission spectrum of the reaction products formed on the gold plate covers the same wavelength range it might be possible that also in this non-volatile deposit products with the same or similar molecular structure occur as in the fume deposit, which seem in the latter more selectively concentrated. The most striking result is that the TCL emission occurs in the same spectral range as the new steady state fluorescence emission of the products in the fume deposit. Hence, the light emitting molecular species in the TCL might be identical with the species emitting fluorescence between 500 and 600 nm when excited in the UV.

The spectral analysis of TCL and UV excited fluorescence emission indicate to the formation of excited carbonyl or keto singlet and triplet states representing this type of molecular species. Compared to our TCL emission spectra, rather similar chemiluminescence emission spectra were reported for lophine hydroperoxide and its derivates[17] having rather broad emission bands with peaks between 490 and 580 nm.[3,4,17] Light emission in the spectral domain between 450 and 550 nm has been attributed to excited carbonyl triplet and singlet states formed in the decomposition process of peroxidic compounds.[5,6,23] From synthesis of the respective 3-hydroperoxides studied in the present paper it is known that they react with DMSO forming the 3-hydroxy compounds and thermolysis in ethanol results in oxidation to the corresponding 3-ones.[16] Hence, a thermolytic mechanism leading to an excited triplet or singlet keto group in position-3 in the isothiazole heterocycle is possible.

Beside the formation of excited carbonyl and keto singlet and triplet states also the formation of excited singlet oxygen can take place,[24] showing three distinct dimol emission bands at 634, 703 and 785 nm.[25] Due to emission intensities well below 1000 cps in that spectral range of our experiments (Supplementary data; Fig. S-1) the signal is of uncertain origin and cannot be attributed to the formation of considerable amounts of singlet oxygen. However, triplet oxygen is known especially as a strong quencher of excited carbonyl and keto triplet states. But since only two of the seven hydroperoxides showed a significant increase in TCL intensity in oxygen free atmosphere this principal type of quenching mechanism seems to be only of secondary significance in our experiments (Table 1). Furthermore, this observation indicates that the mechanism of thermolytic breakdown obviously yield significant amounts of excited singlet states. This in turn agrees with findings for cyclic ketones in the gas phase, which showed only UV-induced fluorescence on UV-irradiation and a negligible influence of oxygen on the quantum yield.[26] From the results of the latter study[26] also the conclusion might be drawn that at least a considerable part of the TCL in our experiments has its origin in the hot gas phase above the solid phase of the decomposing hydroperoxide on the gold plate, either due to the liberation (vaporization/sublimation) of the excited state species into the gas phase or due to the thermolytic reaction taking place in the gas phase itself. However, since several hydroperoxides have melting points well above the maximum temperature of heating (180 °C, c.f.) and sublimation will contribute far less to such a molecular transfer than vaporization probably all phases in the thermolytic decomposition process might contribute to the TCL signal. In case of hydroperoxides **3**, **5** and **6** the liquid phase is reached before TCL reaches its peak temperature (Table 1). Here, vaporization will probably transfer more material into the gas phase. However, the white fume deposit on the protective filter glass of hydroperoxide **4** obviously shows that sublimation takes place, since this compound has a melting point significantly above the maximal heating temperature.

The TCL curves (Fig. 2, Table 1) are characterized by two important parameters, their peak temperature and their intensity (amplitude, integral). Several factors will influence these parameters shifting the peak to lower or higher temperature and decreasing or increasing the TCL intensity. One major factor is the melting point associated with the molecular weight (MW) of the respective compound but also with the level of symmetry and intra- and intermolecular attractive forces within and between the molecules. The latter often override the primary dependency of the melting point on the MW. Another major factor is the activation energy for the thermolytic decomposition of the hydroperoxy group, which also depends on the above-described molecular qualities governing the electronic configuration of the molecule. A higher activation energy will upshift the TCL peak and a lower one will result in a down-shift. The molecular structure will also determine the mechanism of the chemiluminescent reaction and the lifetimes of excited product states. Different mechanisms for the chemiluminescent decomposition of hydroperoxides have been described in literature, e.g., selfreaction of peroxylradicals ('Russel mechanism'), formation of dioextanes as intermediates and dehydration of allylic hydroperoxides (for review c.f. Cilento and Adam).[24] Competition between radiative and radiationless deactivation pathways of excited states in turn influence the intensity of TCL. However, also the character of the environment can influence the quantum yield of chemiluminescent reactions. Lifetimes of excited states strongly depend on the collisional rate with neighbouring molecules, which strongly differs in the solid, liquid and the gas phases. As already described other molecule species operating as quenchers and present in the reaction environment, e.g., triplet oxygen, can lower the intensity of TCL. This short overview shows that the parameters of TCL curves can be determined in a rather complex way.

However, when viewing the structures of the studied hydroperoxides (Fig. 1) they are displaying two homogeneous structural groups (**1**–**4** and **5**–**7**) with differences in only a few residues. Hence, a good chance is given that differences in the TCL parameters can be explained on the basis of a few predominant factors. When looking at the melting points of the hydroperoxides **5**–**7** these do not follow the order of MWs. Hydroperoxide **7** with an intermediate MW of 324.18 g mol^{-1} has the highest melting point of all. Its structure is the most symmetric of the three compounds favouring a more highly ordered crystal structure. However, the main reason for the rather high mp of compound **7** is the two heavy chlorine atoms resulting in strong dipolaric intermolecular forces. The peak temperature of TCL for this hydroperoxide is well below the mp indicating that the activation energy of thermolytic decomposition of the hydroperoxide moiety is reached much earlier. The decomposition takes either place in the solid or, if sublimation will occur, in the gas phase. When transfer to the gas phase is prerequisite to the decomposition process the low intensity of TCL in compound **7** might be due to the very high mp not allowing significant sublimation. However, another factor might more likely explain the rather low TCL yield of **7**, the so-called 'heavy atom effect'. This effect induced typically in halogenated organic fluorophores lead to a strong quenching of singlet excited states,[27] which might be also responsible for the low TCL intensity in compound **7**. Hydroperoxides **5** and **6** are even more similar to each other differing only in the *p*-CO_2Me and *p*-CO_2Et residues, respectively. Also for these two structures the melting points do not follow the MWs of the two compounds. Compound **6** having a MW of 327.36 g mol^{-1} melts significantly at lower temperature (153–155 °C) than **5** with MW=313.33 g mol^{-1} and mp=166–168 °C. The more symmetric compound **5** seems to form a more stable crystal structure. This is supported by X-ray structure

analysis. Compound **5** forms highly compact centrosymmetric head-to-tail dimers held together by strong C=O···H–OO hydrogen bonds further stabilized by π/π interactions of the phenyl rings.[28] The significantly lower mp of **6** indicates, when dimers are formed at all they must be far less stable. The reason for this behaviour can be seen in the far less symmetric ethoxyester group compared to the methoxyester group in **5**. Unfortunately, no X-ray structures of **6** are available. The highly stable dimers of **5** might also be responsible for the significantly higher peak temperature of TCL. Due to the strong hydrogen bonds and other intermolecular forces the hydroperoxy group is probably better protected from decomposition than in its monomeric form. Also after melting, the dimeric forces will reduce the vaporization of compound **5** to the gas phase. However, according to their rather similar monomeric structures activation energies for the hydroperoxy-decomposition should be very similar. In this example the peak temperatures of the TCL seem to be strongly governed by the differences in intermolecular forces.

Similar considerations can be drawn for the second homogeneous group of hydroperoxides (**1**–**4**). Also in this group the melting points do not follow the order of MWs and the symmetry factor obviously overrides the dependency on MW (c.f. Section 4.1.3 and Fig. 1). The mps follow the order **1**>**2**>**4**>**3**, which also resembles the level of symmetry with **1** being the most symmetric and **3** with its outwardly directed large phenyl group the least symmetric one. However, the differences in peak temperatures and intensities of TCL seem to be determined in this homogeneous group of hydroperoxides in a more complex manner. Especially the extreme differences between **1** and **2** characterized only by the exchange of a methyl- by an ethyl-residue in position-5 of the heterocycle cannot be explained easily. Additionally, due to the lack of X-ray crystal data a discussion of this group is difficult.

3. Conclusions

Most of the synthetic organic hydroperoxides show rather homogeneously shaped TCL curves peaking in the temperature range between 140 and 180 °C. (Fig. 2, Table 1). From the presented results no general reaction mechanisms can be deduced. However, the data clearly show that the hydroperoxide moiety is a prerequisite for thermochemiluminescent light emission. The quantum yield and the peak temperature of this TCL strongly depend on the chemical structure, the type of metal surface and the atmospheric environment in which the reaction takes place. Also the intermolecular forces play a major role for differences in crystal structure and/or the dimerization probability in the liquid phase governing in turn the TCL curve parameters via the character of phase transition. The TCL quantum yield for most hydroperoxides is not influenced by the presence or absence of oxygen and the TCL emission spectrum is dominated by blue-green and green light. Hence, the thermal decomposition most likely results in a singlet excited carbonyl state representing an intermediate or product of reaction.

Concerning the applied area of organic chemistry TCL might be supplementary as a simple and fast method to detect and characterize novel hydroperoxy compounds. For example, the shelf lifetime of novel hydroperoxides could be tested in future. Moreover, TCL has the advantage compared to the popular characterization by FTIR that it can detect the presence of hydroperoxide groups specifically due to their TCL. However, detection by FTIR will be restricted if –OH or –NH groups occur beside the hydroperoxide moiety contributing to the broad wavenumber range between 3000 and 4000 cm^{-1}.

4. Experimental section

4.1. Synthesis and characterization of organic hydroperoxides

Compounds **1**–**7** (Fig. 1) were synthesized by the cyclocondensation of β-thiocyanatovinyl aldehydes with corresponding amine derivative followed by the oxidation of the isothiazolium perchlorate by hydrogen peroxide. 3-Hydroperoxyisothiazole-1,1-dioxides **1**, **4**–**7** and their detailed synthesis were described elsewhere.[29,16] Synthetic procedure and characteristics for new compounds **2**, **3** are presented below.

Melting points were determined on Boetius micro-melting-point apparatus. ^1H and ^{13}C NMR spectra were recorded at 300 or 400 MHz (^1H) and 75 or 100 MHz (^{13}C) using Varian Mercury Plus 300 or 400 NMR spectrometers in DMSO-d_6 or acetone-d_6 solution with TMS as internal standard; *J* in hertz. IR spectra were recorded on a spectrophotometer Genesis FTIR Unicam Analytical System (ATI Mattson) with KBr pellets; values in cm^{-1}. UV/vis spectra were measured on Beckmann DU650, λ_{max} in nanometre (log ε). Elemental analysis was performed on a Heraeus CHNO Rapid Analyser. Mass spectra were determined on Quadrupol-MS VG 12-250; 70 eV.

4.1.1. Preparation of isothiazolium perchlorates; general procedure. To a magnetically stirred solution of corresponding β-thiocyanatovinyl aldehyde (1 mmol) in glacial acetic acid, (2 ml) *N*-aminophthalimide (1 mmol, 0.162 g) was added under argon atmosphere. The reaction mixture was stirred for 15 min and perchloric acid (0.4 ml) was added. After stirring for 50 min, the reaction mixture was diluted with 20 ml of diethyl ether. The precipitate of isothiazolium perchlorate was filtered off, washed several times with diethyl ether and dried on air.

4.1.1.1. 5-Ethyl-4-methyl-2-(phthalimid-1-yl)isothiazolium perchlorate. Yield: 90%; white solid; mp 207–211 °C; IR (KBr): ν=1756 (CO), 1089 (ClO_4) cm^{-1}; UV (CH_3CN): λ_{max} (log ε)=221 nm (4.46), 282 nm (3.98); ^1H NMR (300 MHz, DMSO-d_6): δ=1.32 (t, 3H, *J*=7.5 Hz, CH_3), 2.39 (s, 3H, CH_3), 3.26 (q, 2H, *J*=7.5 Hz, CH_2), 8.04 (d, 2H, *J*=7.8 Hz, 2 arom. CH), 8.14 (d, 2H, *J*=7.8 Hz, 2 arom. CH), 9.34 (s, 1H, CH); ^{13}C NMR (75 MHz, DMSO-d_6): δ=179.3 (C-5), 162.1 (2CO), 160.6 (C-3), 136.1 (2 arom. CH), 130.8 (C-4), 129.3 (2 arom. C), 124.8 (2 arom. CH), 21.6 (CH_2), 14.4 (CH_3), 10.6 (CH_3).

4.1.1.2. 5-Methyl-4-phenyl-2-(phthalimid-1-yl)isothiazolium perchlorate. Yield: 81%; white solid; mp 225–226 °C; IR (KBr): ν=1757 (CO), 1090 (ClO_4) cm^{-1}; UV (EtOH): λ_{max} (log ε)=220 nm (4.67), 299 nm (3.88); ^1H NMR (400 MHz, DMSO-d_6): δ=2.93 (s, 3H, CH_3), 7.31–7.88 (m, 5H, 5 arom. CH), 8.06 (d, 2H, *J*=7.8 Hz, 2 arom. CH), 8.19 (d, 2H, *J*=7.8 Hz, 2 arom. CH), 9.72 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6): δ=173.4 (C-5), 162.0 (2CO), 159.5 (C-3), 136.1 (2 arom. CH), 134.8–128.3 (5 arom. CH, 3 arom. C, C-4), 124.9 (2 arom. CH), 15.4 (CH_3).

4.1.2. Synthesis of 2,3-dihydro-3-hydroperoxy-2-(phthalimid-1-yl) isothiazole 1,1-dioxides 2,3; general procedure. H_2O_2 (2.8 ml, 30%) was added at room temperature to a stirred suspension of 5-ethyl-4-methyl-2-(phthalimid-1-yl)isothiazolium perchlorate or 5-methyl-4-phenyl-2-(phthalimid-1-yl)isothiazolium perchlorate (0.7 mmol) in AcOH (4.2 ml). After 24–48 h the solution was diluted with cold water, colourless crystals of compounds **2**,**3** were filtered off and recrystallized from ethanol/water.

*4.1.2.1. 5-Ethyl-2,3-dihydro-3-hydroperoxy-4-methyl-2-(phthalimid-1-yl)isothiazole 1,1-dioxide (**2**).* Yield: 59%; white solid; mp 194–197 °C; IR (KBr): ν=1731 (CO), 1314 (SO_2), 1173 (SO_2) cm^{-1}; UV (CH_3CN): λ_{max} (log ε)=216 nm (4.47), 220 nm (4.46), 295 nm

(3.37); ^{1}H NMR (400 MHz, acetone-d_6): δ=1.26 (t, 3H, J=7.5 Hz, CH_3), 2.10 (s, 3H, CH_3), 2.62 (q, 2H, J=7.5 Hz, CH_2), 5.98 (d, 1H, 3-H), 7.98 (m, 4H, 4 arom. H), 11.67 (s, 1H, OOH); ^{13}C NMR (100 MHz, acetone-d_6): δ=165.9 (CO), 165.4 (CO), 139.4 (C-5), 138.4 (C-4), 136.3 (2 arom. CH), 130.6 (arom. C), 130.5 (arom. C), 124.8 (arom. CH), 124.6 (arom. CH), 94.5 (C-3), 18.0, 12.9, 12.4; EIMS: m/z=320 ([M−H_2O]$^{+\bullet}$); $C_{14}H_{14}N_2O_6S$ (338.34); calcd (%): C 49.70, H 4.17, N 8.28, S 9.48; found (%): C 50.05, H 4.09, N 8.52, S 9.71.

*4.1.2.2. 2,3-Dihydro-3-hydroperoxy-5-methyl-4-phenyl-2-(phthalimid-1-yl)isothiazole 1,1-dioxide (**3**).* Yield: 58%; white solid; mp 170–172 °C; IR (KBr): ν=1739 (CO), 1329 (SO_2) 1190 (SO_2) cm^{-1}; UV (CH_3CN): λ_{max} (log ε)=218 nm (4.64), 221 nm (4.63), 292 nm (3.55); ^{1}H NMR (400 MHz, acetone-d_6): δ=1.27 (s, 3H, CH_3), 6.50 (s, 1H, 3-H), 7.53–7.63 (m, 5H, 5 arom. H), 7.98 (m, 4H, 4 arom. H), 11.70 (s, 1H, OOH); ^{13}C NMR (100 MHz, acetone-d_6): δ=165.7 (CO), 165.6 (CO), 139.2 (C-5), 136.4 (2 arom. CH), 135.9 (C-4), 130.9 (arom. C), 130.8 (2 arom. CH), 130.6 (arom. C), 130.5 (arom. C), 129.7 (arom. CH), 129.5 (2 arom. CH), 124.9 (arom. CH), 124.9 (arom. CH), 94.8 (C-3), 9.32 (CH_3); EIMS: m/z=368 ([M−H_2O]$^{+\bullet}$). $C_{18}H_{14}N_2O_6S$ (386.39); calcd (%): C 55.95, H 3.65, N 7.25, S 8.30; found (%): C 56.38, H 3.71, N 7.42, S 8.04.

4.1.3. Melting points of hydroperoxides and sample preparation. Since several hydroperoxides have melting points lower than the peak temperature of their respective TCL glow curve the mps of all hydroperoxides are listed below together with their molecular weights (MW) in overview:

(1) mp=215–217 °C (MW 324.32);
(2) mp=194–197 °C (MW 338.34);
(3) mp=170–172 °C (MW 386.38);
(4) mp=184–187 °C (MW 350.35);
(5) mp=166–168 °C (MW 313.33);
(6) mp=153–155 °C (MW 327.36);
(7) mp=207–209 °C (MW 324.18).

Whereas hydroperoxides (1), (2), (4) and (7) will stay in the solid state, hydroperoxides (3), (5) and (6) will undergo a solid/liquid phase transition during the heating process to 180 °C.

Sample preparation:

Fig. 1, Table 1: 0.5 μmol of each hydroperoxide dissolved in acetonitrile (Prolabo HPLC grade VWR 20060.320) was applied to round gold or copper plates of 0.65 cm^2 resulting in a micro-crystalline cover after evaporation of the solvent.

Fig. 4A, B: for recording of TCL emission spectra amounts of 6 μmol were applied (c.f. Section 4.3.).

Fig. 4C, D: for measurements of fluorescence emission and excitation spectra four heated gold plates each covering 0.5 μmol were rinsed with acetonitrile (Prolabo, VWR 20060.320, HPLC grade) and diluted to a final volume of 2.6 ml. For controls non-heated gold plates were rinsed and diluted in the same manner. For analysis of the fume deposit covering the protective glass filter, seven gold plates, each covering 0.5 μmol were heated to 180 °C and then rinsed from the glass filter with acetonitrile to a final volume of 2,6 ml.

4.2. Thermochemiluminescence measurements

Thermochemiluminescence (TCL) was measured in a set-up already described according to Gilbert et al.[30] The artificial hydroperoxides were dissolved in acetonitrile (spectroscopic grade); 30 μL of the solution was applied to gold or copper plates forming a micro-crystalline layer of 0.5 μmol after evaporation of the solvent. The hydroperoxide covering plates were transferred to the sample holder and incubated at 20 °C for 5 min in air or N_2 atmosphere and then heated in the respective atmosphere from 20 to 180 °C at a rate of 20 °C min^{-1}. Light emission was detected by means of channel photomultipliers (CPM). The different TCL curves were characterized by their peak temperatures and maximal amplitudes (relative quantum yield).

4.3. Emission spectra of thermochemiluminescence and fluorescence emission and excitation spectra

Emission spectra were recorded with a Spectrofluorometer Fluoromax-4 (Horiba Jobin Yvon, Edison, NJ, U.S.A). Sample heating was performed with a watercooled Peltier unit detached from a TL measuring device described by Ducruet.[31] This unit was mounted on the original solid sample holder of the spectrofluorometer positioned with the sample plate centrally at the focus position of the fluorometer's excitation/emission beam. The sample plate was in vertical position and its surface plane facing at an angle of 90° to the emission beam and the entrance gate of the detector unit. The sample plates were kept in place on the Peltier element by means of a Teflon diaphragm. The wavelength range was scanned in parallel to the heating gradient (20 °C min^{-1}), the latter covering the range from 160 to 175 °C and therefore also the peak of the TCL. The wavelength range covered 150 nm for different spectral areas, e.g., 370–520 nm or 450–600 nm. The instrument settings were: slit 20 nm, increment 2 nm and integration time 0.6 s. It has to be emphasized that this procedure is a compromise due to the lack of enough material, which would have allowed otherwise to perform several measurements at constant different emission wavelengths. The applied procedure results in a convolution of the TCL, being a function of temperature/time, and the emission scan being a function of time. An exact determination of the emission spectrum localizing true peaks is therefore not possible. The applied procedure can only deliver an estimate of the spectral range where the main light emission occurs (see also Results and discussion).

Due to the large distance between the detector and the sample plate as well as to the omnidirectional chemiluminescent light emission the absolute amount of hydroperoxide had to be increased to 6 μmol to achieve a good signal-to-noise ratio (c.f. Section 4.1.3.).

Steady state fluorescence emission and excitation spectra were also performed with the Spectrofluorometer Fluoromax-4 (Horiba Jobin Yvon, Edison, NJ, U.S.A). To avoid Raman peaks of the solvent acetonitrile, excitation was restricted to the range of 220–340 nm and emission scanned from 390 to 750 nm. To avoid distortions by higher order light a cut-on order filter opening at 350 nm having a constant transmission of 91% from 390 to 750 nm was placed in front of the emission site. Settings were: slit width 5 nm, increment 1 nm, integration time 0.1 s.

4.4. Fourier transform infrared (FTIR) spectroscopy

Samples of 1 μL from re-dissolved hydroperoxide (acetonitrile) prepared before and after heating (solid state, gold plates) were placed on a microtiter plate and dried at room temperature for 10 min. Infrared (IR) spectra (Vector 22, Bruker Optics, Karlsruhe, Germany) were recorded in the range of 4000–700 cm^{-1} in transmission mode with 32 scans oversampling to enhance the signal-to-noise ratio (Vector 22 laser unit, HTS-XT microtiter

module, OPUS and OPUSLab v5.0 software, Bruker Optics, Karlsruhe, Germany).

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Supplementary data

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