Weak chemiluminescence emission during base induced rearrangement of G-factors†

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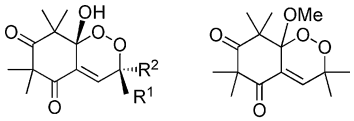
A rearrangement in basic medium of the natural endoperoxide G3-factor extracted from *Eucalyptus grandis* is described. Evidence to support a 1,2-dioxetane intermediate that decomposes with weak luminescence emission (quantum yield) is presented.

G-Factors are natural endoperoxides first extracted from mature leaves of *Eucalyptus grandis* in much larger quantities after a cold period. They act as phytohormones and growth regulators (Fig. 1).[1]



R^1	R^2	G
Et	Me	G1
Me	Et	G2
Me	Me	G3

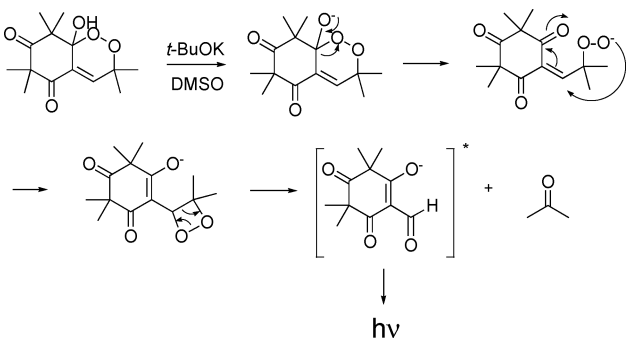
G3Me

Fig. 1 Structures of G-factors and G3Me.

It seems that G-factors cannot be present in the plant in their physiologically active form. Their function in the plant is not well elucidated but they reduce water loss, contribute to root inhibition and are involved in frost resistance in *Eucalyptus grandis*. Their structures are interesting because of the presence of a peroxide function providing potential antimalarial properties. We have previously reported an optimized synthesis of G-factors and analogs.[2] The parent compounds are obtained in a two step procedure, *i.e.* Mannich reaction between syncarpic acid and the corresponding aldehyde, then acidic fragmentation of the Mannich base followed by spontaneous oxygen uptake leading to the expected endoperoxides. Indeed some G-factor analogs are active against *Plasmodium*, in particular compounds alkylated on the peroxy-hemiketal function.[3] The crucial role of the peroxy-ketal function for anti-plasmodial activity has been reported. The methyl ether of G3 (G3Me) was found to be one hundred times more active than G3 (IC_{50} (G3) = 36 μM and IC_{50} (G3Me) = 0.28 μM on Nigerian strains).[3]

Alkylation of this position proved to be very difficult. The methyl moiety group was introduced in good yield (with BuLi–THF followed by methyl triflate or with K_2CO_3–MeI) but the yield decreased when using other alkyl iodides or benzyl bromide (30–40%).[4]

To explain these difficulties, we decided to study the stability of G3-factor in basic media. Depending on the base used, it decomposed with different kinetics giving a polar compound. We tried to monitor the reaction in d6-DMSO in the presence of *t*-BuOK (1 eq.), but the transformation was very rapid and ^{1}H NMR immediately showed an 80% conversion of G3 into an aldehyde and acetone (Scheme 1). In the presence of K_2CO_3–DMF, the reaction was carried out overnight, and led to the same products. This aldehyde was isolated and fully characterized by 2D NMR‡ and its structure was found to be that of aldehyde **A**. The aldehyde was then crystallized and X-ray diffraction analysis was performed to get additional information. Surprisingly, this analysis allowed us to unveil a dimeric hydrated magnesium salt, the magnesium originating from $MgSO_4$ used as desiccant (Fig. 2). X-Ray crystal analysis showed that the magnesium atom lies on an inversion center and the structure is nearly planar due to the extended conjugated system: the negative charge on the carbonyl of the aldehyde, the C=C double bond and the keto C=O bond. The distances C_{11}–C_5 and C_5–C_6 are equivalent (1.42 and 1.44 Å), and the same occurs for C_{11}–O_5 and C_6–O_4 (both 1.24 Å) and Mg–O_5 and Mg–O_4 (2.03 and 2.04 Å).§ The planarity of the structure was confirmed by the ^{1}H NMR spectrum of the magnesium dimeric salt, which is particularly simple: both *gem*-dimethyl protons are equivalent at 1.34 ppm (12H) in CD_3OD while aldehydic proton resonates at 9.66 ppm.



Scheme 1 Rearrangement of G-factor in basic medium.

Aldehyde formation involves the decomposition of an intermediate 1,2-dioxetane which is formed by Michael addition of ROO^- to the conjugated double bond after cleavage of the peroxyl-carbon bond. Such a rearrangement has already been described by us[5] on an analog of the G-factor but in acid media. 1,2-Dioxetanes are known to be highly reactive molecules which decompose into electronically excited carbonyl compounds

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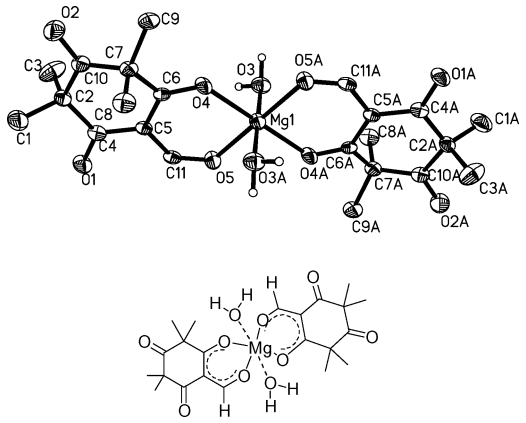

Fig. 2 ORTEP drawing of magnesium dimeric salt of **A**. (50% probability chosen for the ellipsoids.) A central symmetry is implied: magnesium lies on an inversion center.

responsible for chemiluminescence. Few examples in the literature describe endoperoxide–dioxetane rearrangements, and the associated chemiluminescence. For instance, such a rearrangement has been documented in the photooxygenation of heteroarenes.[6,7] Here we report on the chemiluminescence emitted during treatment in basic media of natural G3-factor endoperoxide.

First the luminescence properties of G3-factor were examined in the presence of *t*-BuOK in DMSO and the light emission profile was recorded. The maximum intensity was obtained at a wavelength of 410 nm (Fig. 3). The peak intensity decreased quickly.

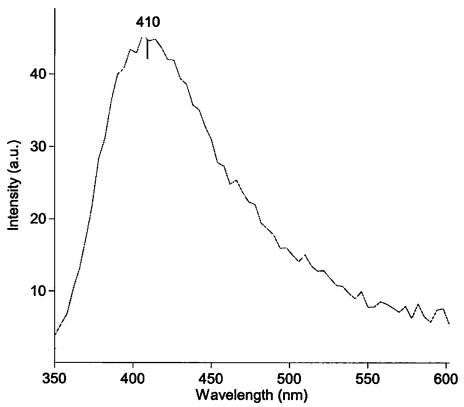

Fig. 3 Direct emission spectrum for base-catalyzed decomposition of G3-factor (corrected spectrum).

A kinetic study (Fig. 4) based on chemiluminescence decay was recorded for the reaction of G3 with *t*-BuOK (1 eq.) in DMSO at room temperature. The reaction was followed by monitoring the intensity $I_{CL}(t)$ of the emitted light at 410 nm, which reached a maximum immediately after *t*-BuOK addition, and then decayed following pseudo-second order kinetics.

The chemiluminescence intensity is expressed by equation (1) in which v is the reaction rate and Φ_{CL} represents the chemiluminescence yield. Φ_{CL} corresponds to the total amount of light ($N_{photons}$) divided by the number of moles of dioxetane (n_D).[8] The expression for $N_{photons}$, which is the area under the chemiluminescence intensity curves, is given by integration of

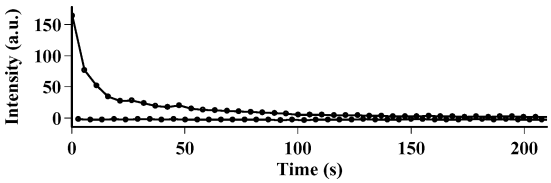

Fig. 4 Emission profile for the chemiluminescence of the reaction of *t*-BuOK (1 eq.) with G3 (1 eq.) in DMSO, and blank curve.

$I_{CL}(t)$ over the reaction time (eqn (2)) in which

$$\int_0^\infty v\,\mathrm{d}t$$

is the number of moles of decomposed dioxetane. It follows that Φ_{CL} is indeed experimentally defined by equation (3).

$$I_{CL} = \Phi_{CL} v \tag{1}$$

$$N_{photons} = \int_0^\infty I_{CL}\,\mathrm{d}t = \Phi_{CL}\int_0^\infty v\,\mathrm{d}t = \Phi_{CL} n_D \tag{2}$$

$$\Phi_{CL} = \frac{N_{photons}}{n_D} \tag{3}$$

The Φ_{CL}^{G3} value was determined as the quantum yield with respect to the Φ_{CL}^{L} value (0.0124) of luminol[9] in DMSO containing *t*-BuOK–*t*-BuOH under air.¶

Φ_{CL}^{G3} was found to be $(1.9 \pm 1) \times 10^{-8}$ E mol^{-1}. This value is to be compared to the thermal decomposition of organic peroxides which are also weakly chemiluminescent with quantum yield as low as 10^{-8} in general.[10]

A mechanistic alternative is proposed:[10] this could be the thermolysis of dioxetanes or the intramolecular CIEEL "chemically initiated electron exchange luminescence" decay process.

The thermal decomposition of rather simple dioxetanes affords predominantly a triplet-excited carbonyl along with a small amount of a singlet excited carbonyl so that direct emission of bright light is scarcely expected. Many reports[11–13] dealt with the experimental and theoretical studies of chemiluminescence of 1,2-dioxetanes. When 1,2-dioxetanes bear an electron rich substituent, they become labile and display CIEEL. This happens for 1,2-dioxetanes containing substituents with low oxidation potentials, such as aryl–O$^-$ or aryl–RN$^-$ functionalities.[8]

As the basic treatment of G3 only generated a faint light emission, we decided to add the fluorescent additive DBA (9,10-dibromoanthracene, a triplet energy acceptor) or DPA (9,10-diphenylanthracene, a singlet energy acceptor) and we observed the evolution of chemiluminescence. Generally, obtaining a linear correlation in the Stern–Volmer plot of the double reciprocal of the fluorescer concentration and the chemiluminescence quantum yields gives evidence for a bimolecular process between the excited species and the fluorescer. 9,10-Dibromoanthracene (DBA) is capable of accepting the excitation energy from a triplet excited carbonyl group *via* triplet to singlet energy transfer. This energy transfer results in the formation of the fluorescent singlet state of DBA. 9,10-Diphenylanthracene (DPA) is *ca.* 1000 times less efficient as an acceptor of triplet energy *via* triplet to singlet energy transfer than DBA.[14] An estimate of the ratio of the triplet

to singlet excited species resulting from the decomposition of the dioxetane can be obtained from the intensity of DBA and DPA chemo-excited emission intensity. The observed intensities of DBA and DPA emission were extrapolated at infinite dye concentration.[14] Corrections were applied for differences in energy transfer efficiency, fluorescence efficiency of the dyes and cross activation (triplet–singlet energy transfer to DBA).

The double reciprocal plot of quantum yield and the DBA and DPA concentration during treatment of G3 is shown in Fig. 5. It is linear which allows $\Phi_{CL}^{Flu.(\infty)}$ to be estimated. The $\Phi_{CL}^{DBA(\infty)}$ value at infinite concentration of DBA could be estimated from the intercept[14] and is expressed by equation (4).

$$\Phi_{CL}^{DBA(\infty)} = \Phi_r \times \Phi_T^* \times \Phi_{TS} \times \Phi_F^{DBA} \quad (4)$$

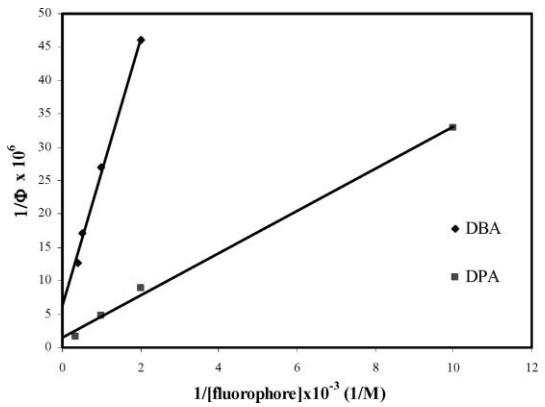

Fig. 5 Reciprocal plot of chemiluminescence quantum yield against concentration of fluorophore. In basic medium G-factor endoperoxide rearranges with weak chemiluminescence emission at 410 nm.

This total chemiluminescence quantum yield is estimated to be $(1.60 \pm 0.40) \times 10^{-7}$ E mol^{-1}. The yield of triplet aldehyde Φ^*_r can then be calculated from eqn (4), where Φ_r is the chemical yield (equal to 0.8), Φ_{TS} the triplet singlet energy transfer (estimated to 0.2),[13] and Φ_F^{DBA} the fluorescent quantum yield of DBA (0.1).[15,16] Thus, the triplet state quantum yield is $(1 \pm 0.25) \times 10^{-5}$ E mol^{-1}. With DPA, the $\Phi_{CL}^{DPA(\infty)}$ value at infinite concentration of DPA is estimated as $\Phi_{CL}^{DPA(\infty)} = (1 \pm 0.3) \times 10^{-6}$ E mol^{-1}. In the singlet case, $\Phi_{CL}^{DPA(\infty)} = \Phi_r \times \Phi^*_s \times \Phi_F^{DPA}$ where Φ_F^{DPA} is the fluorescent yield of DPA is equal to 0.89.[16] Thus $\Phi^*_S = (1.4 \pm 0.4) \times 10^{-6}$ E mol^{-1}

A yield ratio of triplet to singlet excited aldehyde from dioxetane of *ca.* 7 was observed. It is smaller than the reported values for thermolysis of dioxetanes which give mainly an excited triplet species.[17] In our case, it seems likely that intramolecular CIEEL could be invoked, though the oxidation potential of C=C–O$^-$ is not as low as that of Ar–O$^-$. Moreover, the 1,2-dioxetane intermediate could not be isolated under these conditions (pH, temperature), and so appear as particularly unstable (reactive). Theoretical investigations are ongoing to try to explain this CL property.

In conclusion, we have reported here a novel example of chemiluminescence which is specifically induced from G-factor by using *t*-BuOK–DMSO as a triggering agent. We describe here a natural endoperoxide as a novel particularly stable chemiluminescent precursor. This endoperoxide, obtained by autoxidation, is able to emit low chemiluminescence when pH increases. It is strong evidence of the presence of a 1,2-dioxetane intermediate in this rearrangement.

Acknowledgements

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Notes and references

‡ (**A**) ^{1}H NMR (400 MHz, CD_3OD) δ = 1.32 (12H, 4Me), 9.39 (1H, C*H*O) ppm. ^{13}C NMR (100.61 MHz, CD_3OD) δ = 24.86, 25.18 (4CH_3), 55.20, 55.82 (2C, CH_3CCH_3), 112.91 (C, CO*C*(CHO)=C), 190.73 (CH, *C*HO), 199.00 (C, *C*(OH)=C), 201.17 (C, *C*OC=C), 214.74 (C, *C*O) ppm. MS: (DCI–NH_3, CH_2Cl_2–MeOH, negative mode, m/z(%)): $[M]^-$ = 210 (100). IR (neat in compressing cell) ν: 3498, 3390, 3260 (O–H stretching), 2979, 2943, 2876 (CH_3 stretching), 2746, 2793 (C–H stretching of aldehyde), 1723, 1707 (C=O saturated and αβ-unsaturated), 1650 (CH=O, β ketoaldehyde in enol form), 1592 (C=C, conjugated with αβ-unsaturated carbonyl), 1051 (C–O stretching) cm^{-1}.

§ Crystal data for dimeric magnesium salt of **A**: $C_{22}H_{30}MgO_{10}$, M = 239.38, triclinic, $P\bar{1}$, a = 8.279(3) Å, b = 8.724(3) Å, c = 9.245(4) Å, α = 97.164(8)°, β = 94.153(8)°, γ = 116.289(8)°, V = 587.8(4) Å^3, Z = 2, ρ_{calcd} = 1.352 Mg m^{-3}, F(000) = 254, λ = 0.71073 Å, T = 173(2) K, μ($Mo_{K\alpha}$) = 0.130 mm^{-1}, crystal dimensions 0.1 × 0.3 × 0.4 mm^3, 2627 reflections (1656 independent, R_{int} = 0.1123) were collected at low temperatures using an oil-coated shock-cooled crystal on a Bruker-AXS CCD 1000 diffractometer. The structure was solved by direct method[18a] and 163 parameters were refined using the least-squares method on F^2.[18b] Largest electron density residue 0.261 eÅ^{-3}, R_1 (for $I > 2\sigma(I)$) = 0.0600 and $wR2$(all data) = 0.1347 with $R1 = \sum||F_o| - |F_c||/\sum|F_o|$ and $wR2 = w(\sum w(F_o^2 - F_c^2)^2/\sum w(F_o^2)^2)^{0.5}$. CCDC reference number 663261. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b715491g.

¶ The chemiluminescence quantum yields were measured using a Cary Eclipse fluorimeter in chemiluminescence mode equipped with a Hamamatsu photomultipler tube R955. (Get Time 700 ms.) Calculation method: the area of emission can be converted from arbitrary units (a.u.) to Einstein (E, "moles" of photons) using the luminol conversion factor (f_{lum}), which is calculated by:

$$f_L = \frac{\Phi_L n_L}{S_L} [\text{Ea.u.}^{-1}]$$

where Φ_L is the luminol quantum yield (1.24%), n_L is the number of moles of luminol (4.65×10^{-9}) and S_L is the area under the curve of emission (3.98×10^5), which is obtained by integration of the light emission intensity as a function of the reaction time. The quantum yield for a certain chemiluminescence reaction is obtained by the following equation:

$$\Phi_{CL} = \frac{S f_L f_{photo}}{n}$$

where S is the area under the curve of the CL reaction to be calibrated, f_L is the luminol factor, n is the number of moles of the limiting reagent, and f_{photo} is the photomutiplier tube wavelength sensitivity factor.

Hamamatsu photomultipler tube R955: $f_{420}^{438} \approx f_{420}^{430} = 72/70 = 1.03$; $f_{420}^{410} = 72/73 = 0.99$.

In this study f_{photo} will be taken as equal to 1.03 as the spectral response will be quite the same between 430 (DPA emission) and 438 (DBA emission) and 0.99 for direct emission during G3 treatment. The emission intensity (I, a.u. s^{-1}) can also be calibrated: I_C[E s^{-1}] = I[a.u. s^{-1}] × f_L × f_{photo}[E a.u.$^{-1}$]; $f_L = 1.45 \times 10^{-16}$[E a.u.$^{-1}$]

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