Journal of Photochemistry and Photobiology A: Chemistry 218 (2011) 41–47

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

Fenchyl substituted 1,2-dioxetanes as an alternative to adamantyl derivatives for bioanalytical applications

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ARTICLE INFO

Article history:
Received 8 September 2010
Received in revised form
18 November 2010
Accepted 6 December 2010
Available online 13 December 2010

Keywords:
Chemiluminescence
1,2-Dioxetane
Fenchyl group
Organic peroxides
Intramolecular CIEEL

ABSTRACT

The synthesis and study of the chemiluminescence parameters and thermal stability of 1,2-dioxetanes containing a spirofenchyl substituent are reported. Three fenchyl-substituted 1,2-dioxetanes were synthesized by photooxygenation of the corresponding alkenes, obtained by Barton–Kellogg olefination of the readily available (−)-fenchone. The fenchyl-substituted 1,2-dioxetanes showed thermal stabilities similar to those of the corresponding spiroadamantyl-substituted derivatives, although being slightly more labile with respect to unimolecular decomposition than the latter derivatives, which are widely utilized as labels in a great variety of chemiluminescent immunoassays. Fluoride induced decomposition of one triggerable fenchyl 1,2-dioxetane derivative showed kinetic parameters similar to those of the corresponding adamantyl-substituted derivative. The chemiluminescence quantum yields in the one percent range are also similar to that of other widely utilized chemiluminescence systems as the luminol reaction. These results indicate that fenchyl-substituted 1,2-dioxetanes can potentially be utilized as a cheaper alternative to substitute the corresponding spiroadamantyl derivatives in bioanalytical applications.

1. Introduction

1,2-Dioxetanes are four-membered cyclic peroxides whose thermal or catalyzed decomposition can result in chemiluminescence. They are proposed as key intermediates in several chemi- and bioluminescent reactions [1]. The unimolecular thermal decomposition of these high-energy molecules generates two carbonyl fragments, one of which can be formed in the triplet excited state with yields (Φ_T) of up to 30%. Singlet excited states are also formed in this decomposition, however, their yields (Φ_S) are much lower, usually less than 0.1% [2,3]. As triplet excited states are preferentially deactivated in a non-radiative way, these processes show very low chemiluminescence quantum yields (Φ_{CL}) [4], contrarily to bioluminescence processes that can have quantum yields (Φ_{BL}) of up to 40% [5].

Surprisingly, when a 1,2-dioxetane derivative bears an electron-rich substituent it becomes thermally labile and cleaves rapidly producing singlet excited states in high yields (Φ_S up to 100%) [6]. This phenomenon can be rationalized by the "chemically initiated electron transfer chemiluminescence" (CIEEL) mechanism, first proposed by Koo and Schuster for the catalyzed decomposition of several peroxides in the presence of easily oxidizable activators [7]. In the case of the above-mentioned 1,2-dioxetane derivatives, an intramolecular electron transfer from a group with low oxidation potential, particularly aryl-O^- and aryl-RN^-, induces the reduction of the peroxidic ring, resulting finally in the production of singlet excited states in high yields [8].

Exploring this feature, many research groups worked on the synthesis of thermally stable 1,2-dioxetanes that can lead to light emission, in a process initiated by a chemical or enzymatic transformation. The most successful design is based on *meta*-substituted phenol groups protected with a "trigger", which can be removed by the action of a chemical or enzymatic reagent. The formerly stable 1,2-dioxetane becomes labile due to the presence of the phenolate moiety, which induces decomposition and light emission [9]. The development of triggered stable 1,2-dioxetanes with various protecting groups and high emission quantum yields resulted in many successful analytical and bioanalytical applications, most prominently immunoassays, with very high specificity and sensibility [10–13].

The most successful application of such triggered chemiluminescent reagents is based on the spiroadamantyl-substituted 1,2-dioxetane AMPPD (**1**), bearing a phosphate moiety as removable protective group, being able to detect concentrations as low as 10^{-21} mol L^{-1} of the enzyme alkaline phosphatase [14]. The *tert*-butyldimethylsilyl analogue of AMPPD,

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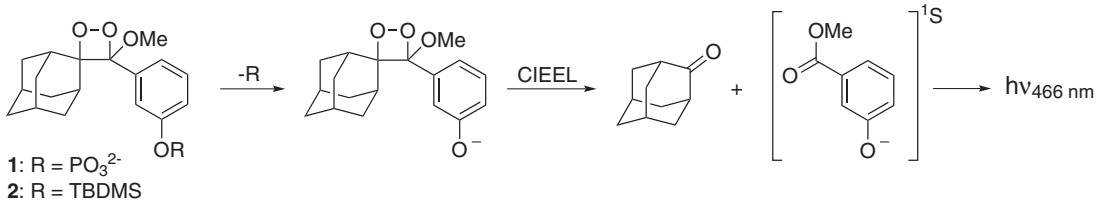
doi:10.1016/j.jphotochem.2010.12.001



Scheme 1. Excited singlet state formation induced by deprotection of appropriate phenolate-substituted 1,2-dioxetanes with alkaline phosphatase from phosphate-protected 1,2-dioxetane **1** and fluoride from silyl-protected derivative **2**.

the 4-(3-*tert*-butyldimethylsilyloxyphenyl)-4-methoxyspiro[1,2-dioxetane-3,2′-adamantane] (**2**), is the most often used model compound for investigations on the mechanisms of chemical singlet excited state generation by catalyzed decomposition of triggered 1,2-dioxetanes [15]. The chemiluminescence of this stable cyclic peroxide is initiated by the addition of tetrabutylammonium fluoride (TBAF) as a F^- source (Scheme 1).

The widespread use of spiroadamantyl substitution as stabilizing group for 1,2-dioxetanes [16,17] is due to its structural characteristics (Fig. 1), which results in a remarkable stabilizing effect: for example, bis-(adamantyl)-1,2-dioxetane, the most stable 1,2-dioxetane ever prepared, has a $\Delta^{\ddagger}G^0$ value of 137.6 kJ mol^{-1} for its thermal decomposition [18]. Despite the extensive effective application of the specific deprotection followed by light emission of thermally stable 1,2-dioxetanes, there are still mechanistic questions that remain unanswered and are subject of recent theoretical studies [19]. There is also until now no definitive quantitative explanation for the thermal stability of 1,2-dioxetanes, but the empirical knowledge in this field showed that the degree of thermal stability is closely related to the substitution pattern at the peroxidic ring [20]. The fenchyl group (1,3,3-trimethylbicyclo[2.2.1]heptyl) is a bulky, very hindered substituent (Fig. 1) [21], and is supposed to exercise a stabilizing effect when attached by a *spiro* connection to the dioxetane ring, similar to the effect arising from the adamantyl group [22], with the advantage of involving simpler and cheaper synthetic procedures, recently developed by our workgroups [23], together with a much lower price of the starting material. Therefore, the synthesis and study of the chemiluminescence properties of fenchyl-derived 1,2-dioxetanes may contribute to improve the knowledge on the chemiexcitation mechanism operating in the induced decomposition of 1,2-dioxetanes, as well as offer easily available and cheaper alternatives for detection systems in immunoassays and other biotechnological applications.

2. Materials and methods

2.1. General

Radial chromatography was made on round glass plates covered by a layer 1, 2 or 4 mm thick of Kieselgel 60 F_{254} with 10% gypsum, on an equipment Chromatotron model 7924T (Harrison Research). Thin-layer chromatography was conducted on Kieselgel 60 F_{254} plates (Merck) over polystyrene or aluminum. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker spectrometer model DRX 500 in $CDCl_3$. The measurement of light intensity was made in a spectrofluorimeter Varian Cary Eclipse equipped with a photomultiplier tube Hamamatsu R928, in a zero order measurement mode that is sensitive to photons of all wavelengths. The 1,2-dioxetane **2** and the olefins **4** and **5** (Scheme 2) were synthesized according to the literature procedures [8,23].

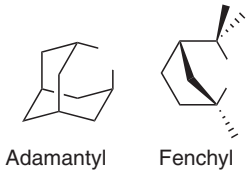


Fig. 1. Carbon skeletons of the adamantyl and fenchyl groups.

2.2. Synthesis of 2-(methoxymethylene)-1,3,3-trimethylbicyclo [2.2.1]heptane (**3**)

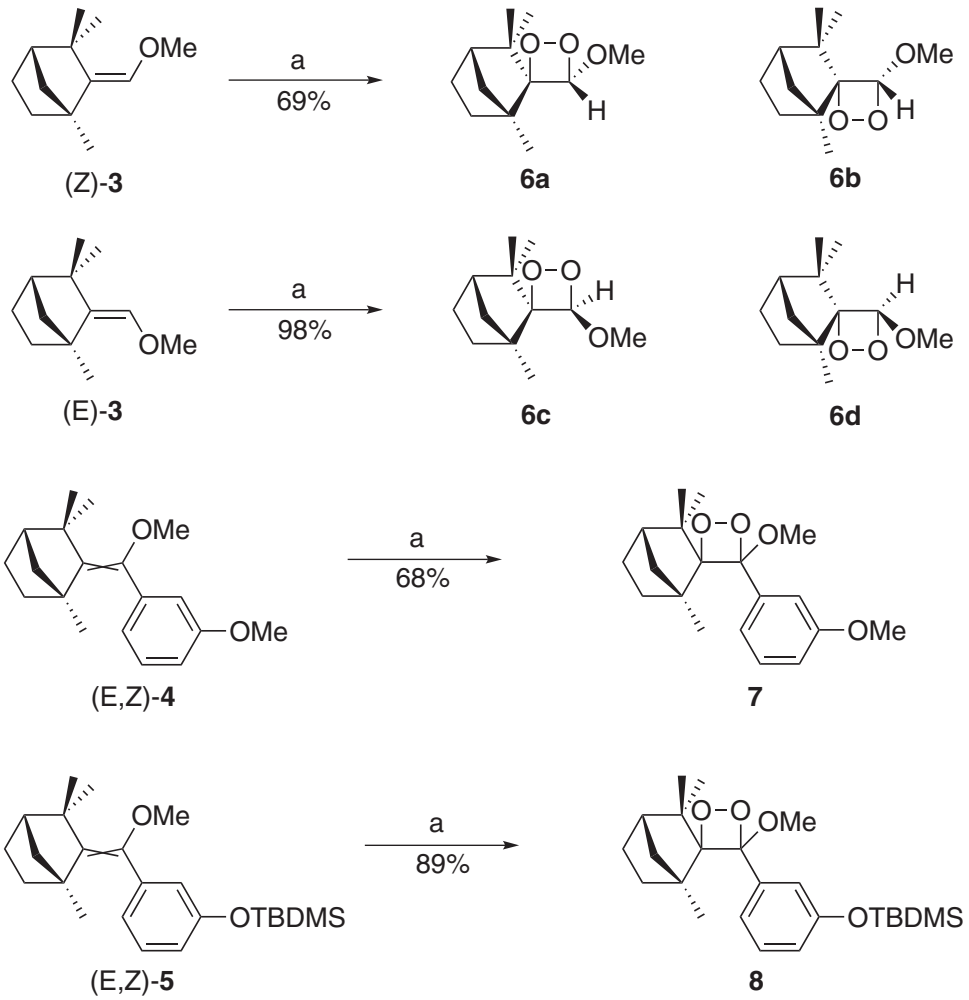
In a vacuum-flamed round-bottom flask, 5.7 g (66 mmol) of a dispersion of NaH in mineral oil (60%) was added to a suspension of 21.6 g (63 mmol) of methoxymethyltriphenylphosphonium chloride in 80 mL dry DME. The suspension was refluxed for 2 h, forming a deep red solution, to which 10 mL (9.8 g, 63 mmol) of (−)-fenchone (**9**) was added by means of a syringe. After 48 h of reflux, the resulting black solution was cooled to room temperature and added to 350 mL of water and extracted with CH_2Cl_2 (4× 50 mL), the organic phases were separated and dried over $MgSO_4$. After evaporation of the solvent, the oily black residue obtained weighted 22.8 g, and to this methyl iodide was added to eliminate the triphenylphosphine present. When the TLC analysis showed that all triphenylphosphine was eliminated, the residue was filtered and purified by column chromatography (SiO_2, heptane/ethyl acetate 9:1) and 1.7 g (9.4 mmol, 15%) of **3** as a colorless oil were obtained as a mixture of *E* and *Z* isomers. This mixture could be separated in the constituting isomers by radial chromatography (SiO_2, hexanes). Isomer *E* (1.16 g, 6.4 mmol, 68% of the mixture of isomers): ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.52 (s, 1H, C=CH); 3.48 (s, 3H, OCH_3); 1.04–1.77 (m, 16 H, fenchyl system with singlets at 1.18 (3H, CH_3) and 1.12 (6H, 2 CH_3)). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 136.73, 135.68, 59.32, 48.71, 47.67, 44.85, 42.28, 36.69, 26.11, 25.69, 23.89 and 18.67. Isomer *Z* (0.54 g, 3.0 mmol, 32% of the mixture of isomers): ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.58 (s, 1H, C=CH); 3.45 (s, 3H, OCH_3); 0.98–1.73 (m, 16H, fenchyl system with methyl singlets at 1.03, 1.04 and 1.41). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 138.40, 135.37, 59.51, 49.59, 48.14, 45.87, 41.95, 36.09, 30.48, 27.22, 25.55 and 20.16.

2.3. General procedure for the synthesis of the 1,2-dioxetanes

A solution of the chosen olefin in dry $CDCl_3$ (20 mL), tetraphenylporphyrin (*ca.* 10 mg) and a drop of pyridine *d*-5 were placed in a photooxygenation flask, cooled to −20 °C and irradiated with two halogen lamps (2× 500 W), under a flux of dry oxygen. After *ca.* 2 h, the starting material has been completely consumed (determined by TLC) and the solvent was evaporated *in vacuo* at 0 °C. The crude product was purified by radial chromatography at room temperature.

2.4. Synthesis of (3S,4R)- and (3R,4S)-4′-methoxy-1,3,3-trimethylspiro[bicyclo[2.2.1] heptane-2,3′-[1,2]dioxetane] (**6a–b**)

Synthesized from 137 mg (0.76 mmol) of (*E*)-**3**, purified by radial chromatography with the mixture CH_2Cl_2/heptane/ethyl acetate 10:9:1 yielding 111 mg (0.53 mmol, 69%) of a slightly yellow oil as a mixture of two diastereoisomers. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.54 (s, 1H, OOCH, 26%), 5.47 (s, 1H, OOCH, 74%), 3.46 (s, 3H,



Scheme 2. Synthesis of 1,2-dioxetanes **6**, **7** and **8** from the corresponding olefins by photooxygenation (a: O_2, $CDCl_3$, TPP, $h\nu$, −20 °C).

OCH_3, 26%), 3.45 (s, 3H, OCH_3, 74%), 0.93–1.65 (m, 16H, fenchyl system with singlets at 0.99, 1.15 and 1.48 (3× 3H, 3× CH_3)). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 111.26, 109.46, 105.10, 98.74, 98.17, 68.61, 66.79, 55.56, 50.78, 49.79, 48.66, 45.03, 44.02, 40.66, 39.74, 32.43, 28.10, 27.56, 27.09, 25.76, 24.80, 24.16, 23.43, 20.93, 17.96, 15.91.

2.5. Synthesis of (3R,4R)- and (3S,4S)-4′-methoxy-1,3,3-trimethylspiro[bicyclo[2.2.1] heptane-2,3′-[1,2]dioxetane] (**6c**–**d**)

Synthesized from 38 mg (0.21 mmol) of (*Z*)-**3**, purified by radial chromatography with the mixture CH_2Cl_2/heptane/ethyl acetate 10:9:1 yielding 43 mg (0.20 mmol, 98%) of off-white crystals as a mixture of two diastereoisomers. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.11 (s, 1H, OOCH, 20%), 5.87 (s, 1H, OOCH, 80%), 3.47 (s, 3H, OCH_3, 20%), 3.45 (s, 3H, OCH_3, 80%), 0.80–1.71 (m, 16H, fenchyl system with singlets at 1.08, 1.11 and 1.54 (3× 3H, 3× CH_3)). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 108.07, 106.94, 100.72, 99.94, 57.70, 56.88, 50.63, 48.04, 47.64, 44.15, 41.81, 40.96, 40.52, 28.48, 28.41, 25.97, 25.54, 25.40, 23.88, 23.82, 20.92, 18.11, 17.27.

2.6. Synthesis of (3S,4R)-, (3R,4S)-, (3R,4R)- and (3S,4S)-4′-methoxy-1,3,3-trimethylspiro [bicyclo[2.2.1]heptane-2,3′-[1,2]dioxetane] (**6a**–**d**)

Synthesized from 390 mg (2.2 mmol) of the mixture of isomers *E* and *Z* of **3** (*E* 68%, *Z* 32%). Purified by radial chromatography with the mixture heptane/ethyl acetate 5:1 yielding 150 mg (0.7 mmol, 33%) of a yellow oil as a mixture of four diastereoisomers. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.02 (s, 1H, OOCH, 1.3%), 5.82 (s, 1H, OOCH, 7.8%), 5.52 (s, 1H, OOCH, 27.9%), 5.43 (s, 1H, OOCH, 63.0%), 3.40 (s, 4× 3H, 4× OCH_3), 0.7–2.0 (m, 16H, fenchyl system). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 111.2, 109.4, 107.9, 99.7, 98.8, 98.2, 77.5, 77.0, 76.5, 56.5, 55.5, 54.1, 50.9, 50.6, 50.0, 48.9, 47.7, 47.29, 45.4, 45.3, 44.2, 44.0, 41.6, 40.9, 40.8, 39.9, 37.0, 32.7, 31.9, 31.8, 30.0, 29.6, 29.3, 28.4, 28.2, 27.9, 27.8, 27.1, 25.9, 25.4, 24.9, 24.1, 23.7, 23.7, 23.5, 23.3, 22.6, 21.6, 21.0, 19.7, 18.0, 17.1, 15.9, 14.5, 14.1.

2.7. Synthesis of 4′-methoxy-4′-(3-methoxyphenyl)-1,3,3-trimethylspiro[bicyclo[2.2.1] heptane-2,3′-[1,2]dioxetane] (**7**)

Synthesized from 350 mg (1.2 mmol) of the mixture of isomers *E* and *Z* (*E* 69%, *Z* 31%) of **4**. Purified by radial chromatography with the mixture heptane/ethyl acetate 5:1 yielding 252 mg (0.8 mmol, 68%) of a slightly yellow oil as a mixture of diastereoisomers. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.89–7.69 (m, 4H, ArH), 3.93 (s, 3H, OCH_3), 3.21 (s, 3H, OCH_3, 33%), 3.12 (s, 3H, OCH_3, 66%), 0.2–1.9 (m, 16H, fenchyl system with singlets at 0.81, 1.03, 1.14, 1.32, 1.49 and 1.67, 6× 3H, 6× CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 160.0, 159.3, 140.3, 138.73, 136.6, 129.6, 129.4, 129.0, 128.5, 128.3, 126.3, 122.0, 121.1, 120.4, 115.3, 114.8, 114.5, 114.4, 114.2, 114.0, 111.9, 111.7, 104.0, 103.0, 102.4, 55.3, 54.1, 53.7, 53.0, 51.2, 50.8, 48.9, 48.7, 48.6, 48.4, 47.3, 45.5, 45.3, 44.6, 43.7, 42.2, 41.6, 41.3, 40.9, 33.6, 31.8, 31.1, 29.7, 28.7, 27.0, 26.2, 26.0, 25.7, 25.3, 25.0, 23.0, 21.6, 20.1, 18.0, 17.7, 14.6.

2.8. Synthesis of tert-butyl(3-(4′-methoxy-1,3,3-trimethylspiro [bicyclo[2.2.1]heptane-2,3′-[1,2]dioxetane]-4′-yl)phenoxy) dimethylsilane (**8**)

Synthesized from 500 mg (1.29 mmol) of the mixture of isomers *E* and *Z* of **5**. Purified by radial chromatography with the mixture CH_2Cl_2/heptane/ethyl acetate 10:9:1 yielding 481 mg (1.15 mmol, 89%) of a slightly yellow oil as a mixture of diastereoisomers. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.82–7.80 (m, 4H, ArH), 3.02 (s, 3H, OCH_3, 36%), 3.08 (s, 3H, OCH_3, 35%), 3.12 (s, 3H, OCH_3, 28%), 0.8–2.0 (m, 16H, fenchyl system), 0.99, 1.00 and 1.02 (s, 3× 9H, 3× $(CH_3)_3$), 0.20, 0.21, 0.24 and 0.25 (s, 4× 6H, 4× $Si(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 156.24, 155.58, 155.34, 138.83, 136.68, 129.58, 129.05, 128.51, 122.74, 121.84, 121.35, 121.24, 121.00, 120.83, 120.19, 114.08, 111.69, 103.91, 102.96, 102.30, 53.70, 53.05, 51.28, 50.87, 48.96, 48.81, 48.62, 48.52, 48.49, 45.51, 44.64, 42.19, 41.42, 40.91, 35.43, 31.87, 31.09, 29.00, 28.36, 27.09, 26.43, 26.18, 26.02, 25.71, 25.68, 25.01, 24.96, 24.59, 24.52, 24.33, 22.67, 21.45, 20.32, 18.23, 18.19, 17.56, 14.08, −4.25, −4.30, −4.36, −4.39.

2.9. Kinetic assay of the thermal decomposition of 1,2-dioxetanes

In a quartz cuvette of capacity 3.0 mL and 1.0 cm of optical path, under magnetic stirring, 2.0 mL of toluene or 0.025 mM DBA solution in toluene was added, the cuvette was inserted in a thermostatized cell holder inside a spectrofluorimeter and allowed to stabilize at the desired temperature. The assay was initiated by injection of 10–30 μL of a solution of the chosen 1,2-dioxetane in toluene, and the emission intensity decay accompanying 1,2-dioxetane decomposition is followed over at least three half-life times.

2.10. Kinetic assay of the induced decomposition of 1,2-dioxetanes

In a quartz cuvette of capacity 3.0 mL and 1.0 cm of optical path, under magnetic stirring, was added enough dry THF to obtain a final volume of 3.0 mL. The cuvette was inserted in a thermostatized cell holder inside a spectrofluorimeter and allowed to stabilize at the desired temperature. Then 10–30 μL of a solution of **8** in THF were injected, followed by 10–300 μL of a TBAF solution (Acros, 1 mol L^{-1} solution in THF), and the emission intensity decay accompanying the induced 1,2-dioxetane decomposition was followed over at least four half-life times.

3. Results and discussion

3.1. Synthesis of the 1,2-dioxetanes **6**, **7** and **8**

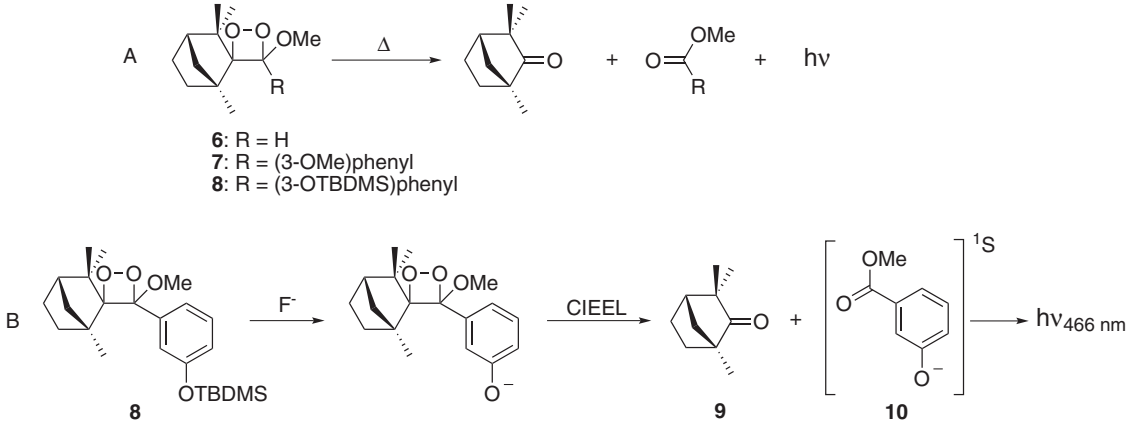
The fenchyl-substituted olefin **3** was prepared in analogy to the literature procedures [24] by means of a Wittig reaction, however in low yields (15% overall yield for the mixture of isomers, *E*:*Z* = 68:32) because of the bulkiness of the fenchyl moiety. The *E*/*Z* mixture could be separated in the constituting isomers by radial chromatography (SiO_2, hexanes). The olefins **4** and **5** were prepared by coupling between a substituted thionoester and 2-diazofenchone followed by nitrogen and sulfur extrusion, a modified Barton–Kellogg procedure that affords highly hindered olefins or enol-ethers in good yields (96% for the final reaction step) as an inseparable mixture of *E*/*Z* isomers [23].

The photooxygenation of the mixture of *E* and *Z* isomers of the olefin **3** in $CDCl_3$ and with tetraphenylporphyrine as sensitizer at −20 °C rapidly formed a mixture of four 1,2-dioxetane diastereoisomers in a total yield of 33%. The formation of four diastereoisomers is expected because the *syn* cycloaddition of singlet oxygen to the olefin can produce, with each isomer of the olefin, two different products, from an *endo* or an *exo* addition. The ^{1}H NMR spectrum of the mixture obtained showed four peaks, with distinct intensities, between 5.4 and 6.1 ppm, in the region expected to show the signal of the hydrogen atom directly attached to the 1,2-dioxetane ring. The photooxygenation of the pure olefins (*E*)-**3** or (*Z*)-**3**, which were separated by radial chromatography, produced two 1,2-dioxetanes for each case, with yields of 69% and 98%, respectively (Scheme 2). In the case of (*E*)-**3**, the ^{1}H NMR signals attributed to the 1,2-dioxetanes were integrated and the intensities determined as 74% and 26%; for the case of (*Z*)-**3**, the intensities are 80% and 20%.

The photooxygenation of **4** and **5** (as a mixture of *E* and *Z* isomers that could not be separated) in the same conditions of **3** proceeded smoothly producing the corresponding 1,2-dioxetanes **7** and **8**, with total yields of 68% and 89%, respectively (Scheme 2); the ^{13}C NMR spectra indicated the formation of four diastereoisomers in each case, that could not be separated by chromatographic methods.

3.2. Thermal decomposition of the 1,2-dioxetanes **6**, **7** and **8**

The thermal decomposition of all 1,2-dioxetanes prepared in this work produced the expected carbonyl compounds (Scheme 3, A), identified on the basis of their ^{1}H-NMR spectra. 1,2-Dioxetanes **6** were obtained in considerable quantities as mixture of the four possible diastereoisomers **6a**, **6b**, **6c** and **6d** (originating from the



Scheme 3. Chemiluminescent decomposition of 1,2-dioxetanes **6**, **7** and **8**. (A) Thermal decomposition of 1,2-dioxetanes; (B) fluoride induced decomposition of the silyl-protected derivative **8**.

Table 1
Activation parameters for the thermal decomposition of 1,2-dioxetanes **6** to **8**.

1,2-Dioxetane	E_a (kJ mol^{-1})	$\Delta^{\ddagger}G^0$ (kJ mol^{-1})	k_1 at 298.15 K (s^{-1})[b]	Condition
6	113 ± 9	103 ± 4	5.68×10^{-6}	A
6a–b	111 ± 2	104 ± 1	3.85×10^{-6}	B
6c–d	96 ± 7	104 ± 7	4.05×10^{-6}	C
7[a]	110.1	115.8	3.24×10^{-8}	D
8[a]	112.8	116.5	2.52×10^{-8}	D
2	119	120	5.74×10^{-9}	E

A: toluene, [**6**] = 2.2×10^{-3} mol L^{-1}. B: toluene, [DBA] = 2.5×10^{-5} mol L^{-1}, [**6a–b**] = 1.8×10^{-5} mol L^{-1}. C: toluene, [DBA] = 2.5×10^{-5} mol L^{-1}, [**6c–d**] = 8.8×10^{-4} mol L^{-1}. D: DSC in 1-acetylnaphtalene, heating rate 8 K min^{-1}. E: in *o*-xylene [8].

[a] Values shown correspond to the predominant diastereoisomer.

[b] Values obtained from E_a and log$A$ or $\Delta^{\ddagger}H$ and $\Delta^{\ddagger}S$ at 298 K.

photooxygenation of the *E*/*Z* mixture of **3**); and, in lower quantities, as the separate mixtures of **6a** and **6b** as well as of **6c** and **6d** (originating from the photooxygenation of the isolated alkenes *E*-**3** and *Z*-**3**). The activation parameters for each mixture were determined by the method of isothermal kinetics, in the presence or not of 9,10-dibromoanthacene (DBA) as a triplet energy acceptor. The thermal stability of these fenchyl-substituted 1,2-dioxetanes **6** proofed to vary only slightly with the configuration of the derivatives (Table 1). For 1,2-dioxetanes **7** and **8**, which are considerably more stable than derivative **6**, the activation parameters were determined by a method using Differential Scanning Calorimetry (DSC) [25], as for isothermal kinetic measurements the temperatures would have to be higher than 200 °C, not achievable in our conventional spectrofluorimeter. The interpretation of the data produced by this method required the presence of two simultaneously decomposing substances of different thermal stabilities for a precise fitting, which can indicate the presence of diastereoisomers, reinforcing the information obtained from the ^{13}C spectra. For **7**, a mixture of substances with the ratio 64.2% and 35.8% was observed, and for **8** it was 61.9% and 38.1% (Table 1).

Comparing the activation parameters of 1,2-dioxetane **8** to the analogue derivative substituted with the spiroadamantyl group **2**, the spirofenchyl 1,2-dioxetanes possess a $\Delta^{\ddagger}G^0$ value *ca.* 4 kJ mol^{-1} lower than the corresponding spiroadamantyl derivative. This result indicates the stabilizing effect of the spirofenchyl group, although this effect proofed to be somewhat lower than that of the corresponding adamantyl group. However, although the effect of the spirofenchyl group is less pronounced, the sterical hindrance introduced by this group is high enough to increase the thermal stability of these fenchyl-substituted 1,2-dioxetanes sufficiently for their possible use in analytical applications.

3.3. *Fluoride catalyzed decomposition of **8***

The deprotection of the silyl group of **8** in aprotic medium by fluoride anions produces a phenolate ion, which is capable of affecting an intramolecular electron transfer to the 1,2-dioxetane ring, inducing the dioxetane decomposition finally producing two carbonyl fragments (**9** and **10**), one of them being possibly formed in its singlet excited state, which decays to the ground state with light emission (Scheme 3, B).

In effect, addition of a solution of tetrabutylammonium fluoride (TBAF) to the solution of 1,2-dioxetane **8** leads to an intense blue light emission, easily visible by the naked eye even in daylight, with a maximum emission intensity at 466 nm. The chemiluminescence emission spectrum obtained from the induced decomposition of **8** is the same as that observed in the decomposition of the adamantyl substituted derivative **2** and corresponds to the fluorescence emission spectrum of the methyl 3-hydroxybenzoate phenolate (**10**) (Fig. 2) [26].

This result indicates that the only reaction product formed in the singlet excited state is the phenolate **10**, which is also in agreement with the commonly formulated reaction mechanism for the induced decomposition of phenolate-substituted 1,2-dioxetanes (Scheme 4). Excited state formation occurs in the back-electron transfer step from the carbonyl anion radical to the phenoxyl radical, which can occur in an intermolecular (Scheme 4, path A) or in an intramolecular (Scheme 4, path B) manner. In any of the cases, the excited states formed will be in the benzoate moiety and not in the aliphatic carbonyl compound (Scheme 4). This observation is in contrast to the results obtained in the unimolecular decomposition of the 1,2-dioxetanes **6** to **8**, where the observed direct chemiluminescence emission from thermal decomposition is due to the fluorescence of fenchone, as judged by the chemiluminescence emission spectra (data not shown). The emission from the optically active fenchone in direct 1,2-dioxetane decomposition could produce the circular polarization of luminescence (CPL) effect [28], however as the emitting species in the much more efficient triggered decomposition from 1,2-dioxetane **8** originates from the achiral phenolate moiety, we did not make efforts to detect the occurrence of CPL in this system.

The observed rate constants of emission intensity decay, as well the values for Φ_S and Φ_{CL}, were determined for the decomposition of **8** in the presence of different concentrations of TBAF (see SI, Table S1). The rate constants show linear correlation with the TBAF concentration in the low concentration range ([TBAF] < 0.01 mol L^{-1}) with a bimolecular rate constant of $\underline{k}_F = 1.08 \pm 0.02$ mol L^{-1} s^{-1} (Fig. 3). For TBAF concentrations higher than 0.2 mol L^{-1}, the rate constants proofed to be independent of the TBAF concentration, with a mean value of $k_{obs} = (3.9 \pm 0.1)\,10^{-3}$ s^{-1} (Fig. 3). The rate constant determined in these conditions should correspond to the initial intramolecular electron transfer from the phenolate ion to

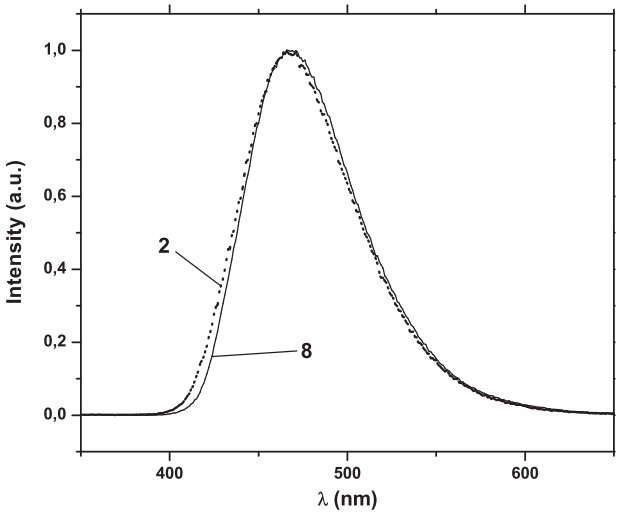
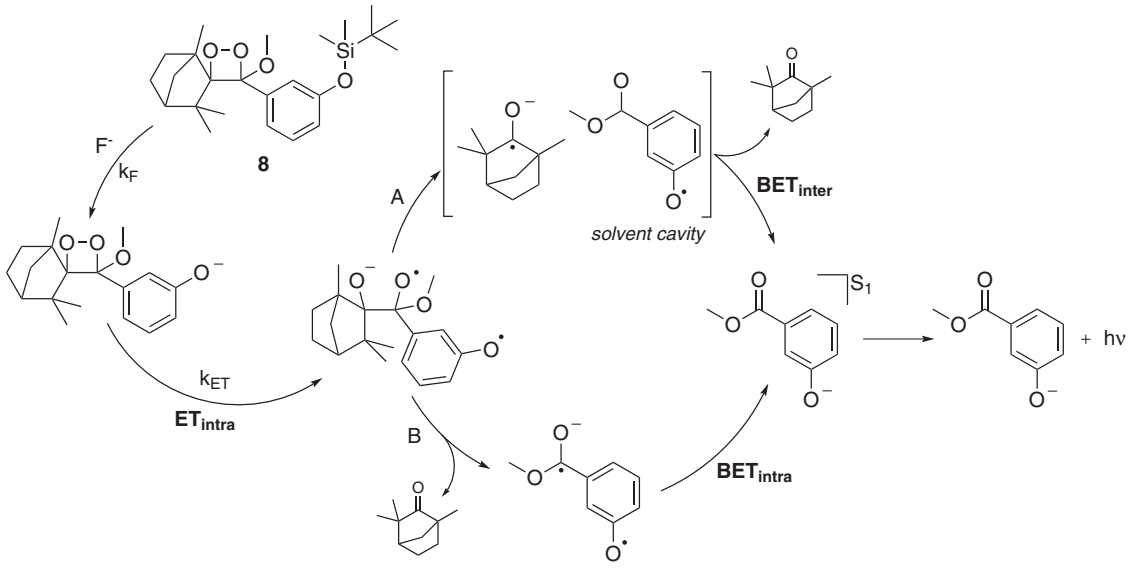


Fig. 2. Chemiluminescence emission spectrum of the fluoride-induced decomposition of **2** and **8**. Conditions: THF, 20 °C, [1,2-dioxetane] = 2.0×10^{-5} mol L^{-1}, [TBAF] = 5.0×10^{-2} mol L^{-1}.



Scheme 4. Mechanism for the induced decomposition of phenoxyl-substituted 1,2-dioxetanes.

the peroxidic ring (k_{ET}), as at high [TBAF] the bimolecular deprotection step can become faster than the unimolecular electron transfer step, resulting in a change of the rate-limiting step (Scheme 4). The singlet quantum yields (Φ_S) and chemiluminescence quantum yields (Φ_{CL}) proofed to show a slight increase with increasing [TBAF], reaching values of up to 6% for Φ_S and up to 1.5% for the emission quantum yields Φ_{CL} (see SI, Table S1).

The activation parameters for the induced decomposition of 1,2-dioxetane **8** were determined at high TBAF concentrations, by measuring the emission intensity decay at different temperatures, obtaining an Eyring plot with excellent linear correlation (Fig. 4). The activation enthalpy ($\Delta^{\ddagger}H$), the activation entropy (reported as $-T\Delta^{\ddagger}S^0$) and the free activation energy values ($\Delta^{\ddagger}G$) calculated, as well as the singlet excitation and chemiluminescence quantum yields obtained in different conditions, are shown in Table 2, in comparison with the corresponding values for the adamantyl-substituted derivative **2**. Whereas the activation enthalpy values for **8** proofed to be more than 10 kJ mol^{-1} higher than for **2**, the calculated free activation energy values showed to be very similar. The low positive activation entropy value obtained in the present work for the decomposition of **8** is in agreement with the assumption that the observed reaction step is the intramolecular electron transfer, which initiates the peroxide decomposition (Scheme 4). Contrarily, the much higher negative entropy value reported in the literature for the induced decomposition of **2** might indicate a contribution of the deprotection step or the necessity, in the case of this derivative, of a specific conformation for the occurrence of the electron transfer process (Table 2).

The similarity of the free activation energy values for the induced decomposition of **2** and **8** indicates a very similar kinetic behavior of these derivatives, suggesting that derivative **8** could substitute the spiroadamantyl derivative **2** in bioanalytical applications. The singlet excitation and chemiluminescence quantum yields for derivative **8** proofed to increase with increasing fluoride concentrations, however, constant values were obtained in the high concentration range (Table 2, see also SI, Table S1). Although quantum yields for **8** showed to be one order of magnitude lower than for **2**, the more convenient access to the olefinic precursor of **8** might at least partially compensate this fact. Anyway, chemi-

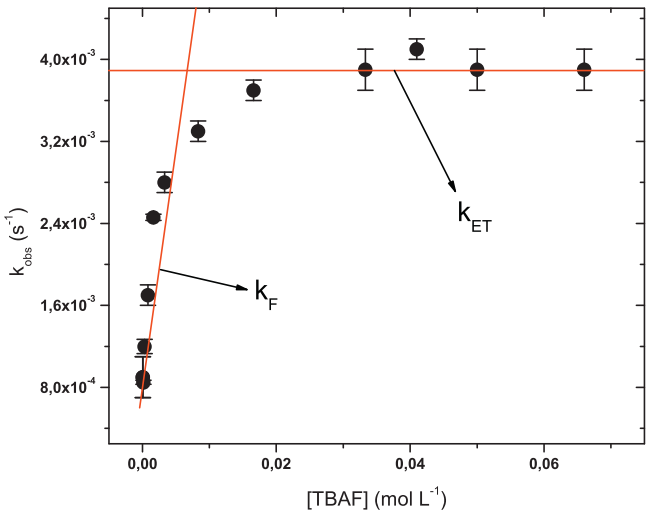


Fig. 3. Dependency of the observed emission intensity decay rate constant in the fluoride induced decomposition of **8** on the TBAF concentration. Conditions: THF, 20 °C, [**8**] = 2.0 × 10^{-5} mol L^{-1}.

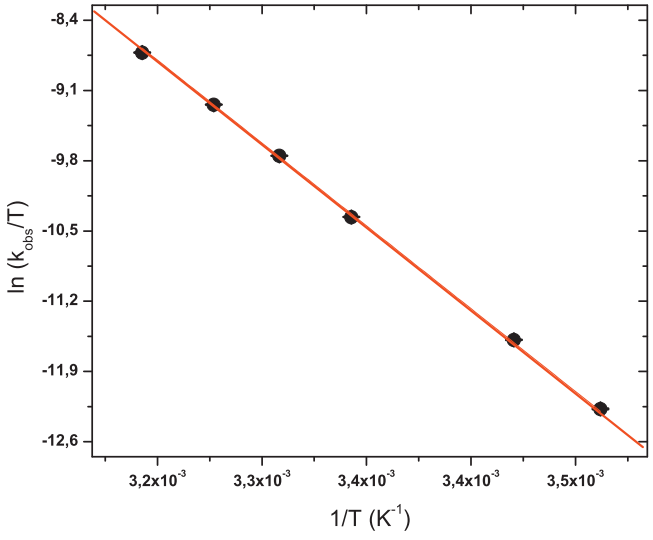


Fig. 4. Eyring plot of the observed emission intensity decay rate constants in the fluoride induced decomposition of **8**. Conditions: THF, [**8**] = 2.0 × 10^{-5} mol L^{-1}, [TBAF] = 5.0 × 10^{-2} mol L^{-1}.

Table 2
Activation parameters and quantum yields for the fluoride induced decomposition of 1,2-dioxetane **8**, as well as the corresponding literature values for derivative **2** [4].

1,2-Dioxetane	$\Delta^{\ddagger}H$ (kJ mol^{-1})	$-T\Delta^{\ddagger}S^{0}$ (kJ mol^{-1})	$\Delta^{\ddagger}G$ (kJ mol^{-1})	Φ_S ($\times 10^2$ E mol^{-1})	Φ_{CL} ($\times 10^2$ E mol^{-1})
8	85.4 ± 0.4	−0.82 ± 0.01	84.6 ± 0.8	3.1 ± 0.5[a] 6.3 ± 0.1[b]	0.7 ± 0.1[a] 1.46 ± 0.03[b]
2	72.8 ± 0.4[g]	9.2 ± 0.1[g]	82.0 ± 0.8[g]	55 ± 7[c] 66 ± 9[d] 45[e] 57[f]	12 ± 2[c] 29 ± 4[d] 9.4[e] 25[f]

[a] THF, 20 °C, [**8**] = 2.0×10^{-5} mol L^{-1}, [TBAF] = 3.3×10^{-3} mol L^{-1}.
[b] THF, 20 °C, [**8**] = 2.0×10^{-5} mol L^{-1}, [TBAF] = 1.6×10^{-2} mol L^{-1}.
[c] Acetonitrile, 25 °C, [**2**] = 1.0×10^{-7} mol L^{-1}, [TBAF] = 3.3×10^{-3} mol L^{-1}.
[d] DMSO, 25 °C, [**2**] = 1.0×10^{-7} mol L^{-1}, [TBAF] = 3.3×10^{-3} mol L^{-1}.
[e] Acetonitrile, 25 °C, [**2**] = 1.0×10^{-7} mol L^{-1}, [TBAF] = 1.0×10^{-3} mol L^{-1}.
[f] DMSO, 25 °C, [**2**] = 1.0×10^{-7} mol L^{-1}, [TBAF] = 1.0×10^{-3} mol L^{-1}.
[g] See Refs. [27,4] for detailed information on chemiluminescence quantum yields obtained for **2**.

luminescence quantum yields in the one percent range are usual for several much utilized chemiluminescence systems like the well known and widely applied luminol reaction [4].

4. Conclusions

In this work it is shown that fenchyl-substituted 1,2-dioxetanes can potentially be utilized to substitute the corresponding spiroadamantyl derivatives in bioanalytical applications. Although those fenchyl-derivatives show slightly reduced thermal stability, kinetic data for its induced decomposition proofed to be similar to the ones obtained with the established adamantyl substituted 1,2-dioxetanes. Chemiluminescence emission quantum yields in the one percent range classify the induced decomposition of the fenchyl-substituted 1,2-dioxetane containing a triggerable phenolate unit as an efficient chemiluminescence system, comparable in efficiency to the well known luminol reaction.

The most important advantage of the fenchyl derivatives as compared to the spiroadamantyl-substituted 1,2-dioxetanes is the advantage of the much easier synthesis and the use of the inexpensive (−)-fenchone instead of the expensive 2-adamantanone as starting product.

Acknowledgements

The authors would like to thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Arbeitsgemeinschaft industrieller Forschungsvereinigungen “Otto von Guericke” e.V. (AiF) for financial support. L.F.M.L.C thanks Deutscher Akademischer Austauschdienst (DAAD) and Coordenadoria de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) for a doctoral fellowship (BEX 1132/04-0).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jphotochem.2010.12.001.

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