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Studies on the Mechanism of the Peroxyoxalate Chemiluminescence Reaction: Part 1; Confirmation of 1,2-dioxetanedione as an Intermediate using $^{13}$C Nuclear Magnetic Resonance Spectroscopy

Richard Bos, Neil W. Barnett*, Gail A. Dyson, Kieran F. Lim, \( \text{Richard A. Russell and Simon P. Watson}^\dagger \).

Centre for Chiral and Molecular Technologies,
School of Biological and Chemical Sciences,
Deakin University, Geelong, Victoria 3217, Australia.

Abstract
A simple model peroxoalxate chemiluminescence system was monitored directly across a range of temperatures (from -80°C to +20°C) using $^{13}$C nuclear magnetic resonance spectroscopy. These experiments were made possible by the utilisation of $^{13}$C doubly labelled oxalyl chloride, which was reacted with anhydrous hydrogen peroxide in dry tetrahydrofuran. *Ab initio* quantum calculations were also performed to estimate the $^{13}$C NMR shift of the most commonly postulated key intermediate 1,2-dioxetanedione and this data, in concert with the spectroscopic evidence, confirmed its presence during the reaction.

$^\dagger$Present Address: John Curtin School of Medical Research, Australian National University, Canberra, ACT 0200, Australia.

Keywords
peroxoalxate chemiluminescence intermediate, 1,2-dioxetanedione, nuclear magnetic resonance spectroscopy, *ab initio* quantum calculations
Introduction

Some forty years ago, Chandross [1] first observed intense blue chemiluminescence from the reaction between oxalyl chloride and hydrogen peroxide in the presence of 9,10-diphenylanthracene, the emission had a wavelength distribution that was identical to the fluorescence spectrum of the polyaromatic hydrocarbon (see Scheme 1).

\[
\begin{align*}
\text{O} & \quad \text{Cl} \\
\text{Cl} & \quad \text{O} & \quad \text{H}_2\text{O}_2 & \quad \text{Chemically induced fluorescence}
\end{align*}
\]

Scheme 1

This study [1] was to be the precursor of, so-called, peroxyxalate chemiluminescence, which has subsequently enjoyed extensive utility in analytical chemistry and as a portable light source, due to its capacity to efficiently excite a wide range of fluorophores [2]. Elucidation of the peroxyxalate reaction mechanism has been the subject of numerous studies [3-17] one of the earliest being that of Rauhut [3] who noted, that after four minutes the reagent concentration had been depleted by 98% whereas the chemiluminescence emission was still at 58% of its maximum intensity. Consequently, Rauhut [3] suggested that a transient key intermediate with some degree of stability was responsible for the excitation of the fluorophore and further postulated that 1,2-dioxetanedione was the most likely candidate (see Scheme 2). Stigbrand et al. [2] critically reviewed much of the work undertaken on mechanistic studies [3-17] and concluded that neither the light-producing pathway nor the key intermediate had been definitively characterised. Likewise, recent studies [18-26] designed to probe the reaction pathways of peroxyxalate chemiluminescence have not afforded any direct evidence for the existence of the postulated key intermediates.
This paper describes, for the first time, the use of $^{13}$C doubly labelled oxalyl chloride, as a reagent to facilitate the efficient monitoring of a peroxoxtalate chemiluminescence reaction using $^{13}$C NMR spectroscopy. The chemistry described by Chandross [1] was selected as a model system because of its high efficiency at low temperatures and relative simplicity with respect to the nature and number of the possible intermediates.

Experimental

Instrumentation

Variable temperature NMR experiments were performed using a Varian Unity plus 300 MHz NMR spectrometer. A 5 mm ID NMR tube, fitted with a Young valve connection, was adapted to accept a 5-meter long 2 mm ID PTFE tube. This modification minimised the risk of damage to the NMR probe should an accidental temperature excursion occur, consequently the tube was not spun during the course of the experiments.

Reagents

The oxaly-1,2-$^{13}$C$_2$ chloride was synthesised from oxalic-1,2-$^{13}$C$_2$ acid ($^{13}$C 99%, Cambridge Isotope laboratories, Novachem, Victoria, Australia), after the method of Galagovsky et al. [27]. The doubly
labelled oxaly chloride was isolated from the reaction mixture using vacuum distillation. Unlabelled oxalyl chloride, phosphorous pentachloride and 9,10-diphenylanthracene were obtained from Aldrich, (Castle Hill, New South Wales, Australia) and HPLC grade tetrahydrofuran from Merck, Science Supply, Oakleigh South, Australia. Deuterated tetrahydrofuran (0.75 ml ampoules 99.8%, Cambridge Isotope laboratories, Novachem, Victoria, Australia) was used as the solvent for all NMR experiments.

The preparation of anhydrous hydrogen peroxide was performed cautiously using the vacuum distillation method described by Cofré and Sawyer [28]. It should be noted that in the interests of safety, only scrupulously cleaned, polished joint glassware was employed for this procedure. The anhydrous hydrogen peroxide was transferred from the glassware used for its drying to an NMR tube using a short length (ca 150 mm) 0.5 mm ID Teflon tube attached to a 1 ml plastic syringe.

**NMR Procedure**

Two NMR tubes containing the anhydrous reactants were cooled at –95°C in an acetone/liquid nitrogen slush bath, the first (with Young valve) contained the $^{13}$C oxalyl chloride in ~ 0.4 ml of d-8 THF, while the second held the anhydrous hydrogen peroxide and 9,10-diphenylanthracene in ~ 0.3 ml of d-8 THF. The latter solution was carefully transferred into the former via a short length (ca 150 mm) 0.5 mm ID Teflon tube attached to 1 ml plastic syringe with mixing achieved by gently bubbling air through the solution. The NMR spectrometer was stabilised at –95°C prior to the insertion of the pre-cooled sample, the reaction mixture was then allowed to equilibrate for two minutes at each chosen temperature interval prior to the collection of an array of 7 × 128 scans. Spectra were monitored at various temperatures from -80°C up to +20 °C.

**Results and Discussion**

**Preliminary experiments**

Using two 20 ml glass vials, aliquots of oxalyl chloride in tetrahydrofuran were simultaneously reacted with hydrogen peroxide (30%) at –75 °C. One of the vials (A) also contained 9,10-diphenylanthracene while the other (B) did not. The reaction within vial A (fluorophore present) elicited a deep blue emission characteristic of the fluorescence from 9,10-diphenylanthracene, which persisted (visually) for more than thirty minutes. When the chemiluminescence from vial A was all but extinguished an identical amount of 9,10-diphenylanthracene was added to vial B, which immediately exhibited the same intense, long-lived blue emission. These initial results indicated that the species responsible for chemi-excitation was temporally stability under the above reaction condition. Prados and co-workers [13] observed the formation of stable but uncharacterised compounds at -20 °C with either of bis(2,4,6-trichlorophenyl) oxalate or bis(4-nitro-2-(3,6,9-trioxadecyloxycarbonyl)phenyl) oxalate in the presence of hydrogen peroxide and
trifluoroacetic acid. Upon subsequent reaction with a suitable fluorophore both of the proposed intermediates generated chemiluminescence [13].

*Ab initio NMR shift calculations*

It is known that *ab initio* NMR predictions benefit substantially from an accurate geometry and a large basis set [29]. Calculations were performed for several structurally related species including the proposed intermediate 1,2-dioxetanedione using GAUSSIAN 98 [30] and a variety of basis sets (see Table 1). Zhang et al. [26] also employed GAUSSIAN 98 to obtain *ab initio* vibrational spectral calculations of proposed intermediates in the peroxo-oxalate chemiluminescence reaction. Structural data for the compounds studied were only available for the either the gas or solid phase. For the geometry optimisation, RHF/6-31G(O(d)) was chosen because it gives optimised structures that compare well with the available experimental data [32,35,36,42,44]. However, RHF theory could not reproduce either the oxalic acid C=O or C-C bond length [39-41], hence the use of B3LYP theory. Since the structures of oxetane, and cyclobutanone were reproduced well at the RHF/6-31G(C-O(d)) level (ie. d functions on only the singly bonded oxygen atoms) this basis set was used to optimise 2-oxetanone, which has analogous structure. Single-point calculations using B3LYP/6-31++G(2p,d) then yielded the NMR shielding tensors of the optimised structures. This method has been shown to give reliable NMR predictions with the relative chemical shifts of a variety of compounds [29]. In particular, for small molecules related to oxalic acid, or four-membered rings with oxygen heteroatoms, this level of theory yields NMR chemical shifts that are close to experimental values from the literature [31,33] and this work. In Table 1, the calculated NMR shifts used TMS as a single reference point for converting shielding tensors into NMR shifts; however, a better estimation procedure employed a linear conversion, which was a best-fit to the data of all the molecules. The estimated shift was equal to 180.67 – 0.9900273 x (calculated shielding tensor).

*NMR spectroscopy*

As a consequence of the $^{13}$C labelling of the oxalyl chloride, the presence of 9,10-diphenylanthracene, during following experiments did not interfere with the resultant $^{13}$C NMR spectra as the peaks from fluorophore were not observed above the noise level.

As can be seen in Figure 1, at −80°C the major spectral features were a singlet at δ 158.9 assigned to unreacted oxalyl chloride, a pair of doublets at δ 159.3 and δ 153.9 ($J$ 105.3 Hz) and a small singlet at δ 154.5. These latter resonances become dominant in the spectrum at −70°C. The pair of doublets can be assigned to the carbonyl resonances of the unsymmetrical intermediate (2) shown in Scheme 3, resulting from the initial attack of hydrogen peroxide on the oxalyl chloride whilst the singlet must arise from a symmetrical species containing only one type of carbonyl group and this is entirely consistent with the presence of 1,2-dioxetanedione (3). There is reasonable agreement between the observed resonance (δ
154.5) and that of the estimated relative chemical shift (δ 158.4) shown in Table 1. Significantly, at –60°C the resonances due to intermediate (2) have all but disappeared demonstrating that the intramolecular ring closure was largely complete. Additionally, at this temperature the resonance due to 1,2-dioxetanedione (3) also shows some broadening suggesting a dynamic process. As the temperature was increased, this broadening became more evident (see Figures 1 and 2) as did the significance of a new pair of doublets at δ 158.1 and δ 156.7 (J 95.8 Hz) indicative of the formation of another unsymmetrical species.

Interestingly, the broadening of the 1,2-dioxetanedione resonance was reversible with temperature (see Figure 3). The cycling of the system from –70°C to –45°C and back to –70°C also resulted in an increase in the peak heights of the doublets at δ 158.1 and δ 156.7 (J 95.8 Hz) and the loss of resonances due to compound (2) in Scheme 3 as well as those of some minor peaks at δ 162.2 and δ 163.4.

\[\text{(1)} \xrightarrow{\text{H}_2\text{O}_2} \text{(2)}\]

\[\text{(4)} \xrightarrow{\text{H}_2\text{O}_2} \text{(3)} \xleftarrow{} \text{(6)}\]

\[\text{(5)} \xrightarrow{\text{HCl}} \]

Scheme 3

At temperatures above –40°C, line broadening of certain intermediates became significant (see Figure 2) and we interpreted this as evidence of reactions leading to their decomposition, in particular the disappearance of the 1,2-dioxetanedione above –20°C. As yet we have been unable to probe this further, however, a series of 2D EXSY exchange spectroscopy experiments are currently underway and these results will be reported in due course. It was noteworthy that all spectra recorded at temperatures above –70°C (see Figures 1 and 2) show a singlet at δ 160.4. This new entity contains only one carbonyl environment and is relatively thermally stable, partially decomposing overnight at ambient temperature. Attempts to identify
this compound are as yet incomplete, however NMR analysis following overnight storage at ambient
temperature revealed two additional singlet peaks at δ 159.5 and 158.5.
The nature of the second unsymmetrical intermediate attributed to resonances δ 158.1 and δ 156.7 (J 95.8
Hz) is unknown and chemical shift date derived from model compounds [47] such as shown in Figure 4,
would seem to rule out both compound (4) and (5) in Scheme 1. However, the paucity of chemical shift data
makes it difficult to categorically discount compound (6) but any definitive statement will require further
experimentation.
Finally, we observed that in all spectra monitored below room temperature there were significant resonances
at δ 125.6 and δ 184.5 (not shown in Figures 1 and 2) assigned to carbon dioxide and carbon monoxide
respectively which is consistent with the literature [43,48]. The generation of carbon dioxide from this
chemistry is well understood [2] (see Scheme 2) however; this result confirms the more obscure finding [3]
that carbon monoxide is also a significant final product of the peroxyxalate chemiluminescence reaction.

Conclusion
A combination of direct NMR spectroscopic evidence and ab initio calculations, have confirmed Rauhut’s
[3] long held postulation that one of the key intermediates in the peroxyxalate chemiluminescence reaction
mechanism is indeed 1,2-dioxetanedione. Moreover, this study has revealed that several other transient
species were stabilised long enough for spectroscopic interrogation and these entities will be the subject of
ongoing investigations.

Acknowledgements
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 provision of a postdoctoral research fellowship for S.P.W. from the Centre for Chiral and Molecular
Technologies at Deakin University.
References
Table 1

<table>
<thead>
<tr>
<th>Molecule(s)</th>
<th>Basis set for geometry optimisation</th>
<th>Calculated shielding tensor ((\delta))</th>
<th>Calculated Relative Chemical Shift ((\delta))</th>
<th>Estimated Relative Chemical Shift ((\delta))</th>
<th>Experimental Chemical Shift</th>
<th>Reference</th>
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<tr>
<td>THF [35-37] C1/4 C2/3</td>
<td>RHF/6-31G(O(d))</td>
<td>154.1499</td>
<td>28.1</td>
<td>26.5(^{\text{vii}})</td>
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<td>Oxalic acid [39-41]</td>
<td>B3LYP/6-31G(C-O(2d))</td>
<td>19.7125</td>
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<td>160.13(^{\text{vii}})</td>
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<td>Carbon Dioxide [32,42]</td>
<td>RHF/6-31G</td>
<td>52.0296</td>
<td>129.2</td>
<td>125.6(^{\text{v}})</td>
<td>this work</td>
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<tr>
<td>Oxetane [44] C1/3 C2/4</td>
<td>RHF/6-31G(O(d))</td>
<td>110.0506</td>
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<td>72.6(^{\text{vii}})</td>
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<td>Cyclobutanone [45,46] C1 C2/4 C3</td>
<td>RHF/6-31G</td>
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<td>208.8</td>
<td>208.9, 47.8, 9.9(^{\text{vi}})</td>
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<td>2-Oxetanone C1 C2 C3</td>
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<td>168.6</td>
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<td>1,2-dioxetanediene</td>
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<td>22.4751</td>
<td>158.4</td>
<td>58.7(^{\text{vi}})</td>
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</table>

(i) Including references for structural data.
(ii) All NMR shielding tensors (carbon) obtained from B3LYP/6-31++G(2p,d) single-point calculations.
(iii) \(\text{Calculated}\) NMR shifts use TMS as a single reference point for converting shielding tensors to NMR shifts. TMS shielding tensor is 182.4560 using B3LYP/6-31G(d)//B3LYP/6-31++G(2p,d).
(iv) \(\text{Estimated}\) NMR shifts use all the molecules listed above as best-fit reference set. \(\text{I.e., linear conversion of shielding tensors to NMR shifts:}\)
\(\text{Estimated NMR shift} = 180.67 -0.9900273 \times (\text{calculated shielding tensor}).\)
(v) Experimental NMR shifts measured using THF solvent, at ambient temperature.
(vi) Experimental NMR shifts measured using CDCl\(_3\) solvent, at ambient temperature.
(vii) Gas-phase shielding tensor measurement of 58.8 relative to \(^{13}\text{C}\)O in the zero-pressure limit, with TMS at 188.1.
Figure 1
Figure 2
Figure 4
Captions to Tables and Figures

Table 1
Experimental and calculated NMR shift data for a number of model compounds, and compounds present in the peroxoxygenate chemioluminescence reaction between oxalyl chloride and hydrogen peroxide in the presence of 9,10-diphenylnaphthacene in tetrahydrofuran.

Figure 1
Expanded $^{13}\text{C}$ NMR spectrum of the reaction between $^{13}\text{C}$ labelled oxalylchloride and anhydrous hydrogen peroxide in d-8 tetrahydrofuran in the presence of 9,10-diphenylnaphthacene, over the range –80 °C to -50 °C.

Figure 2
Expanded $^{13}\text{C}$ NMR spectrum of the reaction between $^{13}\text{C}$ labelled oxalylchloride and anhydrous hydrogen peroxide in d-8 tetrahydrofuran in the presence of 9,10-diphenylnaphthacene, over the range –40 °C to 20 °C.

Figure 3
Expansion of $^{13}\text{C}$ NMR spectrum of the reaction between $^{13}\text{C}$ labelled oxalylchloride and anhydrous hydrogen peroxide in d-8 tetrahydrofuran in the presence of 9,10-diphenylnaphthacene, showing the reversible temperature dependant broadening of the 1,2-dioxetanedione resonance at δ 154.5.

Figure 4
Structure of 3-isopropyl-5-chloro-5-methoxy-oxazolidine-2,4-dione, the chemical shift of the indicated carbon being 109.7 ppm [47].