

## 由草酸酯、光敏靈到芳香雙醚烯系列的氧化化學發光之研究

### Study on the Oxidizing Chemiluminescence of Luminols, Oxalates and Aromatic Dioxenes

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#### 摘要

本篇論文為回顧本實驗室研究化學發光的成果，共分三部份：(1)草酸酯系統化學發光，分析發光棒(Cyalume)的成分，並藉由合成一系列的對位取代之苯基草酸酯來探討此系統的化學發光。(2)光敏靈系統化學發光，利用能鑑別能量的單重態-叁重態電子能量傳遞螢光劑(DPA、DBA、BPEA及BPEA<sub>Br</sub>)，來研究光敏靈與螢光劑間能量傳遞的情形。與(3)雙醚烯系統化學發光，探討本實驗室三大螢光烯化合物：萘系(A)、連苯系(B)、蒽系(C)，針對其臭氧化反應及伴隨的化學發光性質為主軸進行研究。雙醚烯系統化學發光研究結果發現化萘系(A)、蒽系(C)均有化學發光的現象，而連苯系(B)則無。化合物(A)進行單態氧氧化反應，研究結果經證實可產生高能中間體 1,2-雙氧烷(1,2-dioxetane)化合物，再經熱分解成激發態的雙酯化合物，當激發態的雙酯化合物回到基態時伴隨化學發光，其高能中間體 1,2-雙氧烷(1,2-dioxetane)化合物形成之證據為可觀察到化學發光光譜與激發態雙酯化合物的螢光光譜一致。

關鍵詞：化學發光、草酸酯、光敏靈、雙醚烯

#### ABSTRACT

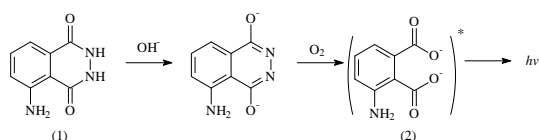
Three chemiluminescent systems are reviewed in this thesis. Part one is the chemiluminescence of the oxalate system. Analyze the contents of the Cyalume light sticks, and study on the chemiluminescence of the phenyl oxalate system whose para-position of the benzene ring is substituted by a series of substituents. Part two is the chemiluminescence of the luminol system. Study on the energy transition between luminol and fluorescent agents (DPA, DBA, BPEA, and BPEA<sub>Br</sub>). The major focused on in this review (Part three) is the chemiluminescence of the tricyclic dioxetane system. Study on the ozonization of the 3 fluorescent compounds (naphthalene A, biphenyl B, and anthracene C), and the properties of the concomitant chemiluminescence. Results show the compound A and C which emit the chemiluminescence, but the compound B doesn't. The compound A is oxidized by reaction with singlet-oxygen (<sup>1</sup>O<sub>2</sub>) in dichloromethane. Evidence for formation of the dioxetane compound is the chemiluminescence spectra that correspond to emission from the electronically excited diester compound, the expected products from thermal decomposition of the dioxetane.

Keywords: chemiluminescence, oxalate, luminol, dioxene

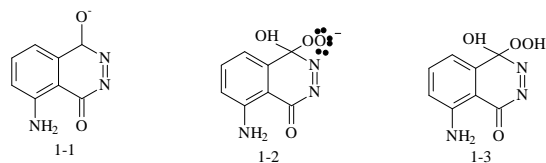
#### 一、緒論

化學發光則純粹藉由化學反應產生激發態分子，當回到基態時，以放光而非放熱的形式釋放能量，所產生的發光現象[1]。自從 1928 年，德國 Albrecht 首先發表光敏靈(luminol)的化學發光現象以來[2]，很多化學研究者深為此華麗奇特現象所吸引，過去七十餘年，不下數百篇論文做這方面研究，尤其是如何產生化學發光的步驟；已

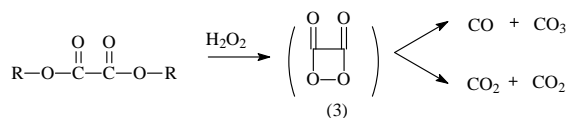
知光敏靈(luminol, 5-amino-2,3-dihydro-1,4-phthalazinedione)化合物(1)在鹼性條件下產生天青色螢光的化學發光，且已確認是由化合物(1)脫去氮而氧化為鄰雙酯基鹽化合物(2)(3-aminophthalate, 簡稱 3-APA)所發出的螢光，如果有催化劑如赤血鹽(K<sub>3</sub>Fe(CN)<sub>6</sub>)，則效果更好。



光敏靈(luminol)系統的化學發光，當推美國 Johns Hopkins 大學 E. H. White 研究群，在這方面所下的功夫最多[3]；光敏靈(luminol)在水溶液或非質子溶液(如 DMSO)已有數條通路達到產物，很明顯的，其激態是化合物(1)在鹼性中和氧作用產生某一種中間高能物，以達到激態化合物(2)，Wurzberg[4~6] 研究群、Merenyi[7] 研究群和 Baxendale[8,9]研究群以電子束(e-beam)、脈衝輻解(pulse radiolysis)研究本系統，在鹼性水溶液中，中間體化合物(1-1)受 pH 值影響，和 O<sub>2</sub> 結合成中間體化合物(1-2)，化合物(1-2)被認為產生高能中間體，而兩個化合物(1-2)互毀(annihilation)，脫去氮形成激態化合物(2)，另一條路成不發光之中間體化合物(1-3)。

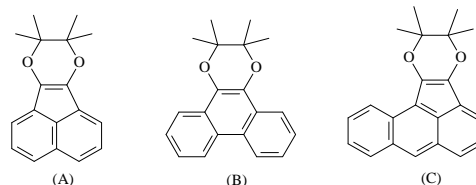


一般非生物的化學發光中，當首推草酸酯系統最有效；在適當的條件下，光量子效應大約可達 0.3 左右。最早文獻可考的草酸酯的化學發光報告，為 Chandross 於 1963 年發現氯化乙二醯(oxalyl chloride)與過氧化氫(H<sub>2</sub>O<sub>2</sub>)反應，在有螢光劑存在下會產生光[10]，Rauhut 亦於 1965 年進行氯化乙二醯(oxalyl chloride)、過氧化氫(H<sub>2</sub>O<sub>2</sub>)與螢光物質的化學發光研究[11]，激發了美國氘胺公司走向一實用化學發光光源的研究；其間不斷的改良化學發光反應劑的成分，以期達到最佳組成及發光之條件。事實上，雖然化學發光現象很耀眼且已製成商品，但於此系統中的重要高能中間體至今仍舊是一個謎。無論如何幾乎可確定是由草酸酯和過氧化氫(H<sub>2</sub>O<sub>2</sub>)反應，形成高能的中間體，最後產生相當量 CO<sub>2</sub> 氣體，其如下之方程式。其中化合物 (3) 為環狀的過氧環乙烷雙醯(1,2-dioxetanedione)被認為可能是高能中間體，但此中間體至今尚未曾成功地觀察到與分離過。



在我們有機光化學實驗室的主要研究方向，為有機化合物可見-紫外光發色團(chromophore)在凝相的光物理與光化學；以及芳香化合物之雙鍵氧化，包括臭氧化、單態氧化及其他氧化化學發光等；而將其聚焦於有機化合物的氧化化學發光之三大系統-草酸酯(oxalate)、光敏靈(luminol)和雙醯烯(dioxene)系統，其中「氧」更是相當重要的媒介。特別要指出的是，此處所謂「氧化」化學發光並非指一般有機物與基態氧反應所產生非常微弱的發光現象，而是指以特殊氧化劑-單態氧、臭氧，與有機化合物反應所產生的波長在可見光範圍內，強度為肉眼可辨的發光現象。

本實驗室長久致力於芳香族雙醯烯化合物(dioxene)相關之光化學反應，其中著墨於臭氧化反應和單態氧化反應尤甚，並研究其化學發光。再探究本實驗室三大主題化合物(A)、(B)、(C)，針對其光化學反應其伴隨的反應性質，推究其反應機構並探討其化學發光性質。

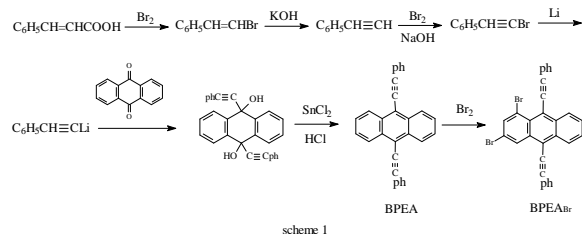


## 二、實驗方法與步驟

### 2.1 BPEA 及 BPEA<sub>Br</sub> 的合成

BPEA [ 9,10-bis(phenylethynyl) anthracene ] 其合成方式為取 0.5 莫耳的肉桂酸(cinnamic acid) 溶於 500 毫升四氯化碳中，加熱回流下，逐滴加入 0.5 莫耳的溴，並反應直到紅棕色完全消失為止。將溶液迴旋濃縮，加入水及碳酸鈉，進行蒸氣蒸餾，棄置水層，以氯化鈣脫水乾燥，再經減壓蒸餾可得約 40 克的 b-bromostyrene。將 30 克的 b-bromostyrene 以 50 克的氫氧化鉀進行脫水反應，經減壓蒸餾可得約 11.5 克的苯乙炔。取 5 克的苯乙炔於冰浴下加入溴進行溴化反應可得約 2g 的溴代苯乙炔。以 3.5 克的蔥醌、1.25 克的鋰溶於無水乙醚，再加入 2g 的溴代苯乙炔反應 48 小時，

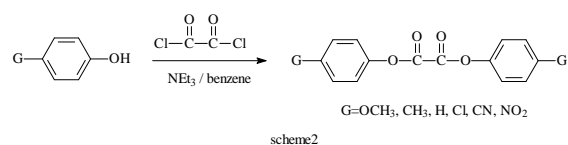
停止反應後取出未反應之鋰，將溶劑抽乾，再滴入氯化亞錫的醋酸溶液可得橘紅色固體之 BPEA，BPEA<sub>Br</sub>[1,3-bibromo-9,10-bis (phenyl ethynyl) anthracene]的合成則繼續將 BPEA 進行溴化反應即可得到，整個反應如 scheme 1 所示。



scheme 1

## 2.2 對位取代苯基草酸酯的合成

將 0.2 克的對位取代苯酚(取代基分別為 -OCH<sub>3</sub>、-CH<sub>3</sub>、-NO<sub>2</sub>、-CN、-Cl、-H)溶於 500 毫升苯中冰浴冷卻至 10°C，加入 0.2 莫耳新蒸餾過的三乙胺，在攪拌下慢慢滴入稍過量的氯化乙二醯，水洗掉三乙胺鹽類，生成物以乙酸乙酯或硝基苯作再結晶，即可得到對應之對位取代苯基草酸酯，如 scheme 2 所示。

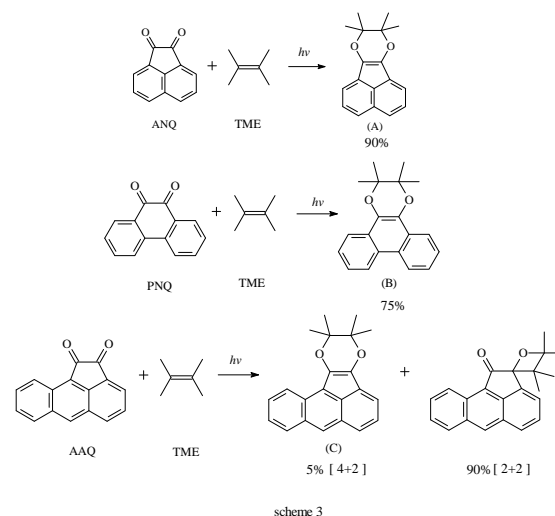


scheme 2

## 2.3 主題化合物(A~C)的合成

取純化後的 acenaphthenequinone (ANQ) 0.5 克，置於照光玻璃儀器內，加入 600mL 光譜級苯溶劑，通氮氣並且充分攪拌。再加入等莫耳數四甲基乙烯(tetramethylethene, TME)，攪拌完全後，以 450W 中壓汞燈照射反應，約 35 分鐘後溶液顏色逐漸由黃色變為紅色，收集溶液以迴旋濃縮儀濃縮，再以矽膠管柱分離產物，收集紅色部分的產物，迴旋濃縮後，用甲醇再結晶得到粒狀紅色晶體，即為主題化合物(A)，產率約 90%。主題化合物(B)的合成是使用 phenanthrenequinone(PNQ)與 TME 進行照光反應，其照光時間約 90 分鐘，溶液顏色逐漸由橘紅色變為淡黃色，結晶後晶體為無色，產率約 75%。主題化合物(C)的合成是使用 aceanthrenequinone(AAQ)與 TME 進行照光反應，其照光時間約 8 小時，溶液顏色逐漸由淺黃色變為暗褐色，結晶後晶體為紫黑色，產率約 5%，光環化主產物為[2+2]加成產物，產率約

90%，如 scheme 3 所示。



scheme 3

## 2.4 臭氧化反應

由臭氧發生器產生的臭氧導入經過丙酮-乾冰之杜耳瓶(-78°C)的螺旋管中冷卻，再導入裝有 0.1g 主題化合物 (A~C)的二氯甲烷溶液之低溫反應瓶中(-78°C)，持續通入臭氧至溶液顏色改變後停止，利用在圍有冰塊的展開槽中的二氯甲烷溶劑作沖提液展開 TLC 片，展開後取出 TLC 片，將不同 R<sub>f</sub>之臭氧化生成產物刮取並鑑定之。另亦可以吹風機吹向 TLC 片背面，檢驗是否有化學發光的產生。

## 2.5 單態氧化反應

本實驗使用三苯基亞磷酸酯-臭氧法產生單態氧以進行單態氧化反應[12]，取 10 克三苯基亞磷酸酯 (Triphenyl Phosphite) 溶於 10mL CH<sub>2</sub>Cl<sub>2</sub> 中，在 -78°C 通入臭氧，因為臭氧溶於二氯甲烷呈藍色，所以當通臭氧時間約兩小時後，溶液會呈微淡藍色，此時表示已通入過量臭氧，以冷卻的氮氣趕掉沒有反應的臭氧；之後加入事先冷至 -78°C，溶有 0.5 克主題化合物(A)的二氯甲烷溶液，移去低溫冷卻槽，使溫度慢慢升至 -35°C，可見到氣泡產生，並且溶液顏色漸由紅色變成淡黃色。迅速在低溫下抽掉溶劑，殘餘物以低溫 (0°C) 矽膠管柱粗分 (以 n-hexane 為沖提液)，收集最先沖提出來的淡黃色溶液，在低溫下濃縮後再以低溫 TLC 分離 (n-hexane : CH<sub>2</sub>Cl<sub>2</sub> = 1 : 1) 刮取 R<sub>f</sub> 約 0.9 處的矽膠，以 CH<sub>2</sub>Cl<sub>2</sub> 萃取，萃取液經低溫濃縮得到淡黃色固體，即為化合物(A)之 1,2-雙氧烷(1,2-dioxetane)高能中間體。當加熱高濃

度的 1,2-雙氧烷化合物到 50°C 以上，肉眼即可看到顯著的化學發光，另 1,2-雙氧烷可配成適當濃度之二氯甲烷溶液，在螢光儀下測量其化學發光光譜。

### 三、結果與討論

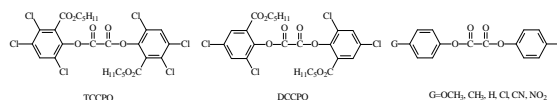
#### 3.1 草酸酯系統化學發光

本實驗室研究分析發光棒(Cyalume)的成分，研究結果得知發光棒之構造是由內含玻璃管之塑膠管所組成，其分別的成分是由 2 種反應試劑所組成：反應試劑(1)為約 5%之草酸酯 TCPPO [ bis-(2,4,5-trichloro-6-(pentylloxycarbonyl) phenyl) oxalate ]， $5 \times 10^{-3}$ M 螢光劑 BPEA 與約 95%溶劑鄰苯二甲酸二丁酯(dibutyl phthalate)；反應試劑(2)為約 5%過氧化氫(H<sub>2</sub>O<sub>2</sub>)，少量的催化劑水楊酸鈉鹽(sodium salicylate)，以及約 78%鄰苯二甲酸二甲酯(dimethyl phthalate)與約 17%的第三丁醇(t-butyl alcohol)混合而成的溶劑。

BPEA 是一具有高螢光量子產率、發綠色光之螢光劑，由於市面不易購買，因此嘗試合成 BPEA，合成產物由結構鑑定證實與發光棒(Cyalume)的螢光劑成份相同，其橘紅色固體在有機溶劑為綠色之特異現象，在不同濃度與不同溫度之螢光發光光譜與激發光譜結果，推測苯乙炔基(phenylethynyl group)與蒽環(anthracene)在固體時可能為平面狀，而在有機溶劑時為互相垂直狀。此外，繼續嘗試合成發光棒中的反應劑之一的 TCPPO；以水楊酸(salicylic acid)為起始物，合成結果顯示無法在其結構上之苯環接上 3 個氯取代基，只能得到 2 個氯的草酸酯 DCPPO [ bis-(2,4-dichloro-6-(pentylloxycarbonyl) phenyl) oxalate ]，判斷接上第 3 個氯有些許立體障礙，並且太多拉電子基團的引入降低苯環的活性，導致不易合成 TCPPO，但由發光實驗顯示，DCPPO 亦是良好的發光劑[13]。

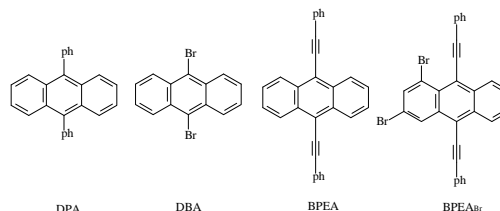
TCPPO 或 DCPPO 的結構皆具有氯及酯類等拉電子基團，為了呈現取代基對化學發光的影響，進行合成一系列的對位取代苯基草酸酯，並探討此系統於室溫下化學發光的動力學。結果顯示取代基為推電子基者(G = -OCH<sub>3</sub>, -CH<sub>3</sub>)，即使在催化劑存在下，亦無法觀察到化學發光現象，而其為拉電子基者(G = -NO<sub>2</sub>, -CN, -Cl, -H)，則

可見到化學發光現象，結果顯示與具有拉電子基團之 TCPPO 或 DCPPO 有同樣的發光現象，判斷拉電子基團有助於高能中間體過氧環乙烷雙醯的形成。



#### 3.2 光敏靈系統化學發光

本實驗室研究光敏靈化學發光系統分子間能量傳遞的化學發光，利用能鑑別高能量單重態-叁重態電子能量傳遞的 DPA(9,10-diphenyl anthracene)、DBA(9,10-dibromo anthracene)和低能量單重態-叁重態電子能量傳遞的 BPEA[9,10-bis(phenylethynyl) anthracene]、BPEA<sub>Br</sub>[1,3-bibromo-9,10-bis(phenylethynyl) anthracene]，來研究光敏靈分子間能量傳遞的情形。所獲得之結論為：光敏靈(luminol)在放入能量低於其化學激發中間體的螢光劑，如 BPEA、BPEA<sub>Br</sub>，及螢光劑 Fluorescein、Rubrene 皆能看到因為能量傳遞而由螢光劑所放出的化學螢光，然而高能的 DPA、DBA，則無可見的化學螢光。比較 BPEA 及 BPEA<sub>Br</sub> 加入光敏靈在過量的 N(Me)<sub>4</sub>OH(0.022M)和過量飽和的溶氧(2×10<sup>-3</sup>M)的條件下，BPEA(~10<sup>-6</sup>M)所得化學螢光約只有 BPEA<sub>Br</sub>(~10<sup>-6</sup>M)的二分之一螢光效應[14]。



另外本實驗室將上述化學發光的兩大系統的研究成果，轉化成兩套「由化學能轉換成光能的示範實驗」之方便教具，並溶入高中教材當中，成果並發表於「化學」期刊 [15]。其中所述「光敏靈系統」的示範方法是：將 0.1 克光敏靈(luminol)溶在 10 毫升 5%NaOH 溶液裡構成甲溶液，另在 10 毫升 3%H<sub>2</sub>O<sub>2</sub> 中加入些許催化劑如赤血鹽構成乙溶液；將甲溶液和乙溶液混合一起，則會激起漂亮的天藍色可見光。而「草酸酯系統」的示範步驟為：準備一張可吸液體的濾紙或衛生紙(宣紙亦可)，並準備約 10<sup>-3</sup>M 的螢光劑溶液-天藍色(如 DPA)、紫色(如 anthracene)、黃綠色(如 BPEA)、

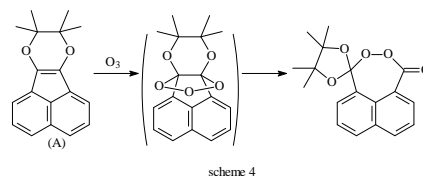
橘紅色(如 Rubrene)和紅色(如 Rodamine B)等分別溶於鄰苯二甲酸二甲酯(dimethyl phthalate), 並準備一可壓擠的氯化乙二醯(oxalyl chloride)純液; 示範時, 先在所預備吸液紙上不同位置各滴 2~3 滴螢光劑溶液, 再於其上各滴 2~3 滴 30% 過氧化氫( $H_2O_2$ )溶液, 再壓擠氯化乙二醯(oxalyl chloride)塑膠滴瓶, 使其滴嘴噴向塗有螢光劑之吸液紙, 此蒸氣就可把紙片點綴得金碧輝煌, 煞是壯觀。

這兩套教具可藉著化學發光的兩個系統說明「直接發光」與「間接發光」; 如光敏靈系統就是直接發光, 由於發光的本身就含有很好的螢光劑-鄰雙酯基鹽化合物(3), 其在 3-位置有胺基(- $NH_2$ ), 可發天藍色螢光。而在草酸酯, 產生高能中間體, 加入能量比較低的各種螢光劑, 可經由能量傳遞而發所置入螢光劑的螢光, 如在氯化乙二醯(oxalyl chloride)的化學發光所置的各種螢光劑, 故此為一種間接發光。

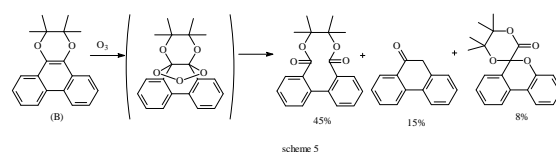
### 3.3 雙醯烯系統化學發光

化合物(A)、(B)、(C)由 ANQ、PNQ 及 AAQ 分別與 TME 進行光加成反應而得。將化合物(A)、(B)、(C)分別溶於二氯甲烷中, 並在 $-78^\circ C$ 下進行臭氧化反應, 當溫度慢慢回復至室溫, 可以發現化合物(A)、(C)均有化學發光的現象, 而化合物(B)則無此現象, 致光物質經實驗證實為 1,2-雙氧烷, 而主產物為經臭氧化重排的穩定過氧化物, 我們亦將三種主題化合物進行臭氧化反應和單態氧化反應的反應機構作深入的探討與比較。

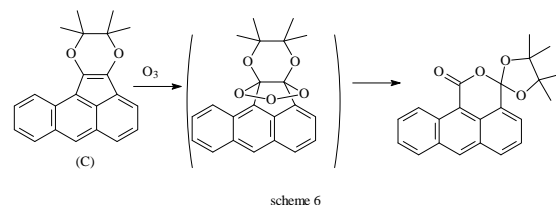
本實驗室進行化合物(A)的合成與臭氧化反應化學發光的研究, 橘紅色的化合物(A), 可以 ANQ 和 TME 經光照進行光合成而得, 產率約 90%, 並利用合成的產物進行臭氧化反應的研究與觀察其化學發光情形, 在暗室中可見到此未經分離的產物放出天青紫色的化學發光, 溫度越高亮度越大, 加入 BPEA 則發生能量傳遞使其放出黃綠色光, 將臭氧產物分離, 可得到主產物為白色固體, 由元素分析、質譜、NMR 及 X-ray 分析, 鑑定為重組的臭氧化過氧化物, 其結構如 scheme 4 所示。



接著將 PNQ 與 TME 進行光加成反應, 形成化合物(B), 其產率約 75%。化合物(B)進行臭氧化之研究, 此未經分離的產物在螢光儀中並未測得化學發光, 分離純化臭氧化產物, 經元素分析、質譜、NMR 及 X-ray 分析, 鑑定主產物為雙酯化合物及少量副產物, 其結構如 scheme 5 所示。雙酯化合物產率高達 45%, 且無 ANQ 系統所呈現穩定的五氧結構之臭氧產物。



接著將 AAQ 與 TME 進行光加成反應, 形成化合物(C), 值得注意的是光加成反應時雙醯烯的產率相當低(約 5%), 而主產物為[2+2]光加成產物, 產率約 90%。將多次收集純化後的化合物(C)進行臭氧化之研究, 如同 ANQ 系統亦可見到化學發光, 而產生的主產物為穩定 spiro lactone, 但此結構為四氧結構異於 ANQ 系統的五氧結構之臭氧產物, 其結構如 scheme 6 所示。



化合物(A)、(B)、(C)在氧化過程中容易由顏色的變化得知反應是否完成, 尤其是反應激烈的臭氧化反應, 即用此易於觀察的溶液顏色變化來決定反應時間。臭氧化的反應機制先經由一個三氧環加成雙鍵的中間體, 再由此三氧環中間體進行複雜的重排反應, 但是化合物(A)所進行的臭氧化反應得到五氧結構之臭氧產物, 卻意料之外的特別安定, 而化合物(B)、(C)卻無五氧結構臭氧化產物的形成, 反而形成四氧結構之重排產物。在低溫之下進行臭氧化反應時, 化合物(A)、(C)有伴隨化學發光的現象, 而化合物(B)則無。推究其原因可能為(1)化合物(B)形成雙酯化合物是經由臭氧化反應後重排而得。(2)化合物(B)因聯苯較易鬆動於

溶液中易因分子扭動而分散能量，以熱的方式釋放。反觀化合物(A)和(C)其臭氧化反應中也都有少量雙酯化合物的生成，推測其在低溫時進行臭氧化反應皆生成 1,2-雙氧烷之致光中間產物。

縱觀上述之三大主題化合物，得到一重要結論，無論是化合物(A)、(B)或(C)在進行臭氧化反應時，產物中均有一雙酯產物(diester)；而由化合物(A)與(C)得到的少量雙酯化合物，推測是由臭氧當中少部分的單態氧參與反應而得，因此雙酯產物的產率並不高。另外化合物(B)進行臭氧化反應時，有大量的雙酯產物生成，推測是由化合物(B)的臭氧化中間體重排所形成，其形成雙酯類產物的路徑有別於化合物(A)和(C)。

由草酸酯系統化學發光與光敏靈系統化學發光都有高能中間體的致光物質，導致「直接發光」與「間接發光」的現象，而在臭氧化反應中，雖有化學發光現象卻無法由產物分析判斷其致光物質，我們假設推斷主題化合物(A)、(C)經臭氧化所產生之化學發光，其原因可能是臭氧中含有少量單態氧，單態氧與化合物(A)、(C)進行單態氧化所產生之化學發光，因此，嘗試將主題化合物(A)進行單態氧氧化反應。

將化合物(A)以三苯基亞磷酸酯－臭氧法所產生的單態氧進行單態氧化反應，於低溫下分離純化得到淡黃色固體，即為接上單態氧之 1,2-雙氧烷化合物。將低溫分離得到的 1,2-雙氧烷化合物以螢光儀測定，得到化學發光光譜如圖 1 所示，由圖中顯現 1,2-雙氧烷化合物的化學發光在 383nm 有最大發射波長。將 1,2-雙氧烷化合物熱分解後的產物純化，並經  $^1\text{H}$  NMR 分析確認為雙酯化合物之結構。將純化後的雙酯化合物配成  $1 \times 10^{-4} \text{ M}$  濃度，以 330 nm 波長激發作放射光譜，由圖可以發現雙酯化合物的激發光譜與 1,2-雙氧烷的化學發光光譜一致，因此可以確認 1,2-雙氧烷熱分解後可產生雙酯化合物的激態，並由激態發光回到基態。化合物(A)單態氧氧化形成 1,2-雙氧烷化合物及其熱分解成雙酯化合物，如 Scheme 7 所示。

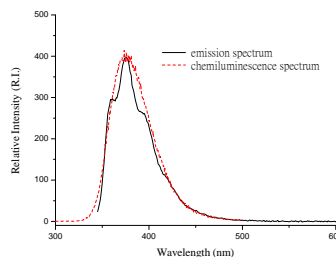
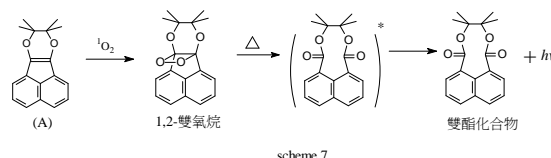


圖 1 雙酯化合物的放射光譜與 1,2-雙氧烷的化學發光光譜



#### 四、結論

草酸酯系統可產生高能中間體，利用所合成的螢光劑（如 BPEA、BPEA<sub>Br</sub>）皆能看到因為能量傳遞而由螢光劑所放出的化學螢光，此外合成一系列的對位取代苯基草酸酯，結果顯示推電子基者(G = -OCH<sub>3</sub>, -CH<sub>3</sub>)，即使在催化劑存在下，亦無法觀察到化學發光現象，而拉電子基者(G = -NO<sub>2</sub>, -CN, -Cl, -H)，則可見到化學發光現象，推測拉電子基有助於環狀的過氧環乙烷雙酮高能中間體的形成。光敏靈系統本身具有螢光發色團，經鹼及過氧化氫催化後能產生高能中間體(3-APA)，可直接化學發光放出天藍色螢光。另利用能量傳遞亦可經由螢光劑間接發光，其中 BPEA<sub>Br</sub> 比 BPEA 有 2 倍的化學螢光量子產率。本實驗室亦將上述化學發光的兩大系統的研究成果，轉化成兩套「由化學能轉換成光能的示範實驗」之方便教具。

主題化合物(A)或(C)在低溫之下與臭氧反應均有化學發光的現象，推究原因可能是臭氧中含有少量單態氧而來，而化合物(B)則因聯苯較易鬆動以熱的方式釋放能量導致無化學發光。將化合物(A)與單態氧進行單態氧化，可得到高能中間體 1,2-雙氧烷化合物，於室溫下熱分解成雙酯化合物並產生化學發光。由雙酯化合物的激發光譜與 1,2-雙氧烷的化學發光光譜呈現一致的實驗結果，顯示化合物(A)或(C)經單態氧氧化產生高能中間體 1,2-雙氧烷化合物，再經熱分解成激發態的雙酯化合物，當激發態的雙酯化合物回到基態時伴隨化學發光。

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**ASIAN JOURNAL OF CHEMISTRY****(An International Peer Reviewed Research Journal of Chemistry)****11/100, Rajendra Nagar, Sector-3, Sahibabad-201 005(Ghaziabad) INDIA****Email: asianjchemistry@gmail.com****Editor-in-Chief****Prof. R. K. AGARWAL****Ph.D., D.Sc.****Circulation/Database Manager****Mrs. Anjul Agarwal****Dated: 20<sup>th</sup> May 2013****Dr. Chung-Wen Sun****Department of Chemistry****National Taiwan Normal University****Taipei 116****Taiwan, R.O.C**

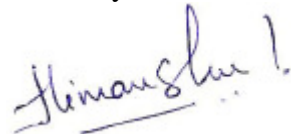
Dear Dr. Chung-Wen Sun

We are pleased to inform you that your research manuscript No. 15477/2013 entitled "*Heavy Atom Effect on the Room Temperature Chemiluminescent Phosphorescence (RTCP) of the Tricyclic 1,2-Dioxetanes*" has been accepted for publication in Asian Journal of Chemistry. The paper will appear in Volume 25 (2013) of Asian Journal of Chemistry. Here, we are enclosing an invoice bill No. 4641 dated 20<sup>th</sup> May 2013 of USD 450=00 (US Dollars Four Hundred and Fifty Only) towards the Printing/Publication and Membership charges.

You are requested to remit the payment at the earliest in favour of Asian Journal of Chemistry. Your early response will be highly appreciated.

Thanks

Sincerely Yours

**Dr. Himanshu Agarwal****Executive Editor****Asian Journal of Chemistry**



1 **Heavy Atom Effect on the Room Temperature Chemiluminescent**  
 2 **Phosphorescence (RTCP) of the Tricyclic 1,2-Dioxetanes**

3 CHUNG-WEN SUN<sup>1</sup>, SHUN-CHI CHEN<sup>2,\*</sup> and TAI-SHAN FANG<sup>1,\*</sup>

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7 1,2-Dioxetane tricyclic compound **1a** and 5-halo substituted of 1,2-dioxetane tricyclic compounds (**2a**, **3a**) were synthesized from 1,4-  
 8 dioxin acenaphthylene compound **1** and 5-halo substituted 1,4-dioxin acenaphthylene compounds (**2**, **3**), by reacting with singlet-oxygen  
 9 (<sup>1</sup>O<sub>2</sub>) at 238 K and their chemiluminescent efficiency both in fluorescence and phosphorescence was studied while upon heating to  
 10 temperatures between 313-353 K in dichloromethane. The chemiluminescent phosphorescence (CP) can be detected along with the  
 11 chemiluminescent fluorescence (CF) when compounds (**2a**, **3a**) are decomposed thermally to the corresponding naphthalene diesters (**2b**,  
 12 **3b**). Experimental evidences for formation of the dioxetanes **1a**, **2a** and **3a** are the observed chemiluminescence spectra which are  
 13 consistent with the photoluminescence spectra of the excited compounds (**1b-3b**). Temperature-dependent kinetic measurements reveal  
 14 an increase of room temperature chemiluminescent phosphorescence (RTCP) efficiency from triplet chemiexcitation processes due to the  
 15 internal heavy atom effect of compound **3a**.

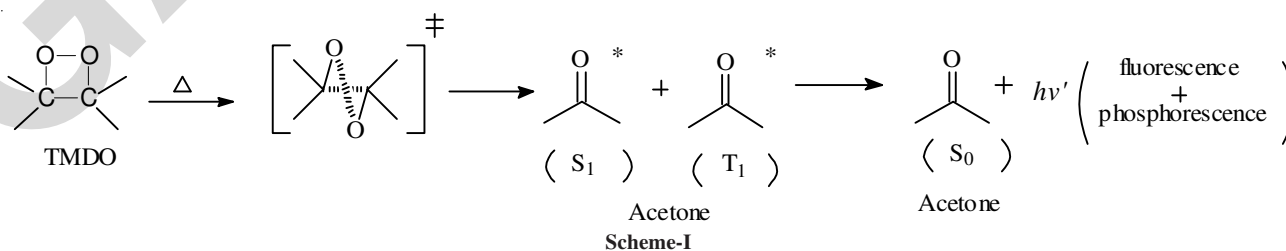
16 **Key Words: Chemiluminescence, Fluorescence, Phosphorescence, Dioxetane, Heavy atom effect.**

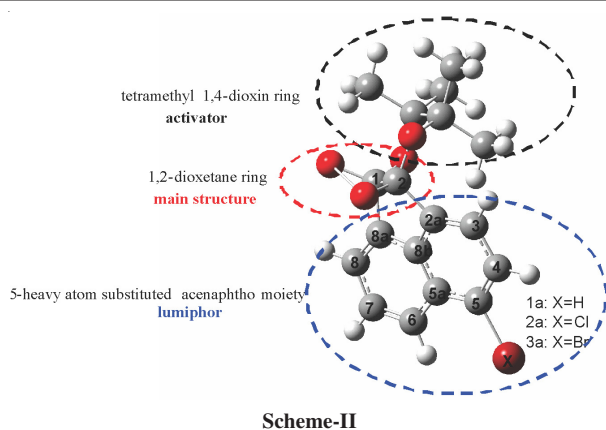
**INTRODUCTION**

17 Photoluminescence is a type of optical spectroscopy in  
 18 which a photoluminescent molecule is promoted to an elec-  
 19 tronically excited state by absorption of incident radiation.  
 20 Chemiluminescence is a type of optical spectroscopy in which  
 21 a chemiluminescent molecule is generated to an electronically  
 22 excited state by chemiexcitation reactions. There are few re-  
 23 ports about the room temperature chemiluminescent fluores-  
 24 cence and phosphorescence. For example, room temperature  
 25 luminescence is rarely observed from the thermal decomposi-  
 26 tion of the simple 1,2-dioxetanes which produce excited car-  
 27 bonyl compounds. The energy in the form of light is released  
 28 from 1,2-dioxetanes due to a chemiexcitation reaction during  
 29 thermal decomposition. A simple example is the thermal de-  
 30 composition of tetramethyl dioxetane (TMDO). In the TMDO

31 structure, the C-O-O-C is a twisted four-membered ring per-  
 32 oxide with high strain energy, which liberates much energy  
 33 during thermal decomposition into two excited carbonyl frag-  
 34 ments (singlet and triplet excited acetone) and then return to  
 35 the ground state by chemiluminescence (**Scheme-I**)<sup>1-5</sup>.

36 The molecular structure of TMDO was used as a base  
 37 template to design and synthesize compounds (**1a-3a**). The  
 38 structure of the 1,2-dioxetane tricyclic compounds (**1a-3a**) are  
 39 comprised of three parts. These are the 1,2-dioxetane ring  
 40 (main structure), the electron-donor dialkoxy activator  
 41 (tetramethyl 1,4-dioxin ring) on one side and the lumiphor (5-  
 42 heavy atom substituted acenaphtho moiety) on the other side  
 43 (**Scheme-II**). The chloro- and bromo- substituents on the  
 44 lumiphor are incorporated to study the internal heavy atom  
 45 effect, which is an important factor for the room temperature





Scheme-II

46 chemiluminescent phosphorescence (RTCP) detection of  
47 chemiluminescent molecule by experimental measurements.

48 It is well known that the 1,2-dioxetane ring with its high  
49 strain energy undergoes simultaneous cleavage of both the O-  
50 O and C-C bonds during thermal decomposition into two excited  
51 intramolecular carbonyl fragments (singlet and triplet  
52 excited intramolecular naphthalene diester compounds **1b\***-  
53 **3b\***), which return to the ground state by chemiluminescent  
54 fluorescence (CF) and chemiluminescent phosphorescence  
55 (CP), respectively<sup>6-9</sup>.

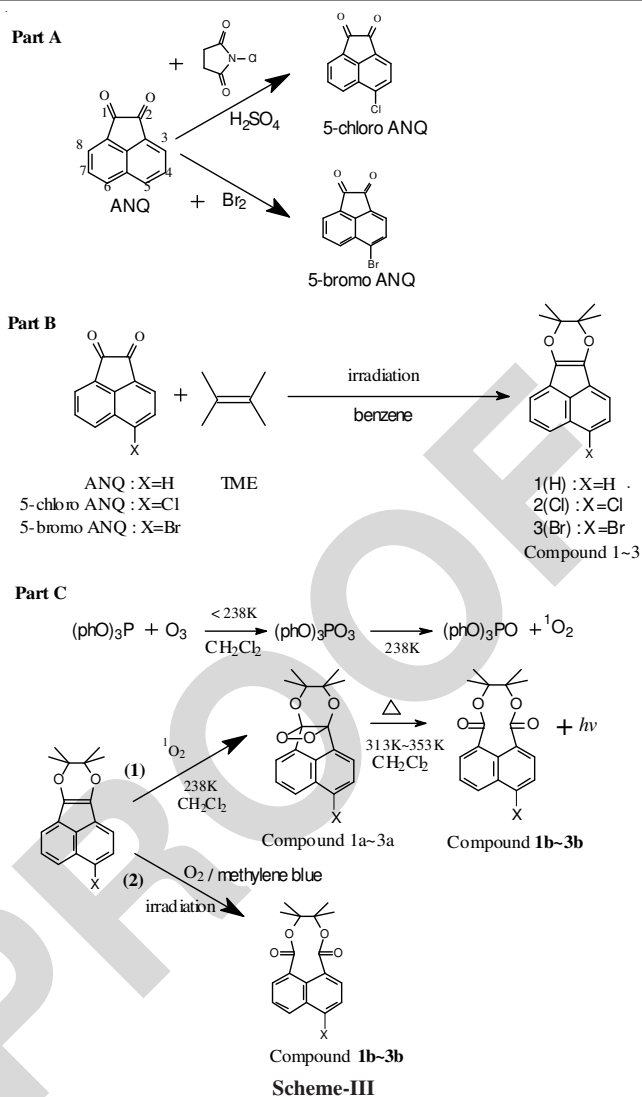
56 While it is difficult to detect the RTP from compounds  
57 (**1b-3b**) under nondegassed condition, because of quenching  
58 by triplet-oxygen (<sup>3</sup>O<sub>2</sub>), the RTCP is detected more easily, be-  
59 cause the RTCP emission derives from the chemiexcitation  
60 reactions of 1,2-dioxetanes. It is well known that the internal  
61 heavy atom effect can enhance the phosphorescence. This pa-  
62 per describes the RTCP of 1,2-dioxetane tricyclic compound  
63 with a 5-heavy atom substituent in the acenaphtho moiety.

## EXPERIMENTAL

64 **1,4-Dioxin acenaphthylene, compounds (1-3):**  
65 Acenaphthenequinone (ANQ) was obtained from Aldrich. The  
66 5-chloro ANQ was synthesized from ANQ and N-  
67 chlorosuccinimide (NCS) in concentrated sulfuric acid. The  
68 reaction mixture was refluxed for 2 h. The 5-bromo ANQ was  
69 synthesized from ANQ and bromine liquid. The reaction mix-  
70 ture was refluxed for 2 h<sup>10,11</sup> (Scheme-III part A). The com-  
71 pounds (**1-3**) were synthesized by the photo-cycloaddition  
72 reaction of acenaphthenequinone (ANQ) with  
73 tetramethylethylene (TME) in benzene<sup>12</sup> (Scheme-III part B).

74 **1,2-Dioxetane tricyclic compounds (1a-3a):** Singlet-  
75 oxygen oxidation of the 1,4-dioxin compounds (**1-3**) to pro-  
76 duce the 1,2-dioxetane tricyclic compounds (**1a-3a**) was car-  
77 ried out using the ozone-triphenyl phosphite procedure given  
78 in ref.<sup>3</sup>. Dioxetane adducts (**1a-3a**) were isolated by ice-chilled  
79 *n*-hexane extraction from the low temperature synthesis of ozo-  
80 nized triphenyl phosphite (Scheme-III part C(1)).

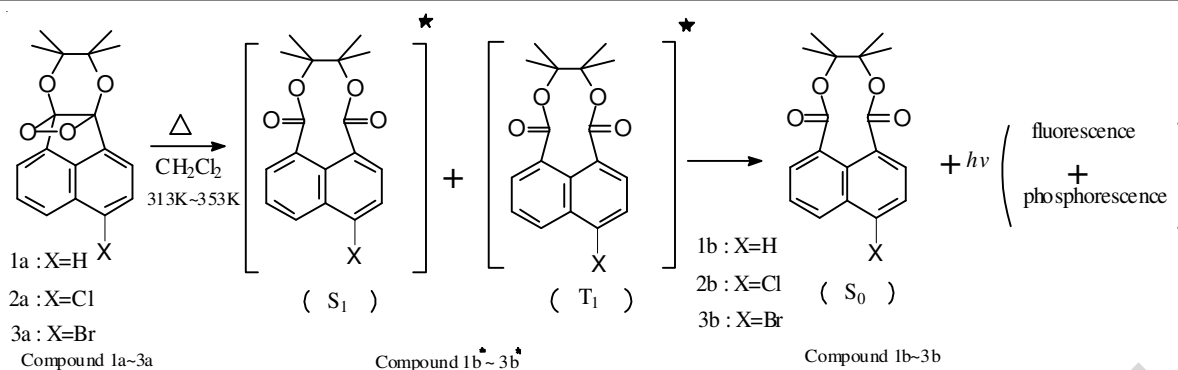
81 **Naphthalene diester compounds (1b-3b):** There are two  
82 methods to synthesize the naphthalene diester compounds (**1b-**  
83 **3b**). (1) The compounds (**1a-3a**) were decomposed thermally  
84 to the naphthalene diester compounds (**1b-3b**) (Scheme-III  
85 part C(1)). (2) The compounds (**1-3**) in benzene solution were  
86 irradiated in the presence of photosensitizer methylene blue  
87 and purged oxygen (Scheme-III part C(2)).



88 **Measurements:** The UV/VIS absorption spectra were  
89 measured on a Hewlett-Packard diode array spectrophotom-  
90 eter. The steady-state emission spectra were obtained using a  
91 Cary Eclipsed Spectrofluorimeter equipped with a tempera-  
92 ture controller. Luminescence lifetimes were measured by a  
93 microsecond flash lamp Luminescence Spectrofluorimeter  
94 (Cary Eclipse) in a temperature range of 313-353 K. For the  
95 temperature-dependent kinetic measurements, the temperature  
96 of the sample solution were controlled to within ± 0.5 K with  
97 an electronically thermostating single cell and monitored with  
98 thermocouples attached to the cell. The room temperature  
99 chemiluminescent fluorescence (RTCF) and the RTCP ther-  
100 mal decay of the compounds (**1a-3a**) were determined using  
101 a photomultiplier tube.

## RESULTS AND DISCUSSION

102 The isolated compounds (**1a-3a**) were formed by react-  
103 ing compounds (**1-3**) with singlet-oxygen (<sup>1</sup>O<sub>2</sub>) in  
104 dichloromethane at 238 K. Thermal decomposition of the 1,2-  
105 dioxetanes produce the electronically excited diesters (**1b\***-  
106 **3b\***) that decayed by chemiluminescent fluorescence or chemi-  
107 luminescent phosphorescence to the ground state compounds  
108 (**1b-3b**). To avoid triplet-oxygen (<sup>3</sup>O<sub>2</sub>) quenching of the trip-



Scheme-IV

109 let excited states, we used the freeze-pump-thaw method to  
 110 degas solutions where the compounds (**1b-3b**) are dissolved.  
 111 Experimental evidences show that the chemiluminescence  
 112 spectral peaks of the compounds (**1a-3a**) are consistent with  
 113 that of the photoluminescence spectral peaks of the  
 114 photoexcited nondegassed and degassed compounds (**1b-3b**).  
 115 These spectroscopic observations suggest that the compounds  
 116 (**1a-3a**) thermally decomposed to the excited state of the com-  
 117 pounds (**1b\*-3b\***), with subsequent radiative decay to the  
 118 ground state of the compounds (**1b-3b**). These processes are  
 119 showed in **Scheme-IV**.

120 As an example, the UV/VIS absorption and photolumi-  
 121 nescence spectra of the compound **3b** and the chemilumi-  
 122 nescence spectrum of the compound **3a** in dichloromethane  
 123 are shown in Fig. 1. To compare the three spectra (chemilumi-  
 124 nescence, nondegassed and degassed photoluminescence) con-  
 125 veniently, the chemiluminescence and photoluminescence  
 126 spectral peaks at  $\lambda_{max}$  are normalized to same relative inten-  
 127 sity. The experimental results show that the RTCF spectral  
 128 peak of the compound **3a** at 378 nm is consistent with that of  
 129 the photoluminescence spectral peaks of the photoexcited  
 130 nondegassed and degassed compound **3b**. The RTCP spectral  
 131 peak of the compound **3a** at 560 nm is consistent with that of  
 132 the photoluminescence spectral peak of the photoexcited de-  
 133 gassed compound **3b**. The photoluminescence spectral peak  
 134 at 560 nm of the photoexcited nondegassed compound **3b**  
 135 is not observed due to quenching of the triplet states by triplet-  
 136 oxygen ( $^3O_2$ ). The lifetimes of the two peaks in the chemilu-  
 137 minescence spectrum of the compound **3a** were measured by  
 138 monitoring the decays at fixed wavelength. The experimental  
 139 results show the fluorescence and phosphorescence decay life-  
 140 times of the degassed compound **3b** in dichloromethane are  
 141 5.3 and 80  $\mu s$ , respectively. The chemiluminescence spectral  
 142 peaks are composed of CF and CP (Fig. 1).

143 We observed quite different RTCP spectra relative inten-  
 144 sity between the nondegassed compounds **1a(H)-2a(Cl)-**  
 145 **3a(Br)**. It is known that the rate of a spin-forbidden process is  
 146 enhanced by the presence of an atom of high atomic number.  
 147 This factor is observed with the RTCP. The 5-heavy atom sub-  
 148 stituted acenaphtho moiety of 1,2-dioxetanes shows enhanced  
 149 nonradiative intersystem crossing from the lowest triplet state,  
 150 resulting in an increase in the intensity of the phosphores-  
 151 cence. This increase of the RTCP spectra relative intensity from  
 152 triplet excited state due to the internal heavy atom substituted  
 153 is  $H < Cl \ll Br$  (Fig. 2).

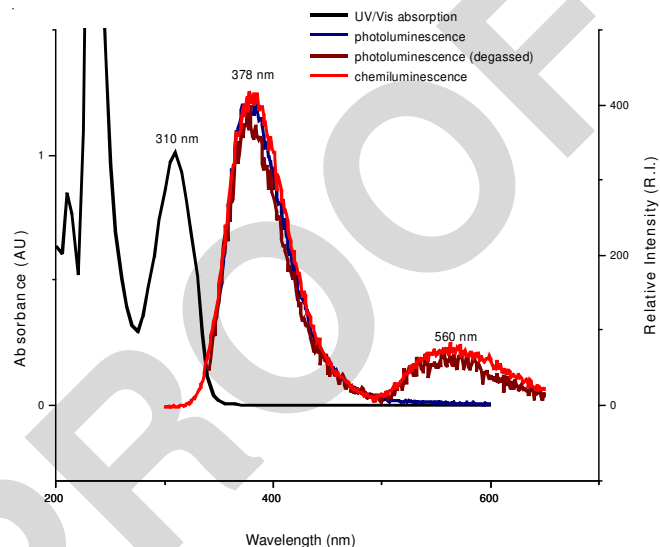


Fig. 1. UV/VIS absorption, photoluminescence spectra of the photoexcited compound **3b** and the RTCF and RTCP spectra of the compound **3a** at 343 K in dichloromethane

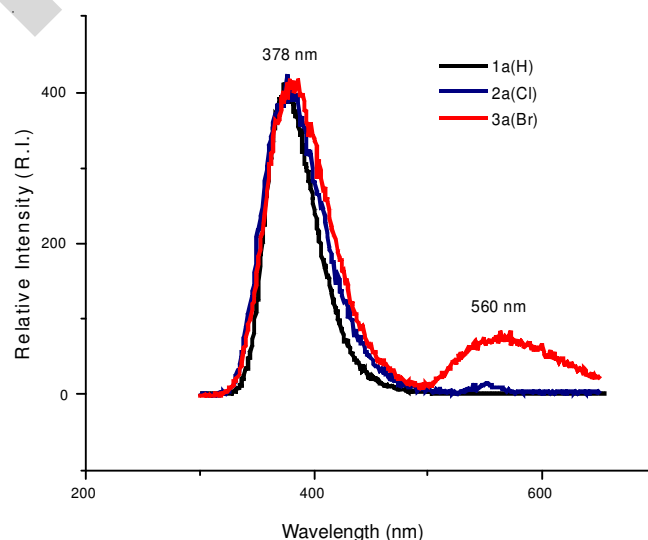


Fig. 2. Chemiluminescence of the compound **1a(H)-2a(Cl)-3a(Br)** at 343 K in dichloromethane

The photoluminescence spectral peaks of the photoexcited 154  
 compounds (**1b-3b**) are little shifted toward longer wave- 155  
 lengths as the solvent is changed from the less polar 156  
 dichloromethane to more polar acetonitrile. The RTCF and 157  
 RTCP derive from singlet and triplet  $^* \pi \rightarrow \pi$  states. An exter- 158

159 nal heavy atom effect was observed with compound **3b** in going  
 160 from benzene to dichloromethane (Fig. 3). The intensity of  
 161 phosphorescence of compound **3b** which dissolved in  
 162 dichloromethane is higher than that dissolved in benzene. The  
 163 ratio ( $RI_{\lambda 560 \text{ nm}}/RI_{\lambda 378 \text{ nm}}$ ) of the degassed compound **3b** in ben-  
 164 zene solvent is 0.10, while this ratio increases to 0.17 in  
 165 dichloromethane solvent.

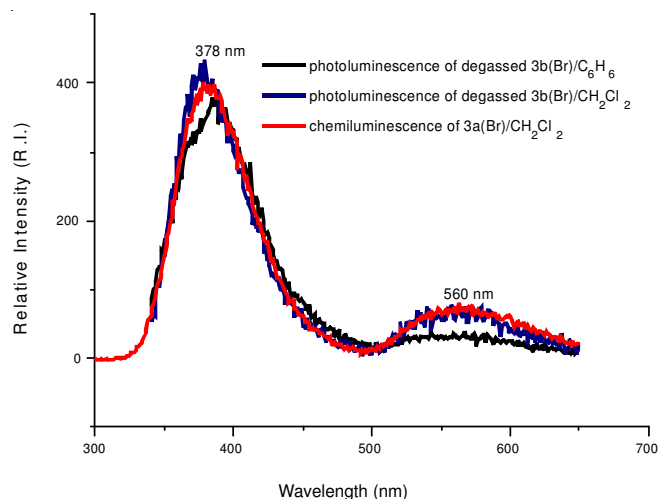


Fig. 3. Photoluminescence spectral peaks of the compound **3b**

166 The rate constants for the decomposition of the compound  
 167 **3a** was obtained at 10 K increments between 313-353 K by  
 168 measuring the decay of the chemiluminescence intensity at  
 169 the maximum wavelength of the chemiluminescence in  
 170 dichloromethane ( $\lambda_{CF,max}$ : 378 nm,  $\lambda_{CP,max}$ : 560 nm). Fig. 4 show  
 171 the CF and the CP thermal decays of the compound **3a** at 343  
 172 K, monitored at 378 and 560 nm, respectively.

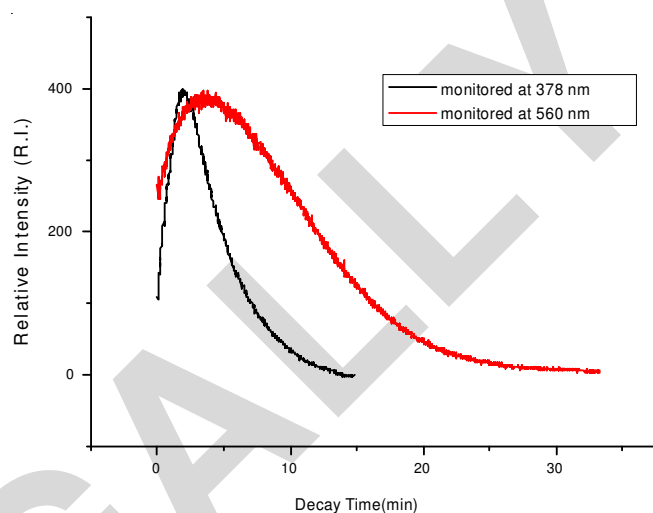
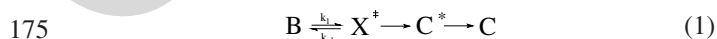


Fig. 4. CF and CP decay of the compound **3a** at 343 K

173 The overall CL mechanism of the 1,2-dioxetane tricyclic  
 174 compound **3a** can be presented as eqn. 1.



176 where B = 1,2-dioxetane tricyclic compound **3a**;  $X^\ddagger$  = the ac-  
 177 tivated complex;  $C^*$  = excited intramolecular diester compound  
 178 **3b**\*.

From the Boltzmann distribution eqn. 2

$$179 \quad k = \left( \frac{k_B T}{h} \right) e^{\left( \frac{-\Delta G^\ddagger}{RT} \right)} \quad (2) \quad 180$$

181 where  $k_B$  = the Boltzmann constant;  $h$  = Planck's constant,  
 182  $\Delta G^\ddagger$  = the activation free energy of the transition state;  $R$  = the  
 183 gas constant and the Gibbs free energy eqn. 3

$$184 \quad \Delta G^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger \quad (3)$$

185 where  $\Delta H^\ddagger$  = the activation enthalpy of the transition state;  
 186  $\Delta S^\ddagger$  = the activation entropy of the transition state, is obtained  
 187 by the Eyring eqn. 4 using transition-state theory<sup>13-15</sup>.

$$188 \quad \ln \frac{k}{T} = \left( \ln \frac{R}{N_A h} + \frac{\Delta S^\ddagger}{R} \right) - \frac{\Delta H^\ddagger}{RT} \quad (4)$$

189 where  $N_A$  = Avogadro's constant;  $h$  = Planck's constant.

190 This equation relates  $\ln(k/T)$  and  $(1/T)$  as a linear func-  
 191 tion. A plot of  $\ln(k/T)$  versus  $(1/T)$  gives a straight line with  
 192 slope  $(-\Delta H^\ddagger/R)$  from which the activation enthalpy of the tran-  
 193 sition state may be derived. The CF and CP decays from 1,2-  
 194 dioxetane **3a** was analyzed by eqn. 4. There are two slopes

195  $\left( \frac{\Delta H^\ddagger_{\text{Singlet}}}{R} \right)$ ,  $\left( \frac{\Delta H^\ddagger_{\text{Triplet}}}{R} \right)$  of the Eyring plots corresponding

196 to the CF decay of compound **3a** at 378 nm and the CP decay  
 197 of compound **3a** at 560 nm, respectively, as shown in Fig. 5.  
 198 The activation parameters and rates of decomposition for comp-  
 199 ound **3a** calculated with eqn. 2 are listed in Table-1.

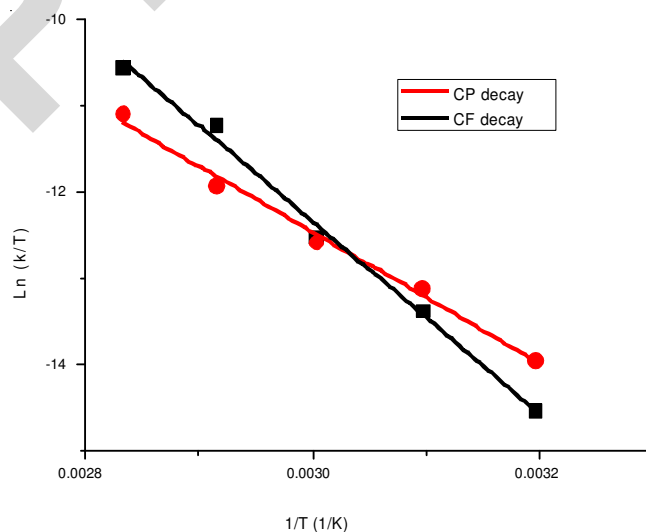


Fig. 5. Eyring plots of the chemiluminescence kinetics of compound **3a**

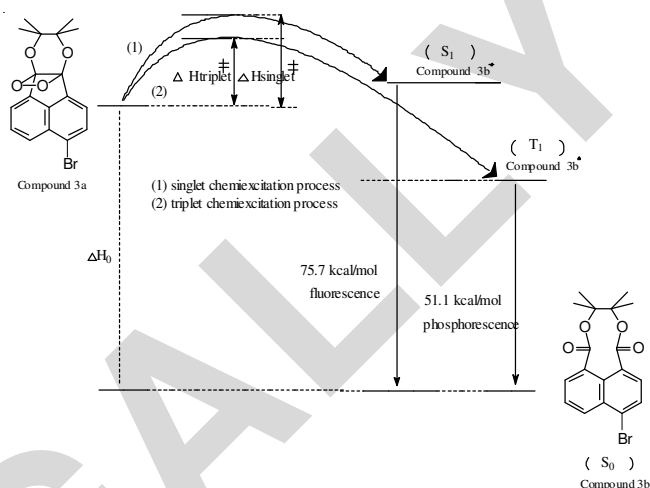
TABLE-1  
 ACTIVATION PARAMETERS AND RATES OF  
 DECOMPOSITION FOR COMPOUND **3a**

$\lambda_{max}$ (nm)	$\Delta H^\ddagger$ (kcal/mol)	$\Delta S^\ddagger$ (cal/mol K)	$\Delta G^\ddagger$ (25 °C) (kcal/mol)	$k_{25}$ °C (s <sup>-1</sup> )
378 (75.7)	22.2	-5.1	23.7	$2.44 \times 10^5$
560 (51.1)	15.2	-26.5	23.1	$7.6 \times 10^5$

200 The results show that there are two transition excited en-  
 201 ergy states in the chemiexcitation processes: (1) The transi-

tion singlet excited state activation enthalpy ( $\Delta H_{\text{Singlet}}^{\ddagger}$ ). (2) The transition triplet excited state activation enthalpy ( $\Delta H_{\text{Triplet}}^{\ddagger}$ ). The singlet excited state activation enthalpy ( $\Delta H_{\text{Singlet}}^{\ddagger}$ ) of compound **3a** has been determined experimentally to be 22.2 kcal/mol. The triplet excited state activation enthalpy ( $\Delta H_{\text{Triplet}}^{\ddagger}$ ) of compound **3a** has been determined experimentally to be 15.2 kcal/mol. The different activation enthalpies show the CP and CF arise by competing path in the decomposition of the dioxetane and the lower enthalpy of activation results from the lower enthalpy (greater stability) of the lower lying triplet state. The rate constant of the triplet state obtained at 298 K is also higher than that of the singlet state. The different activation enthalpies and different rate constants seem to be a reasonable explanation that the triplet state is not formed by way of the singlet state and the chemiexcitation processes favour the pathway for the formation of the CP.

The rate of a spin-forbidden process is enhanced by the internal presence of bromo-substituent, result the increase of the relative intensity of the RTCP of compound **3a**. The enhancement of the phosphorescence of compound **3b** is also observed by the external heavy atom solvent. The calculations of the RTCF and the RTCP decay time at variable temperature obtain the activation parameters for compound **3a**. The enthalpy ( $\Delta H^{\circ}$ ) of reaction for thermal decompositions of typical dioxetanes have been estimated from thermal chemical calculations from -69 to -90 kcal/mol<sup>16</sup>. The energy relationship diagram of the compound **3a** thermal decomposed to compound **3b** can be sketched, if we postulate the enthalpy of reaction for thermal decomposition of compound **3a** symbol for " $\Delta H^{\circ}$ ". The energy relationship diagram is showed in Scheme-V.



Scheme-V

## Conclusion

The unique feature of this work lies in the observed RTCP of the 5-heavy atom substituted acenaphtho lumiphor. In the 1,2-dioxetane ring of compound **3a** which has high strain energy due to the twisted four-membered ring peroxide structure (C-O-O-C), both the O-O and C-C bonds are cleaved almost simultaneously during thermal decomposition into two excited pathways: (1) singlet chemiexcitation processes, (2) triplet chemiexcitation processes. The internal heavy atom effect of 5-heavy atom substituted is  $\text{Br} \gg \text{Cl} > \text{H}$ . The transition triplet excited state activation enthalpy ( $\Delta H_{\text{Triplet}}^{\ddagger}$ ) is lower than the transition singlet excited state activation enthalpy ( $\Delta H_{\text{Singlet}}^{\ddagger}$ ) ca. 7 kcal/mol ( $\Delta H_{\text{Triplet}}^{\ddagger} < \Delta H_{\text{Singlet}}^{\ddagger}$ ). We have shown that the 1,2-dioxetane tricyclic compound-based chemiluminescence especially CP of compound **3a**. We continue to investigate these and other aspects, example as 5-iodo substituted 1,2-dioxetane tricyclic compound.

## ACKNOWLEDGEMENTS

Financial support from the National Science Council, MingChi University of Technology and National Taiwan Normal University, Taiwan, Republic of China, is gratefully acknowledged. Prof. L.A.Singer's (USC) comment is also highly appreciated.

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**May 31, 2013**

**Dear Editor-In-Chief, Prof. X. Zhang attn. Ms. Jeanne Keller (Luminescence:  
The Journal of Biological and Chemical Luminescence):**

**Thank you for your kind and helpful decision letter (reviews) dated on 18-Apr-2013.  
Enclosed please find our response to reviews and the revised manuscript re-entitled  
as "Substituent Effects on the Decomposition of Chemiluminescent Tricyclic  
Aromatic Dioxetanes"**

**Your action on this paper is highly appreciated.**

**We are look forward to hearing from you as soon as possible.**

**With my warm regards,**

**Tai-Shan Fang, Emeritous Professor**

**Department of Chemistry**

**National Taiwan Normal University**

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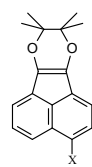
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Referee(s)' comments to author	Response to reviewer's comments
<p><b>Reviewer: 1</b>  Comments to the Author  This manuscript report the intramolecular chemically initiated electron exchange luminescence of tricyclic aromatic dioxetanes. It is an interesting work, but the logic of some paragraph is poor and it is difficult to read. So, this manuscript is not recommend be accepted for publication in current situation.  revision list below:</p>	<p>We are very thankful to the reviewer for his valuable suggestions.</p>
<p><b>(1) The title of the manuscript may be revised.</b></p>	<p>The title of the manuscript was changed to “Substituent effects on the decomposition of chemiluminescent tricyclic aromatic dioxetanes”.</p>
<p><b>(2) The English is not clear and concise. There are many instances of badly constructed sentences. E.g. Page 3, line 15-28; Page 6, line 15-26; Page 7, line 17-20.</b></p>	<p>In the revised manuscript, we modified all the sentences pointed out by the reviewer, and highlighted in yellow color.</p> <p>Page 3, line 15-24: “The CL from ozonization and singlet oxygen oxidation of the tricyclic 1,4-dioxin acenaphthylene compound 1 was reported several years ago from this laboratory[17].Because of the aforementioned current interests, the molecular structure of the compound 1 was used as a base template to design and synthesize 5-methoxy substituted 1,4-dioxin compound 2 and 5-chloro substituted 1,4-dioxin compound 3.” <b>was changed to</b> “The CL from ozonization and singlet-oxygen oxidation of the <b>unsubstituted</b> tricyclic 1,4-dioxin acenaphthylene compound 1 was reported several years ago from <b>our</b> laboratory <b>(17)</b>. <b>In continuation to this work, the 5-methoxy substituted 1,4-dioxin compound 2 and 5-chloro substituted 1,4-dioxin compound 3 were synthesized to study the substituent effects on the decomposition of dioxetanes.</b>”</p> <p>Page 6, line 19-26: <b>The figure 1 was updated, and changed as recommended</b> “Figure 1 shows the</p>

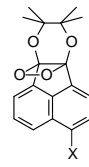
	<p>UV/Vis absorption and photoluminescence spectra of the compounds 1b-3b, and the CL spectra of the compounds 1a-3a in dichloromethane. Therein, the chemiluminescence and photoluminescence peaking at <math>\lambda_{\max}</math> are normalized with the same relative intensity. It shows that the CL spectral peaks of the dioxetanes are consistent with those of the photoluminescence spectral peaks of the photoexcited diesters, respectively.”</p> <p>Page 7, line 14-20: Sentence was rewritten as “From figure 1-2, it can be concluded that the CL as well as the photoluminescence are originated from the singlet excited state, which should be a <math>^1(\pi^* \rightarrow \pi)</math> state. Additionally, the experimental results indicate that the electron-donating methoxy group present in the 5-position is able to stabilize the singlet excited state of the 1,2-dioxetane decomposition products.”</p>
<p><b>(3) There are many English format and grammar format mistakes in the manuscript, such as Page 1, line 29, 34, 44, 47; Page 2, line 28,30,37; Page 6, line 40,51.</b></p>	<p>In revised manuscript, suggested formatting errors as well as grammar mistakes were corrected and highlighted in yellow color.</p> <p>The formatted sentences were given below.</p> <p>Page 1, line 29, 34: “Evidence for formation of the dioxetanes 1a, 2a and 3a is the chemiluminescence that corresponds to the emission from the electronically excited diesters 1b*, 2b* and 3b*, which are decomposed thermally from the dioxetanes 1a, 2a and 3a, respectively.”</p> <p>Page 1, line 44, 47: “Also, a study of the solvent effect on the fluorescence shows a significant red-shift in compound 2b, indicating a more polar excited state. The kinetics of the thermal decomposition of the 1,2-dioxetanes clearly demonstrate that the chemiluminescence characteristics of the compound 2a is quite different from that of compounds, 1a and 3a. These results are consistent with the proposed intramolecular chemically initiated electron exchange luminescence (CIEEL) mechanism which is triggered by the electron-donating group of compound 2a.”</p>





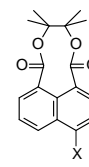
1 : X=H  
2 : X=OCH<sub>3</sub>  
3 : X=Cl

Compounds 1~3



1a : X=H  
2a : X=OCH<sub>3</sub>  
3a : X=Cl

Compounds 1a~3a



1b : X=H  
2b : X=OCH<sub>3</sub>  
3b : X=Cl

Compounds 1b~3b

Page 2, line 28,30: “Although 1,2-dioxetanes have been postulated as reaction intermediates for over 100 years, the charge transfer (CT)/electron transfer (ET) and **electron back-transfer (EBT)** have been suggested as a chemically initiated electron exchange luminescence (CIEEL) mechanism for CL, but the details of the mechanism has not yet been fully clarified (8).”

Page 2, line 37: The sentence “The most common preparation of 1,2-dioxetanes is through the [2+2] cycloaddition of singlet oxygen with electron-rich alkenes (9).” rewritten as “**In general [2+2] cycloaddition of singlet-oxygen with electron-rich alkenes is most convenient method for the synthesis of 1,2-dioxetanes (9).**”

Page 6, line 40: Revised as recommended “Presumably, the methoxy group of compound 2a can donate electrons to the naphthalene ring and extend the conjugation of electrons, causing **the obvious red shift of CL.**”

Page 6, line 51: Revised as recommended “Additional experimental evidence was obtained by examining the solvent effect on the photoluminescence of the **diesters 1b-3b**. The **normalized** fluorescence spectra of **diesters 1b-3b** in different **polar** solvents are shown in **figure 2**. All the emissions from ( $\pi, \pi^*$ ) excited states are seen in the three **diesters**, however, the **fluorescence spectrum of compound 2b** shows a **noticeable** dependence on solvent polarity.”

**(4) Some attributions of FT-IR data are not correct.**

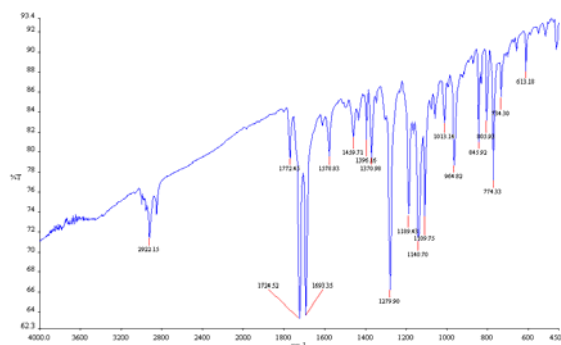
We corrected the FT-IR data in the revised manuscript.

Compound 1b: Page 4, line 30-32: “FT-IR (KBr, **cm<sup>-1</sup>**): 2922, 1725, 1693, 1579, 1280”

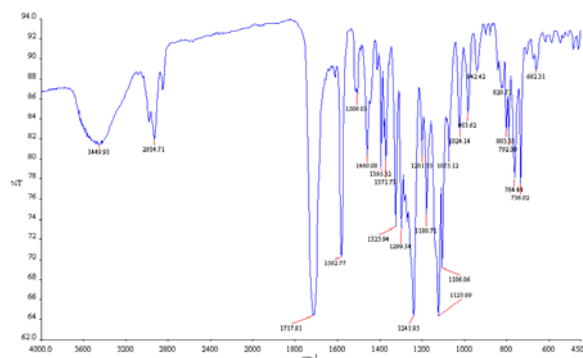
Compound 2b: Page 4, line 39-41: “FT-IR (KBr,  $\text{cm}^{-1}$ ): 2935, 1718, 1583, 1242”

Compound 3b: Page 4, line 47-48: FT-IR (KBr,  $\text{cm}^{-1}$ ): 3005, 1734, 1687, 1570, 1283

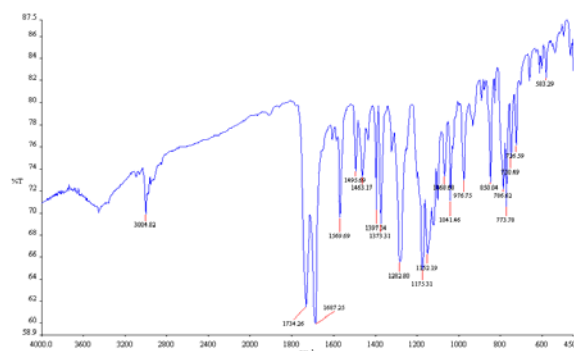
Supplement of FTIR spectra of compounds 1b-3b as follows:



FTIR spectrum of 1b



FTIR spectrum of 2b



FTIR spectrum of 3b

(5) The UV/Vis absorption and photoluminescence spectra of the compound 2a,3a,2b,3b should be listed.

Both UV/Vis absorption and photoluminescence spectra of the compound 2a, 3a, 2b, 3b were listed in the revised manuscript.

Update the figure 1, and erase figure 2. Page 6, line 19-26: Revised as recommended “Figure 1 shows the UV/Vis absorption and photoluminescence spectra of the compounds 1b-3b, and the CL spectra

of the compounds 1a-3a in dichloromethane. Therein, the chemiluminescence and photoluminescence peaking at  $\lambda_{\max}$  are normalized with the same relative intensity. It shows that the CL spectral peaks of the dioxetanes are consistent with those of the photoluminescence spectral peaks of the photoexcited diesters, respectively.”

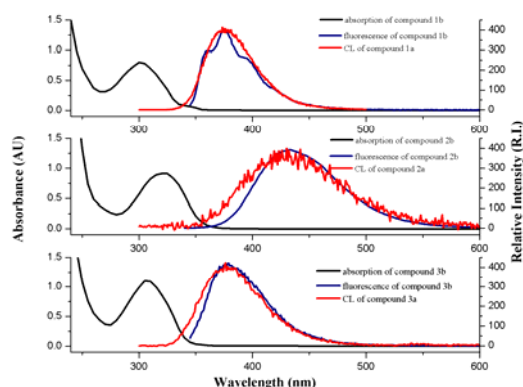
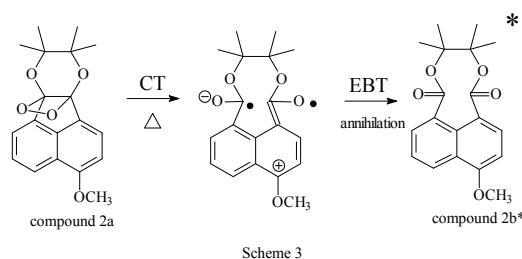


Figure 1. The UV/Vis absorption, photoluminescence spectra of the compounds 1b-3b, and the CL spectra of the compounds 1a-3a in dichloromethane.

(6) The superscript is not correct.

Page 7, line 17,27. Replace “ $^c1(*\pi\rightarrow\pi)$ ” with “ $^c1(\pi^*\rightarrow\pi)$ ”

Page 10, scheme 3. Update scheme 3, and erase the double cross “ $\ddagger$ ” and brackets.



(7) The reference style is not follow the author guidelines.

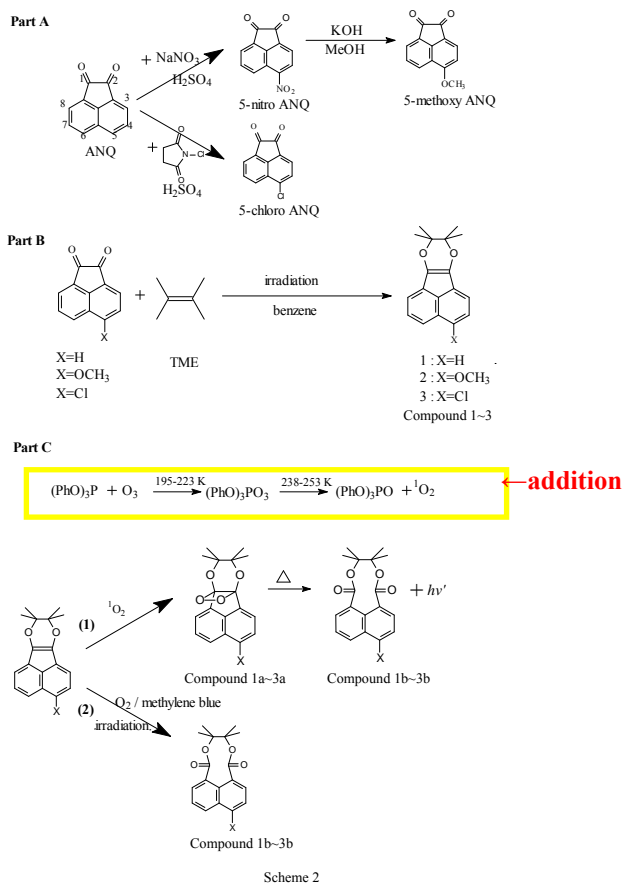
In the revised manuscript, we changed the style of the reference accordingly. E.g. reference 1.

1. Fraga H. Firefly Luminescence: a Historical Perspective and Recent Developments. Photochemical & Photobiological Sciences. 2008; 7: 146-158.

“1. Fraga H. Firefly luminescence: a historical perspective and recent developments. Photochem & Photobiol Sci 2008;7:146-58.”

<p><b>Reviewer: 2</b></p> <p>Comments to the Author</p> <p>The manuscript “Study on the Intramolecular Chemically Initiated Electron Exchange Luminescence of Tricyclic Aromatic Dioxetanes”, submitted by Chung-Wen Suna, Shun-Chi Chenb, Tai-Shan Fanga, shows interesting and important results of a mechanistic study on the electron transfer induced decomposition of highly strained and reactive tricyclic 1,2-dioxetane derivatives. In my opinion, the manuscript is well written, in general and it can be published in Luminescence, however, only after considering the corrections and additions outlined below.</p> <p>Corrections and suggestions outlined in the order of appearance in the manuscript:</p>	<p>We are very thankful to the reviewer for his appreciation.</p>
<p><b>(1) Page 1: Abstract line 25:</b></p> <p><b>Small language correction: Instead of “Three 1,2-dioxetane tricyclic compounds...” I suggest: “Three tricyclic 1,2-dioxetane derivatives...”</b></p>	<p>We thank the reviewer for his valuable suggestion. According to the reviewer suggestion the name of compound “1,2-dioxetane tricyclic compounds,..” replaced by “tricyclic 1,2-dioxetane derivatives”</p>
<p><b>(2) Page 1: Abstract, line 40: Include “higher” as indicated in the following sentence.</b></p> <p><b>“It was observed that the CL from 2a is red-shifted relative to 1a and 3a suggesting a higher degree of stabilization of the excited state by the electron donating methoxy group.”</b></p>	<p>We inserted the word “higher” in the sentence and modified the sentence accordingly.</p>
<p><b>(3) Page 2: Introduction, line 30:</b></p> <p><b>Instead of “Back electron-transfer (BET)” I recommend to use “Electron back-transfer (EBT)”.</b></p> <p><b>The term “Electron back-transfer (EBT)” is preferable to “Back electron-transfer (BET)”, although the latter one has been much utilized in</b></p>	<p>We agree with reviewers comments. In the revised manuscript. The suggested correction was incorporated.</p> <p>Replace “back electron-transfer (BET)” with “electron back-transfer (EBT)”.</p>

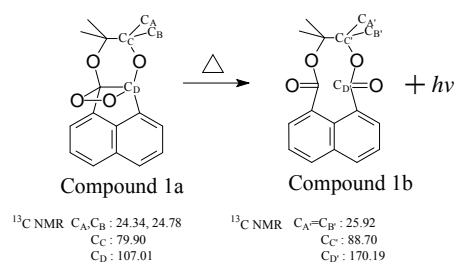
<p>the pertinent literature. For general information on the right terminology see: Glossary of terms used in photochemistry, 3rd edition (IUPAC Recommendations 2006). Pure Appl. Chem., 2007, Vol. 79, No. 3, pp. 293-465.  <a href="http://dx.doi.org/10.1351/pac200779030293">http://dx.doi.org/10.1351/pac200779030293</a>.  For recent use of the more adequate terminology see: Arkivoc, 2012 (iii), 391 – 430, 2012; J. Org. Chem., 77 (23), 10537– 10544, 2012.</p>	
<p><b>(4) Page 4: Materials, lines 5 - 7:</b>  <b>The methodology for singlet oxygen oxidation of dioxins should be better described and a reference given. It would be important to give here also the reaction temperature (238 K, as indicated in Scheme 2) and the reaction time.</b></p>	<p>In the revised manuscript, we have incorporated the scheme for the singlet-oxygen oxidation method of dioxins and mentioned the reaction temperature in the scheme itself. For the information, we have given the scheme below the response.  Update the Scheme 2, and replace “.....was carried out using the ozone-triphenyl phosphite procedure.” with “.....was carried out by using the ozone-triphenyl phosphite procedure (5). The triphenyl phosphite ozonide, (phO)<sub>3</sub>PO<sub>3</sub>, was prepared by passing extra pure oxygen through a commercial Fischer Model 501 ozonizer and bubbling the effluent into solution of the phosphite in the range of 195-223 K (dry ice/acetone bath). The solution was saturated with ozone for about two hours, then the temperature was kept about thirty minutes in the range of 238-253 K to undergo the singlet-oxygen oxidation of the 1,4-dioxin compounds.”</p>



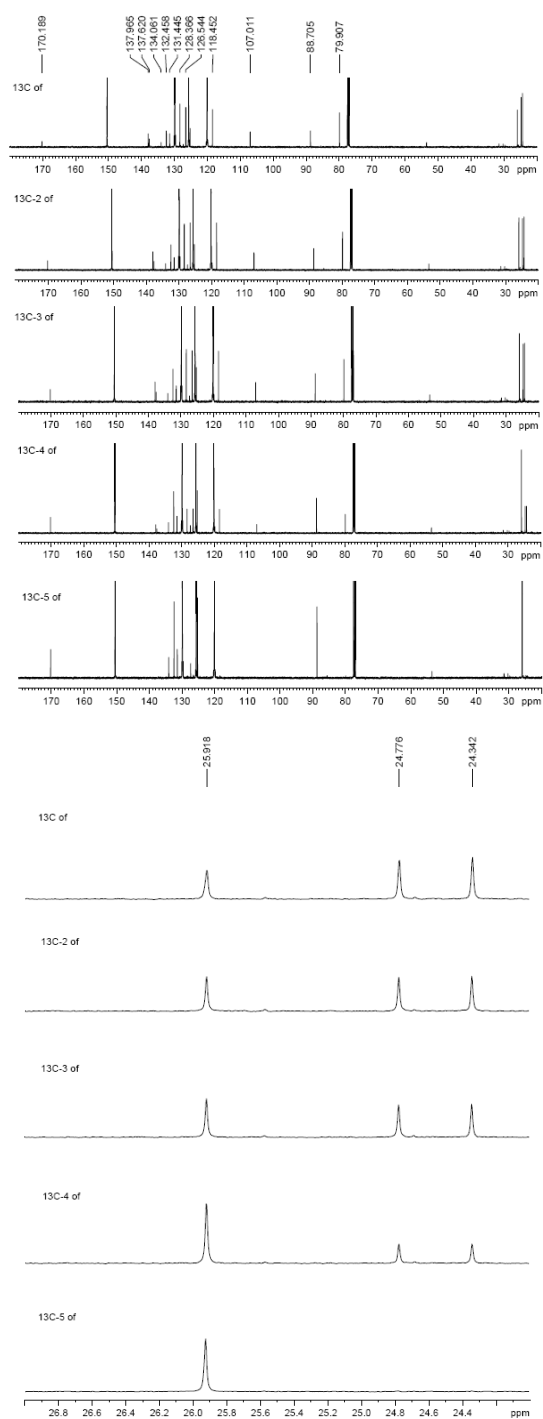
**(5) A more general observation to this part, which does not have to be necessarily considered in the revised manuscript is the following: According to the activation parameter you measured for these 1,2-dioxetane derivatives, it should be possible to isolate them and characterize these compounds by low-temperature  $^{13}\text{C}$  NMR spectroscopy ( $^1\text{H}$  NMR will not give very specific signals). Additionally, I would suggest to use low-temperature photooxygenation for the isolation of these compounds, which can be performed directly in an NMR tube at, for example  $-78$  °C. Dioxetane derivatives with similar or lower stabilities have been characterized by low-temperature NMR; see, for example, characterization of the unsubstituted**

This is very valuable comment. As per the reviewer suggestion we performed the low temperature  $^{13}\text{C}$  NMR experiment.

$^{13}\text{C}$  NMR (ppm) of compound 1a: 24.34, 24.78, 79.90, 107.01, 118.45, 126.54, 128.36, 137.62, 137.96.



derivative, which has a lower thermal stability than your derivatives (Angew. Chem. Int. Ed. Engl.,23 (2), 166-167, 1984.)



<p><b>(6) Page 4: Materials, line 21:</b>  <b>In my opinion you should not use benzene in future preparations, due to its extreme toxicity.</b></p>	<p>We apologize for this typo error. Actually the reactions were carried out in dichloromethane.</p>
<p><b>(7) Page 6: Results and Discussion, lines 8 - 11:</b>  <b>Here again the reaction time should be given.</b></p>	<p>See our response to the reviewer suggestion (4).</p>
<p><b>(8) Page 7: Results and Discussion, lines 15 - 17:</b>  <b>Reformulate the whole sentence. For example: “From Fig, 1 – 3 it can be concluded that the CL as well as the photoluminescence are originated from the singlet excited state, which should be a <math>1(*\pi \rightarrow \pi)</math> state. Additionally, the experimental results indicate that the electron-donating methoxy group present in the 5-position is able to stabilizes the singlet excited state of the 1,2-dioxetane decomposition</b></p>	<p>As per the reviewer suggestion we reformatted the sentence.</p> <p><b>Replaced the sentence</b> “From Fig. 1-3, we conclude the CL and fluorescence come from the transition singlet excited state <math>1(*\pi \rightarrow \pi)</math>. From experimental observations, it appears that the 5-electron-donating substituted group (<math>-\text{OCH}_3</math>) stabilizes the singlet excited state.” <b>with</b> “From figure 1-2, it can be concluded that the CL as well as the photoluminescence are originated from the singlet excited state, which should be a <math>1(\pi^* \rightarrow \pi)</math> state. Additionally, the experimental results indicate that the electron-donating methoxy group present in the 5-position is able to stabilize the singlet excited state</p>



products.”

(9) Page 7: Results and Discussion, lines 30 - 35:

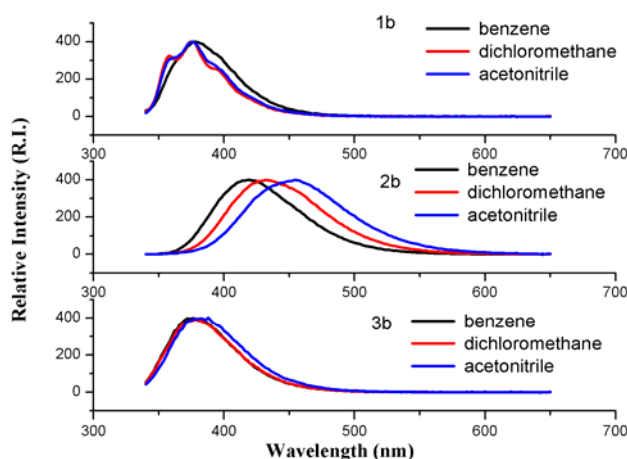
What you say here is true for compound 2a, but not necessarily for the other derivatives. You should comment on this fact.

of the 1,2-dioxetane decomposition products.”

Suggested correction was incorporated in the revised manuscript.

Update the original figure 3 to figure 2, and revised as recommended “Additional experimental evidence was obtained by examining the solvent effect on the photoluminescence of the diesters 1b-3b. The normalized fluorescence spectra of diesters 1b-3b in different polar solvents are shown in figure 2. All the emissions from ( $\pi, \pi^*$ ) excited states are seen in the three diesters, however, the fluorescence spectrum of compound 2b shows a noticeable dependence on solvent polarity. By increasing solvent polarity from benzene to acetonitrile, the fluorescence peak of the compound 2b is red-shift from 420 nm to 455 nm. The diesters 1b and 3b show the emission peak at 375 nm and 378 nm in benzene, and don't display a substantial shift with increasing solvent polarity. We can conclude, therefore, that this long wavelength fluorescence band of compound 2b has a prominent characteristics CT which is stabilized in polar solvent.”

Add comment on Page 7, after lines 35: The compounds 1a and 3a indicate that the CIEEL mechanism is not prominently operating and they are decomposed thermally without involvement of CT or ET.



	<p>Figure 2. The photoluminescence spectral peaks of the compounds 1b-3b in going from nonpolar to polar solvents.</p>
<p>(10) Page 7: Results and Discussion, lines 44 - 46:</p> <p>The sentence: “Figure 4 shows the CL thermal decay of the compound 2a (OCH<sub>3</sub>), measured at variable temperatures (313K~353K), and monitored at 430 nm.” Might be reformulated to: “Figure 4 shows the time course of the CL emission intensity at 430 nm upon thermal decomposition of compound 2a (OCH<sub>3</sub>), measured at different temperatures (313K - 353K).”</p>	<p>Replace “Figure 4 shows the CL thermal decay of the compound 2a(OCH<sub>3</sub>), measured at variable temperatures (313K-353K), and monitored at 430 nm.” with “Figure 3 shows the time course of CL emission intensity at 430 nm upon thermal decomposition of the compound 2a, measured at different temperatures (313-353 K).”</p>
<p>(11) Page 9: Results and Discussion, lines 8 - 10:</p> <p>Use “The Gibbs activation energy ...” instead of “The activation Gibbs energy ...”</p>	<p>Replace “The activation Gibbs energy...” with “the Gibbs activation energy...”</p>
<p>(12) Page 10: Scheme 3:</p> <p>The species you show in brackets is NOT a transition state, as indicated by the “double cross” (‡). This has to be corrected. Additionally, what you mean by “free neutral radical / radical anion species. In this context it is interesting to mention that the occurrence of an electron or charge transfer in induced 1,2-dioxetane decomposition has been recently shown experimentally (J. Org. Chem., 75 (19), 6574 – 6580, 2010.).</p>	<p>We thankful to this reviewer for valuable suggestion. As per the reviewer’s suggestion, the Scheme 3 is corrected as follows.</p> <p style="text-align: center;">Scheme 3</p>

Some more general observations and suggestions to the Results and Discussion section:

(13) (i) The similarity in the behavior of compounds 1a and 1c may indicate that in the case of these compounds the CIEEL mechanism is not operating and they are decomposed thermally without involvement of CT or ET. This should be discussed.

Update the Scheme 3, 1a and 3a are not involved in this scheme.

Page 8, lines 51; Page 9, lines 1 - 11: Revised as recommended “The activation parameters of the unsubstituted compound 1a and the chloro-substituted compound 3a show similar thermal stability at room temperature, with the Gibbs activation energy ( $\Delta G^\ddagger$ ) that different only about 0.1 kcal/mol. However, the methoxy-substituted compound 2a has a  $\Delta G^\ddagger$  value of 22.6 kcal/mol at 25°C, corresponding to a  $\tau_{1/2}$  value of ca. 1.13 h, and shows a much lower thermal stability than compounds 1a and 3a. These results obtained upon intramolecular decomposition of dioxtanes clearly demonstrate that the chemiluminescence characteristics of the compound 2a are quite different from that of the compounds 1a and 3a.”

Page 9, lines 30 - 34: Revised as recommended “The intramolecular CIEEL mechanism is triggered with increasing electron donating ability, with subsequent EBT annihilation leading to an excited state diester and luminescence to the ground state (see Scheme 3).”

**(14) (ii) The CL quantum yields for the decomposition of these compounds should be given. The relative quantum yields can be obtained approximately by integration of the emission curves for the three compounds measured in similar experimental conditions. For determination of quantum yields in CL transformations see for example: Eur. J. Org. Chem., (24) 4037-4046, 2000.**

Actually, the determination of the absolute chemiluminescence quantum yields for the decomposition of the 1,2-dioxetanes is not so easy due to the low thermal stability of these dioxetane compounds. The measurements of relative chemiluminescence quantum yields had been performed in a sealed cuvette containing 2 mL of a methylene blue ( $9.3 \times 10^{-5}$  M) solution and the 1,4-dioxin acenaphthylene compounds 1-3 ( $2 \times 10^{-5}$  M) in acetonitrile. These solutions were irradiated with light of wavelength 655 nm for 100s, the chemiluminescence spectra was integrated after the excitation shutter was closed and the emission shutter opened. (**J. Org. Chem., 75 (19), 6574 – 6580, 2010.**) We found the [2+2] cycloaddition of singlet oxygen and the chloro-substituted dioxin compound 3 was not proceeded in the condition (655 nm, 100s), but the chemiluminescence of compound 3a could be observed when the irradiation time was prolonged to 20 minutes. The following figure A shows the chemiluminescence spectra of the dioxetanes in methylene blue-acetonitrile solution. We can measure the integrated light emission and get the relative quantum yields of CL ( $\phi_{CL,rel.}$ ) which corresponds to the fluorescence quantum yields ( $\phi_{FL,rel.}$ ) of the diesters in figure B except the chloro-substituted dioxin. We supposed the formation yield of the dioxetane 3a was lower due to more electron-poor olefin than others. However, we will continue to study the absolute chemiluminescence quantum yields of CL for purified dioxetanes in future experiments.

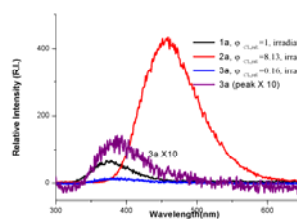


Fig. A. CL spectra of dioxetanes 1a-3a

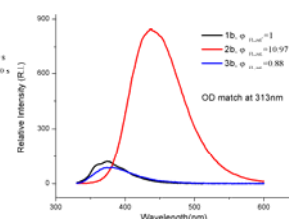
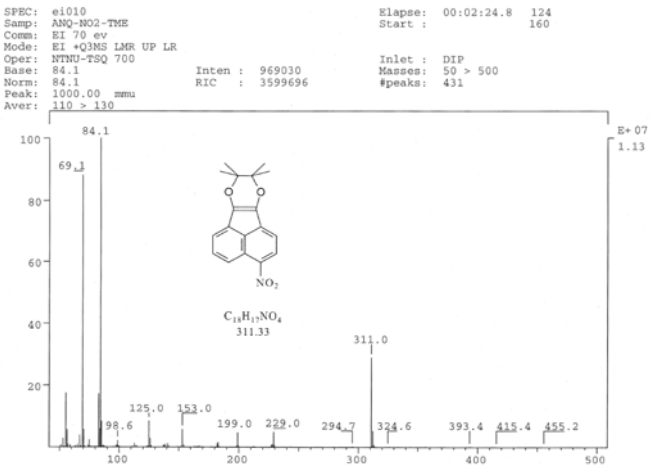
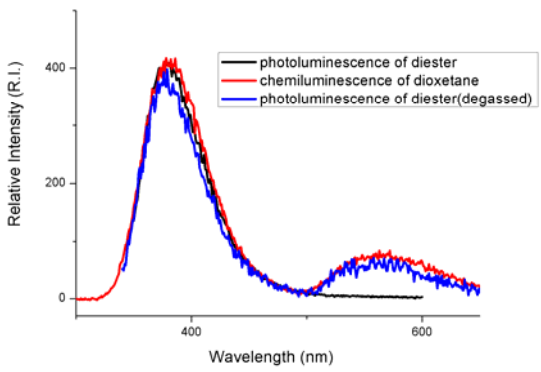


Fig. B. Fluorescence spectra of diesters 1b-3b

<p><b>(15) (iii) In your synthetic sequence, the nitro-substituted derivative is obtained. Did you try to transform it into the corresponding 1,2-dioxetane derivative. This would be an interesting compound, as this substituent is not supposed to be an electron donor at all.</b></p>	<p>This is very interesting point. We synthesized the nitro-substituted 1,4-dioxin acenaphthylene. However, the singlet-oxygen oxidation of the compound was not successful. We couldn't get the nitro-substituted dioxetane. This may be due to the presence of electron-poor olefin that failed to undergo [2+2] cycloaddition of singlet oxygen.</p> 
<p><b>(16) (iv) For 1,2-dioxetane 1a and 1c, the formation of triplet species should be verified, for example using 9,10-dibromoanthracene as an energy acceptor.</b></p>	<p>The phosphorescences of the degassed compound 1b-3b aren't inspected so far, we just only present the singlet species in this study. On the other case, we did see the CL of bromo-substituted ANQ dioxetane as shown in figure C which presents both the singlet and triplet species. We will use the 9,10-dibromoanthracene as a triplet energy acceptor in this system.</p>  <p>Figure C. The CL of 5-bromo ANQ dioxetane and</p>

	PL of 5-bromo ANQ diester
<p><b>(17) Page 10: Conclusions: lines 22 – 25:</b>  <b>This sentence is not right. The color of the CL emission is no modulated by the intramolecular CIEEL mechanism. The color of the CL emission from 1b is just different due to the different fluorescence spectrum of the decomposition product, caused by the stabilization effect of the methoxy group. This sentence has to be reformulated.</b></p>	<p>This sentence was reformulated. According to the reviewer suggestion. “We have shown that the 1,2-dioxetane-based CL color is modulated by a dioxetane bearing a substituted-naphthalene group. The color of the CL emission from compound 2a is just different due to the different fluorescence spectrum of the thermodynamic decomposition product 2b, caused by the stabilization effect of the methoxy group.”</p>
<p><b>(18) Page 13: Table 1:</b>  <b>Indicate the <math>t_{1/2}</math> values are calculated at 25 oC (at least I suppose). These relatively long life times indicate that it should be possible to characterize these compounds by NMR spectroscopy (see my comment above), even at RT.</b>  <b>These activation parameters should be better discussed in the manuscript text. A recent compilation and discussion of activation parameters measured for the decomposition of different cyclic peroxides is given in the literature (J. Org. Chem., 74 (23), 8974 – 8979, 2009).</b></p>	<p>Replace “<math>\tau_{1/2}</math>” with “<math>\tau_{1/2}(25^{\circ}\text{C})</math>”  Also see our response to the reviewer suggestion (13).</p>
<p><b>(19) Pages 14 - 16: Figures 1 - 3:</b>  <b>The experimental condition in which these spectra are obtained should be given, including instrumental parameters like emission slit and scanning time, for example. This might explain the differences in the</b></p>	<p>The photoluminescence of 1b shows the vibronic band in dichloromethane, but the CL of 1a doesn't. In other series, the photoluminescence of 2b or 3b matches the CL of 2a or 3a, as figure 1. In figure 2, the photoluminescence of 1b in acetonitrile also shows the vibronic band.  photoluminescence of 1b: Ex. Wavelength (313nm),</p>

<p><b>CL of 1a and the fluorescence spectrum of 1b.</b></p>	<p>Ex. Slit (5 nm), Em. Slit (5 nm), Scan rate (1200 nm/min), PMT= 500 V.  CL of 1a: Gate time (5ms), Em. Slit (10 nm), Data interval (0.5 nm), PMT= 800 V.  <b>The experiment conditions used in <i>Measurements</i>:</b>  The absorption and emission experiments were performed with prepared solutions containing <math>1.0 \times 10^{-4}</math> M of diesters in dichloromethane.</p>
<p><b>(20) In conclusion, this manuscript should be published in Luminescence, however, major revision is required.</b></p>	<p>We are very thankful to the reviewer for his kind revision and suggestions.</p>



**Substituent effects on the decomposition of  
chemiluminescent tricyclic aromatic dioxetanes**

Journal:	<i>Luminescence: The Journal of Biological and Chemical Luminescence</i>
Manuscript ID:	BIO-13-043.R1
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Date Submitted by the Author:	n/a
Complete List of Authors:	Sun, Chung-Wen; National Taiwan Normal University, Chemistry Chen, Shun-Chi; MingChi University of Technology, Chemical Engineering Fang, Tai-Shan; National Taiwan Normal University, Chemistry
Keywords:	chemiluminescence, tricyclic dioxetane, chemically initiated electron exchange luminescence

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# Substituent effects on the decomposition of chemiluminescent tricyclic aromatic dioxetanes

Chung-Wen Sun<sup>a</sup>, Shun-Chi Chen<sup>b\*</sup>, Tai-Shan Fang<sup>a\*</sup>

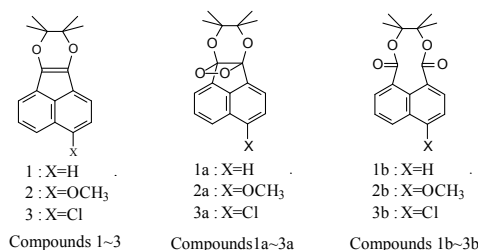
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<sup>b</sup>Department of Chemical Engineering, MingChi University of Technology, Taishan, Taipei 243, Taiwan, ROC

## Abstract

Three tricyclic 1,2-dioxetane derivatives, 1a, 2a and 3a were synthesized from their corresponding 1,4-dioxin acenaphthylene compounds, 1, 2 and 3, by reaction with singlet-oxygen (<sup>1</sup>O<sub>2</sub>) in dichloromethane. Evidence for formation of the dioxetanes 1a, 2a and 3a is the chemiluminescence that corresponds to the emission from the electronically excited diesters 1b\*, 2b\* and 3b\*, which is decomposed thermally from the dioxetanes 1a, 2a and 3a, respectively. The highly strained 1,2-dioxetane ring decomposes from a twisted geometry by simultaneous cleavages of the O-O and C-C bonds producing the electronically excited diester that emits chemiluminescence (CL). It was observed that the CL from compound 2a is red-shifted relative to that of compounds 1a and 3a suggesting a higher degree of stabilization of the excited state by the electron donating methoxy group. Also, a study of the solvent effect on the fluorescence shows a significant red-shift in compound 2b, indicating a more polarity of the excited state. The kinetics of the thermal decomposition of the 1,2-dioxetanes clearly demonstrates that the chemiluminescence characteristics of the compound 2a is quite different from that of compounds, 1a and 3a. These results are consistent with the proposed intramolecular

chemically initiated electron exchange luminescence (CIEEL) mechanism which is triggered by electron-donating group of compound 2a.



## Keywords

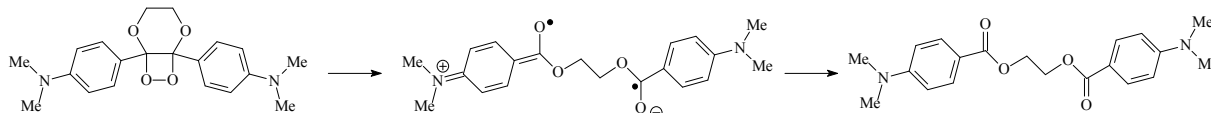
chemiluminescence; tricyclic dioxetane; chemically initiated electron exchange luminescence

## 1. Introduction

The phenomenon of firefly bioluminescence (1,2) has led to the theoretical (3,4) and practical (5-7) study of the chemiluminescence (CL) of 1,2-dioxetanes and has been of interest to chemists for a long time. The high-energy 1,2-dioxetane molecules are the focus of many investigations because of their unique ability to decompose thermally into electronically excited carbonyl products. Although 1,2-dioxetanes have been postulated as reaction intermediates for over 100 years, the charge transfer (CT)/electron transfer (ET) and electron back-transfer (EBT) have been suggested as a chemically initiated electron exchange luminescence (CIEEL) mechanism for CL, but the details of the mechanism has not yet been fully clarified (8). It is likely that the effect of structural changes will help to elucidate the still-debated mechanism of this reaction. In general [2+2] cycloaddition of singlet-oxygen with electron-rich alkenes is most convenient method for the synthesis of 1,2-dioxetanes (9).

The key concept of the intramolecular CIEEL mechanism is that thermal decomposition of the 1,2-dioxetane proceeds through a twisted diradical transition state that yields intramolecular radical ion intermediates. Subsequent exothermic electron back-transfer (EBT) annihilation leads to an excited

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2 state and chemiluminescence (10-15). For example, the intramolecular CIEEL mechanism of the  
3 dioxetane substituted with the electron donating moiety (p-(dimethylamino)phenyl) is illustrated,  
4 where CT from the electron donating moiety to the O-O bond of the dioxetane promotes the  
5 decomposition of the dioxetane into the excited ester (see Scheme 1) (16).  
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Scheme 1

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19 The CL from ozonization and singlet-oxygen oxidation of the tricyclic 1,4-dioxin acenaphthylene  
20 compound 1 was reported several years ago from our laboratory (17). In continuation to this work, the  
21 5-methoxy substituted 1,4-dioxin compound 2 and 5-chloro substituted 1,4-dioxin compound 3 were  
22 synthesized to study the substituent effects on the decomposition of dioxetanes. We report herein the  
23 CL of the rigid tricyclic aromatic dioxetanes compounds, 1a, 2a and 3a, with different functional  
24 groups (-H, -OCH<sub>3</sub> and -Cl) to assess the influence on the intramolecular CIEEL mechanism of 1,2-  
25 dioxetanes.  
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## 35 2. Experimental

### 36 2.1. Materials

#### 37 2.1.1. 1,4-dioxin acenaphthylene, compounds 1-3

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41 Acenaphthenequinone (ANQ) was obtained from Aldrich. The 5-nitro ANQ was synthesized  
42 from ANQ and sodium nitrate in concentrated sulfuric acid by refluxing the reaction mixture for 2h.  
43 The 5-methoxy ANQ was synthesized from the purified 5-nitro ANQ and potassium hydroxide in  
44 methanol by refluxing the reaction mixture for 2h (18). The 5-chloro ANQ was synthesized from ANQ  
45 and N-chlorosuccinimide (NCS) in concentrated sulfuric acid by refluxing the reaction mixture for 2h.  
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(see [Scheme 2 Part A](#)). The compounds 1-3 were synthesized by the photo-cycloaddition reaction of acenaphthenequinone (ANQ) with tetramethylethylene (TME) in benzene (see [Scheme 2 Part B](#)) (19).

### 2.1.2. tricyclic 1,2-dioxetane compounds 1a-3a

Singlet-oxygen oxidation of the 1,4-dioxin compounds 1-3 to produce the tricyclic 1,2-dioxetane compounds 1a-3a was carried out by using the ozone-triphenyl phosphite procedure (5). The triphenyl phosphite ozonide,  $(\text{phO})_3\text{PO}_3$ , was prepared by passing extra pure oxygen through a commercial Fischer Model 501 ozonizer and bubbling the effluent into solution of the phosphite in the range of 195-223 K (dry ice/acetone bath). The solution was saturated with ozone lasting about two hours, then kept the temperature in the range of 238-253 K to undergo the singlet-oxygen oxidation of the 1,4-dioxin compounds for thirty minutes. Dioxetane adducts 1a-3a were isolated by ice-chilled n-hexane extraction from low temperature synthesis of ozonized triphenyl phosphite (see [Scheme 2 Part C \(1\)](#)).

### 2.1.3. naphthalene diester compounds 1b-3b

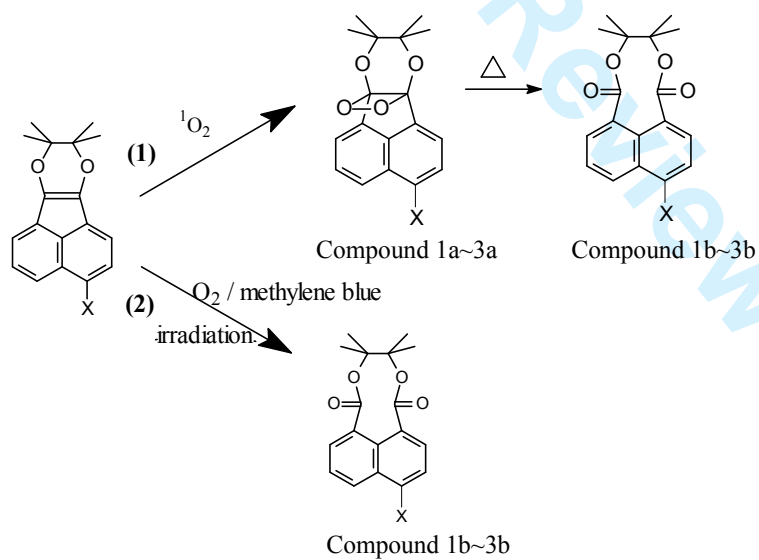
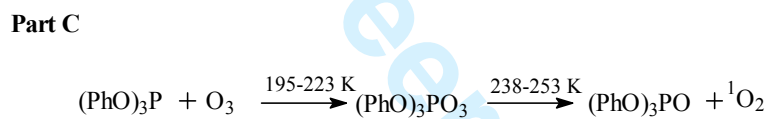
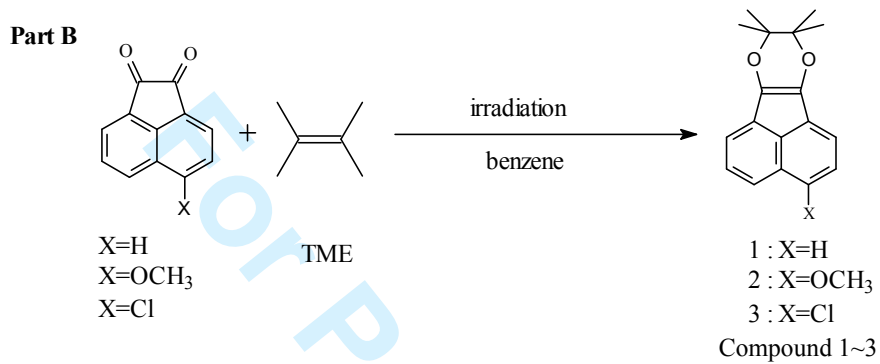
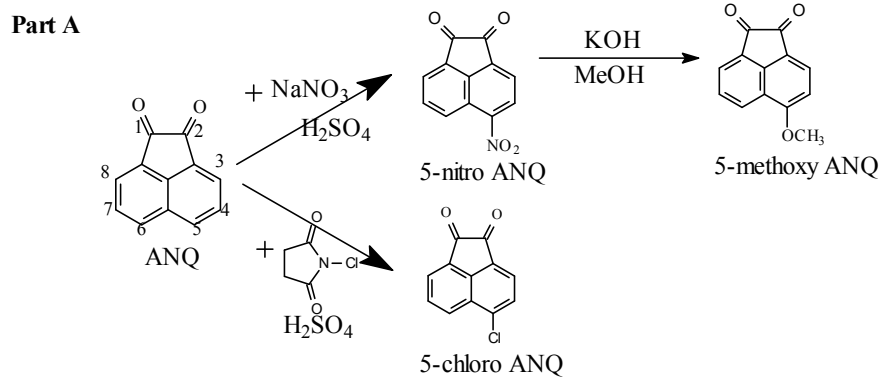
There are two methods were utilized for the synthesis of the naphthalene diester compounds 1b-3b. Among them, the first method involves the thermal decomposition of the compounds 1a-3a to the naphthalene diester compounds 1b-3b (see [Scheme 2 Part C \(1\)](#)). Second procedure was the irradiation of compounds 1-3 in dichloromethane solution in the presence of the photosensitizer (methylene blue) under oxygen atmosphere. (see [Scheme 2 Part C \(2\)](#)). The  $^1\text{H-NMR}$ , IR and MS spectral data for the naphthalene diester compounds 1b-3b are shown below.

Compound 1b:  $^1\text{H-NMR}$  (200 MHz,  $\text{CDCl}_3$ ): 7.99-7.95 (d, 2H, Ar-H,  $J=8$  Hz), 7.90-7.86 (d, 2H, Ar-H,  $J=8$  Hz), 7.56-7.48 (t, 2H, Ar-H,  $J=8$  Hz), 1.74 (s, 12H, 4 $\text{CH}_3$ ), FT-IR (KBr,  $\text{cm}^{-1}$ ): 2922, 1725, 1693, 1579, 1280, SIMS  $m/z$  298 [M].

Compound 2b:  $^1\text{H-NMR}$  (200 MHz,  $\text{CDCl}_3$ ): 8.46-8.42 (d, 1H, Ar-H,  $J=8$  Hz), 7.95-7.91 (d, 1H, Ar-H,  $J=8$  Hz), 7.83-7.79 (d, 1H, Ar-H,  $J=8$  Hz), 7.52-7.44 (t, 1H, Ar-H,  $J=8$  Hz), 6.87-6.83 (d, 1H,

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2 Ar-H,  $J=8$  Hz), 4.03 (s, 3H, OCH<sub>3</sub>), 1.75 (s, 6H, 2CH<sub>3</sub>), 1.68 (s, 6H, 2CH<sub>3</sub>), FT-IR (KBr, cm<sup>-1</sup>): 2935,  
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4 1718, 1583, 1242, SIMS m/z 328 [M].  
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7 Compound 3b: <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>): 8.51-8.47 (d, 1H, Ar-H,  $J=8$  Hz), 7.93-7.89 (d, 1H,  
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9 Ar-H,  $J=8$  Hz), 7.81-7.77 (d, 1H, Ar-H,  $J=8$  Hz), 7.67-7.60 (m, 2H, Ar-H), 1.74 (s, 6H, 2CH<sub>3</sub>), 1.70 (s,  
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11 6H, 2CH<sub>3</sub>), FT-IR (KBr, cm<sup>-1</sup>): 3005, 1734, 1687, 1570, 1283, SIMS m/z 332 [M].  
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Scheme 2

## 2.2. Measurements

The absorption spectra were measured on a Hewlett-Packard diode array spectrophotometer. The steady-state emission spectra were obtained using a Cary Eclipsed Spectrofluorimeter equipped with a temperature controller. The absorption and emission experiments were performed with prepared solutions containing  $1.0 \times 10^{-4} \text{ M}$  of diesters in dichloromethane. For the temperature-dependent kinetic measurements, the temperature of the sample solution was controlled to within  $\pm 0.5 \text{ K}$  with an electronically thermostating single cell and monitored with thermocouples attached to the cell.

## 3. Results and Discussion

The isolated dioxetane compounds 1a, 2a and 3a, respectively, are formed by reacting compounds 1, 2 and 3 with singlet-oxygen ( $^1\text{O}_2$ ) in dichloromethane at 238 K and decomposed thermally into electronically excited diesters 1b\*, 2b\* and 3b\*, which then fluoresced to their corresponding ground state configurations 1b, 2b and 3b. Experimental evidence shows that the CL spectral peaks of the compounds 1a-3a are consistent with that of the photoluminescence spectral peaks of the photoexcited compounds 1b-3b. Figure 1 shows the UV/Vis absorption and photoluminescence spectra of the compounds 1b-3b, and the CL spectra of the compounds 1a-3a in dichloromethane. Therein, the chemiluminescence and photoluminescence peaking at  $\lambda_{\text{max}}$  are normalized with the same relative intensity. It shows that the CL spectral peak of the dioxetane is consistent with that of the photoluminescence spectral peak of the photoexcited diester, respectively.

[Insert figure 1 here]

The CL peak of the dioxetane compound 2a at 430 nm shows an obvious red-shift in comparison with the compounds 1a(375nm) and 3a(378nm). Presumably, the methoxy group of compound 2a can donate electrons to the naphthalene ring and extend the conjugation of electrons, causing the obvious red shift of CL.

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2 Additional experimental evidence was obtained by examining the solvent effect on the  
3 photoluminescence of the diesters 1b-3b. The normalized fluorescence spectra of diesters 1b-3b in  
4 different polar solvents are shown in figure 2. All the emissions from ( $\pi$ ,  $\pi^*$ ) excited states are seen in  
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9 the three diesters, however, the fluorescence spectrum of compound 2b shows a noticeable dependence  
10 on solvent polarity. By increasing solvent polarity from benzene to acetonitrile, the fluorescence peak  
11 of the compound 2b is red-shift from 420 nm to 455 nm. The diesters 1b and 3b show the emission  
12 peak at 375 nm and 378 nm in benzene, and don't display a substantial shift with increasing solvent  
13 polarity. We can conclude, therefore, that this long wavelength fluorescence band of compound 2b has  
14 a prominent characteristics CT which is stabilized in polar solvent.  
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22 **[Insert figure 2 here]**  
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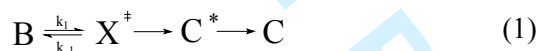
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25 From figure 1-2, it can be concluded that the CL as well as the photoluminescence is originated  
26 from the singlet excited state, which should be a  $^1(\pi^* \rightarrow \pi)$  state. Additionally, the experimental results  
27 indicate that the electron-donating methoxy group present in the 5-position is able to stabilize the  
28 singlet excited state of the 1,2-dioxetane decomposition products. In the chemiexcitation processes,  
29 when both the O-O and C-C bonds are cleaved simultaneously, the electron-donating group ( $-\text{OCH}_3$ ) at  
30 5-position can interact through the  $\pi$  electrons of the naphthalene ring to stabilize the singlet excited  
31 state  $^1(\pi^* \rightarrow \pi)$ . Hence, the chemiluminescence emission of this tricyclic aromatic dioxetane highly  
32 depends on the substitution on 5-position of the naphthalene structure or polarity of solvent system.  
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43 These observations together with the CL spectra are consistent with an intramolecular CIEEL  
44 decomposition initiated by CT from the electron-donating group of compound 2a, followed by EBT to  
45 produce the excited diester. The compounds 1a and 3a indicate that the CIEEL mechanism is not  
46 prominently operating and they are decomposed thermally without involvement of CT or ET.  
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The rate constants for the decomposition of the compounds 1a-3a were obtained at 313-353 K by measuring the decay of the CL intensity. The CL decay of the compounds 1a-3a was monitored at the maximum value of the CL in dichloromethane (compound 1a- $\lambda_{CL,max}$ : 375 nm, compound 2a- $\lambda_{CL,max}$ : 430 nm, compound 3a- $\lambda_{CL,max}$ : 378 nm). [Figure 3](#) shows the time course of CL emission intensity at 430 nm upon thermal decomposition of the compound 2a, measured at different temperatures (313-353 K).

[Insert figure 3 here]

According to the transition state theory, the overall mechanism for the decomposition of the tricyclic 1,2-dioxetane compound can be presented as equation (1).



where B = tricyclic 1,2-dioxetane compound;  $X^\ddagger$  = the activated complex;  $C^*$  = excited intramolecular naphthalene diester compound. The Eyring equation (2) can be obtained by transition state theory (20).

$$\ln \frac{k}{T} = \left( \ln \frac{R}{N_A h} + \frac{\Delta S^\ddagger}{R} \right) - \frac{\Delta H^\ddagger}{RT} \quad (2)$$

where  $N_A$ =Avogadro's constant;  $h$ =Planck's constant

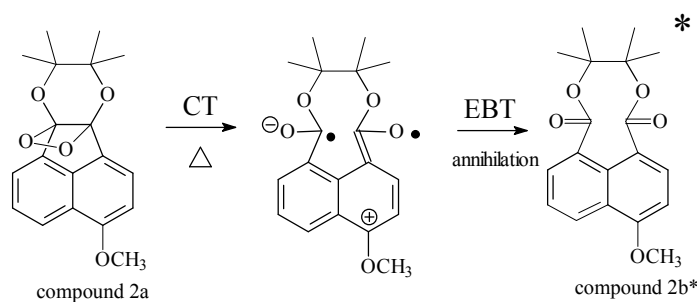
The activation parameters of the dioxetanes 1a-3a were obtained from the temperature dependence of the chemiluminescence decomposition rate constants. The chemiluminescence activation parameters were obtained by plotting the  $\ln(k/T)$  verse  $1/T$  in the Eyring plots, as shown in [figure 4](#). The kinetics of the thermal decomposition of the 1,2-dioxetanes studied at 313-353 K reveal a significantly lower value for the activation enthalpy ( $\Delta H^\ddagger$ ) of 2a. The activation parameters and rates of decomposition for 1,2-dioxetanes 1a-3a are listed in [Table 1](#).

[Insert figure 4 here]

[Insert table 1 here]

Table 1 shows the activation parameters and rates of decomposition for 1,2-dioxetanes 1a-3a. The  $\Delta H^\ddagger$  value of compound 2a is significantly lower than those values for compounds 1a and 3a. The rate constants obtained for compound 2a is about 4-5 times faster than that of 1a, 3a at 298 K. The activation parameters of the unsubstituted compound 1a and the chloro-substituted compound 3a show similar thermal stability at room temperature, with the Gibbs activation energy ( $\Delta G^\ddagger$ ) that different only about 0.1 kcal/mol. However, the  $\Delta G^\ddagger$  value of methoxy-substituted compound 2a is 22.6 kcal/mol at 25°C, corresponding to a  $t_{1/2}$  value of ca. 1.13 h, shows the thermal stability in the behavior are less stable than that of the compounds 1a and 3a. These results obtained upon intramolecular decomposition of dioxetanes clearly demonstrate that the chemiluminescence characteristics of the compound 2a are quite different from that of the compounds 1a and 3a. The more negative activation entropy ( $\Delta S^\ddagger$ ) value for compound 2a, which compensates for the surprisingly low value of  $\Delta H^\ddagger$ , can be understood by the need of a specific conformation for the CT from the methoxy group of the naphthalene ring to the O-O bond of the dioxetane, which promotes the decomposition of the dioxetane into two radicals confined within a solvent cage. The subsequent EBT annihilation between the radicals releases enough energy to excite the naphthalene emitter to its singlet excited state. These results are consistent with the intramolecular CIEEL mechanism where the electron-donating methoxy group promotes O-O bond cleavage by resonance interaction through the  $\pi$  electrons of the naphthalene moiety. The intramolecular CIEEL mechanism is triggered with increasing electron donating ability, with

subsequent EBT annihilation leading to an excited state diester and luminescence to the ground state (see Scheme 3).



#### 4. Conclusions

We have shown that the 1,2-dioxetane-based CL color is modulated by a dioxetane bearing a substituted-naphthalene group. The color of the CL emission from compound 2a is just different due to the different fluorescence spectrum of the thermodynamic decomposition product 2b, caused by the stabilization effect of the methoxy group. The change of substituent on the naphthalene emitter causes a change in the color of the dioxetane-based CL, and also affects the decomposition reaction activation enthalpies of the dioxetanes. The chemiexcitation process of the intramolecular CIEEL decay was rationalized and described as being mainly due to a particular form of electron donating substituent at 5-position of acenaphtho moiety. Further investigation of present and the other aspects of the intramolecular CIEEL mechanism underway in our laboratory.

#### Acknowledgements

Financial support from the National Science Council, MingChi University of Technology and National Taiwan Normal University, Taiwan, Republic of China, is gratefully acknowledged. Prof. L. A. Singer's (USC) comment is also highly appreciated.

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1982;153-189.

**Table 1.** Activation parameters and rates of decomposition for 1,2-dioxetanes 1a-3a

Compound	$\lambda_{\max}$ nm (kcal/mol)	$\Delta H^\ddagger$ kcal/mol	$\Delta S^\ddagger$ cal/mol·K	$\Delta G^\ddagger$ (25°C) kcal/mol	$k_{25^\circ\text{C}}$ s <sup>-1</sup>	$\tau_{1/2}$ (25°C) h
1a(H)	375 (76.3)	20.3	-10.2	23.4	$4.54 \times 10^{-5}$	4.24
<b>2a(OCH<sub>3</sub>)</b>	<b>430 (66.5)</b>	<b>13.1</b>	<b>-31.8</b>	<b>22.6</b>	<b><math>17.1 \times 10^{-5}</math></b>	<b>1.13</b>
3a(Cl)	378 (75.7)	18.8	-15.8	23.5	$3.79 \times 10^{-5}$	5.08

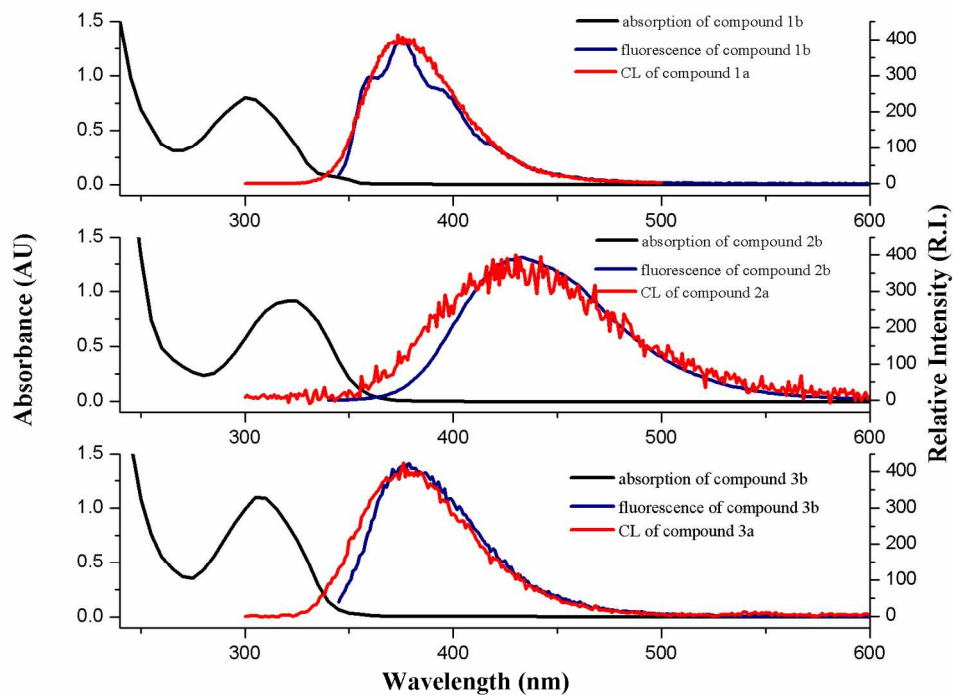


Figure 1. The UV/Vis absorption, photoluminescence spectra of the compounds 1b-3b, and the CL spectra of the compounds 1a-3a in dichloromethane.  
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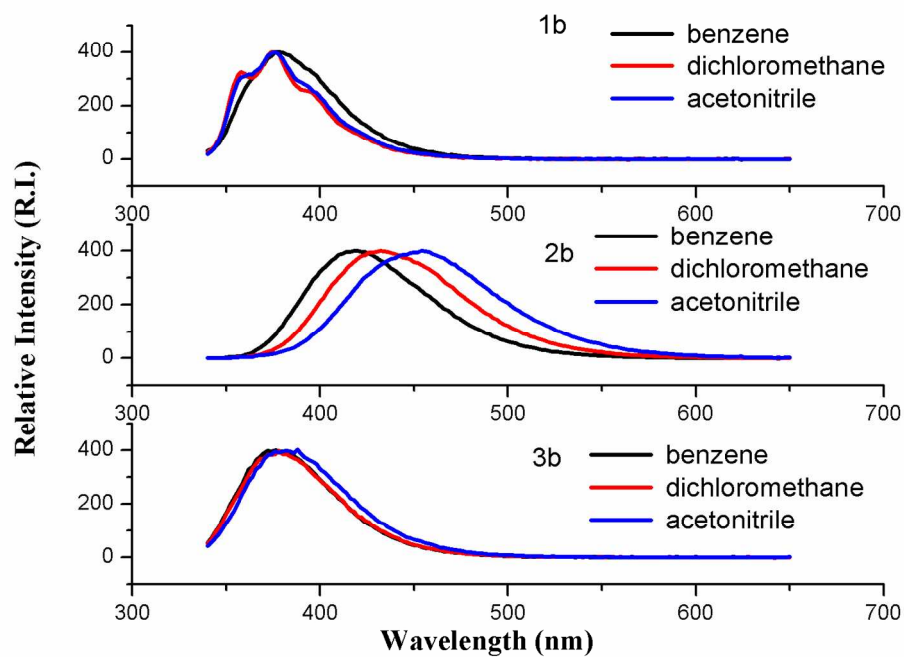


Figure 2. The photoluminescence spectral peaks of the compounds 1b-3b in going from nonpolar to polar solvents.

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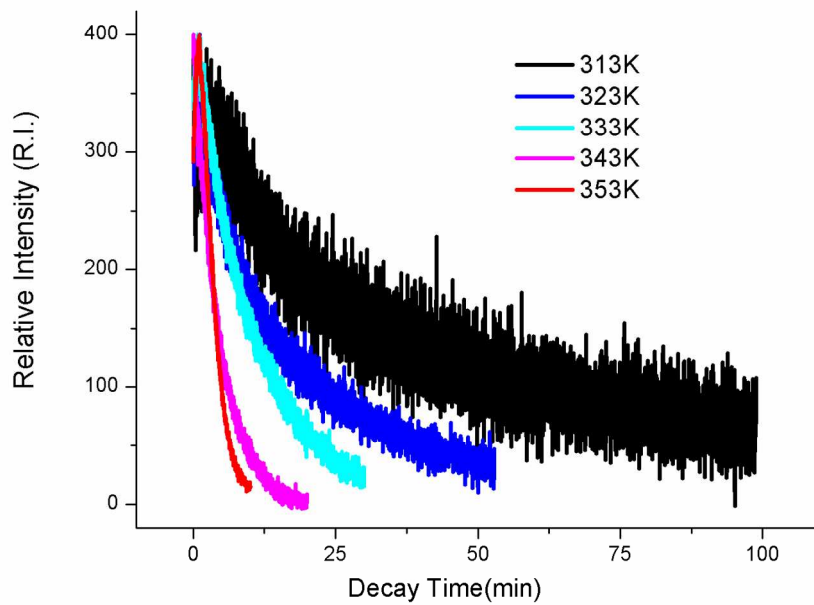


Figure 3. The CL thermal decay of the compound 2a at variable temperatures, monitored at 430 nm.  
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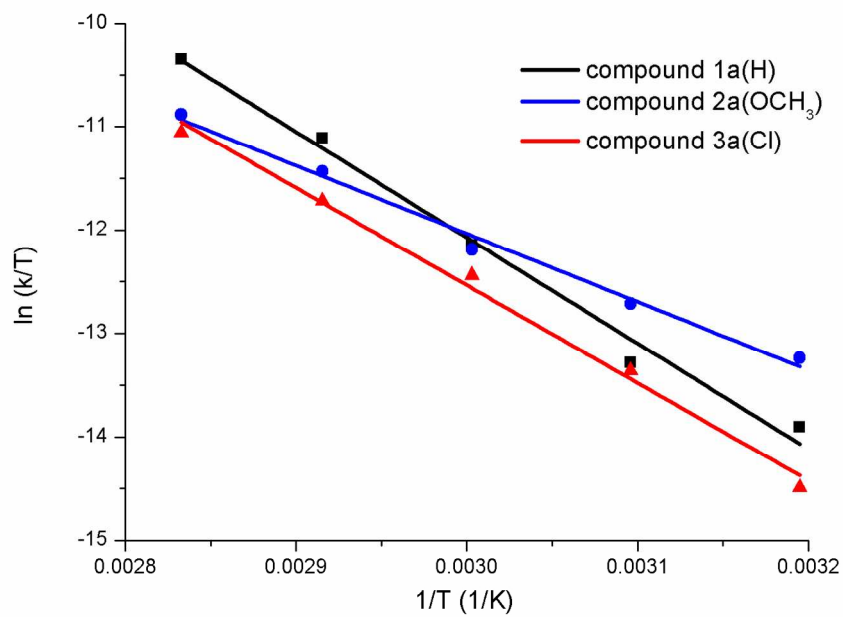


Figure 4. The Eyring plots of the CL kinetics of compounds 1a-3a.  
297x213mm (150 x 150 DPI)

(附錄:孫崇文碩士論文):

## 秋水仙素與甲硫秋水仙素光化學反應的研究

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<sup>1</sup> 國立臺灣師範大學化學系

<sup>2</sup> 明志科技大學化工系

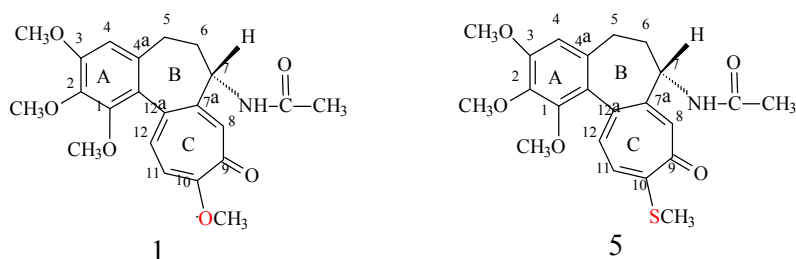
### 摘要

本論文研究秋水仙素與甲硫秋水仙素的光化學反應性質。秋水仙素(1)與甲硫秋水仙素(5)的差異，為化學結構上之環庚三烯酚酮(tropolone)的羥基(-OH)分別被甲氧基(-OCH<sub>3</sub>)及甲硫基(-SCH<sub>3</sub>)取代，因其結構相近使得物理與化學性質相似，為一種有效治療痛風之藥物。然而，其結構上環庚三烯酮(tropone)的構造很容易因照光而進行環合反應，使得藥物耐光性較差而容易變質。秋水仙素(1)照光得到 β-lumicolchicine(2)、γ-lumicolchicine(3)，將 β-lumicolchicine(2)繼續照光，可以得到 α-lumicolchicine(4)。甲硫秋水仙素(5)照光得到 β-lumithiocolchicine(6)、γ-lumithiocolchicine(7)。秋水仙素(1)與甲硫秋水仙素(5)二者在化學動力學的研究，測量其在不同溶劑極性下及不同照光時間下之產物 β/γ 比值，並比較其光化學反應速率，結果顯示化合物(1)與化合物(5)皆進行 Woodward-Hoffmann 的電環反應，化合物(1)因其環庚三烯酮 (tropone) 環上甲氧基 (-OCH<sub>3</sub>)有較高的光化學反應性及產物立體選擇性；化合物(5)則因甲硫基(-SCH<sub>3</sub>) 有較低的反應性及立體選擇性。

關鍵詞：秋水仙素； 環合反應； 立體選擇性

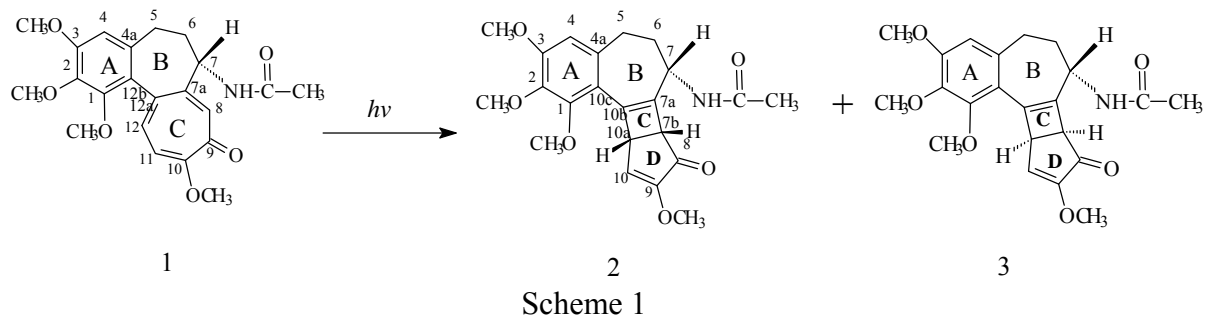
## 一、緒論

百合科植物秋水仙球莖(*colchicum autumnale*)中之環庚三烯酚酮(tropolone)衍生物，在十八世紀時就被發現有治療痛風的效果[1]。由於近年來大家生活水準提高，飲食過好，痛風病人也有逐漸增加的趨勢。它致病的原因是因為攝取過多的蛋白質，導致尿酸結晶積存於關節所形成。後來化學家們才在秋水仙的球根中找到對治療痛風病有效之成份，並將之命名為秋水仙素(colchicine; N-[(7S)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide 化合物(1))，它是一種淡黃色針狀結晶的生物鹼。秋水仙素具有抗炎性(antiinflammatories)，抑制細胞的有絲分裂(mitotic poisons)，抑制癌瘤生長的作用(inhibitors of tumor growth)，因此也作為抗癌藥使用，直到至今，不斷有學者以它作為藥理上的研究，發表它全合成的方法[2]。至於甲硫秋水仙素(thiocolchicine；N-[(7S)-1,2,3-trimethoxy-10-(methylthio)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide 化合物(5))，係化合物(1)C-10 上的氧被硫所取代，其結構如下所示。

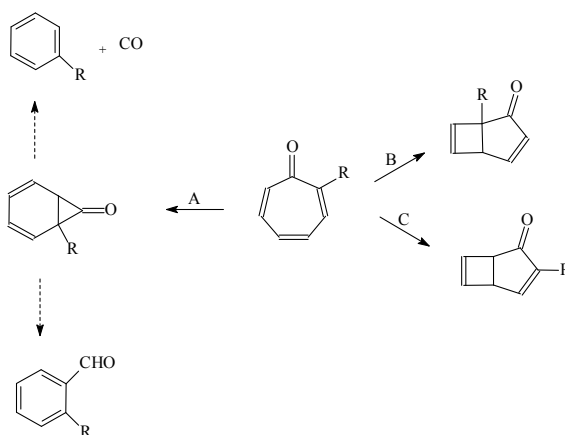


Shiau[3]曾製備了化合物(5)，並發現其抗炎性及抑制細胞有絲分裂的能力與化合物(1)幾近相同。Dustin[4]採用對細胞有絲分裂的抑制作用為基準，作了以下簡要的結論：秋水仙素(colchicine)及其衍生物要有活性需具備三個條件(1)A 環至少須有一個甲氧基(methoxy group)；(2)C 環必須為七環；(3)C 環上的甲氧基可為其他的基團所取代。然而，其結構上環庚三烯酚酮(tropolone)的構造很容易因照光而進行環合反應，使得藥物耐光性較差而容易變質。Cohen[5]探討秋水仙素暴露於陽光下易進行光化學反應，為一光敏感性極高的物質，Grewel[6]利用部分結晶的方法，分離出三種光照產物，分別為  $\beta$ -lumicolchicine(2)、 $\gamma$ -lumicolchicine(3)及  $\alpha$ -lumicolchicine(4)。Forbes、Gardner 及 Chapman [7-9]認為秋水仙素(colchicine；1)經照光後產生 C-D 環之順、反異構物： $\beta$ -lumicolchicine(2)、 $\gamma$ -lumicolchicine(3)(如 Scheme 1)，其最主要原因乃在於化合物(1)C 環上為一環庚三烯酚酮(tropolone)的構造，光照下易進行環合

反應所致。

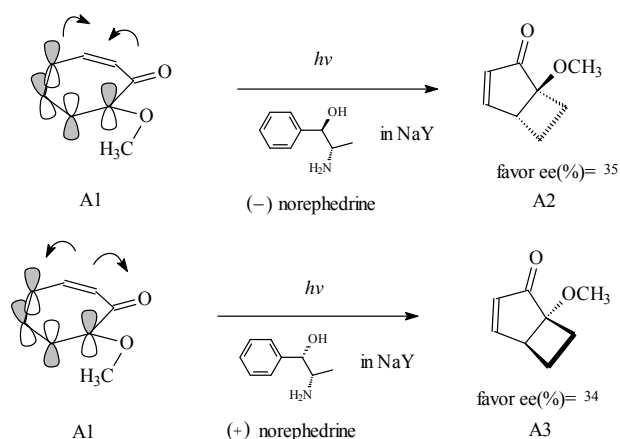


環庚三烯酮(tropone)在光化學與光物理為一重要的發色團(chromophore)，有關“tropone photochemistry”領域的研究很多，Chapman[10]在環庚三烯酮(tropone)上第 2 個碳上有一取代基之化合物經照光後，推測反應情形可能之途徑，如 Scheme 2 所示。



Scheme 2

Joy [11]於其 tropolone methyl ether(A1)在沸石(zeolite)光照反應所發表論文中，可以得知 Woodward-Hoffmann 的反向旋轉電環反應容易受立體選擇性影響，而反應的中間體或產物是否與溶劑或另一溶質形成氫鍵，往往影響產物的立體選擇性，Joy 就是因 Woodward-Hoffmann 反應有較大立體選擇性，利用 zeolite 吸附及加入 norephedrine (chiral inductor)形成氫鍵的特性，來增加其化合物(A2)或(A3)對掌過量的值，如 Scheme 3 所示。

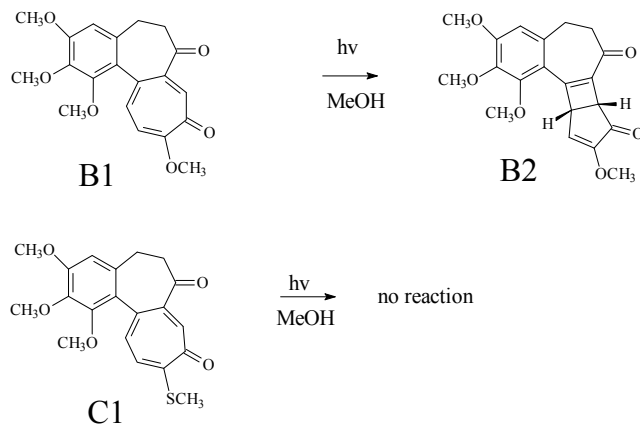


Scheme 3

## 二、研究動機

不論是秋水仙素(1)或者是甲硫秋水仙素(5)，其結構上的差別乃在於環庚三烯酮(tropone) C-10 位置上的取代基的不同；化合物(1)為  $\text{OCH}_3$ ，化合物(5)為  $\text{SCH}_3$ ，它們有一共同特點即光照反應之後，其 tropone 環會進行“七環→四環并五環”的環合反應，秋水仙素(1)照光得到  $\beta$ -lumicolchicine(2)、 $\gamma$ -lumicolchicine(3)，將  $\beta$ -lumicolchicine(2)繼續照光，可以得到  $\alpha$ -lumicolchicine(4)。甲硫秋水仙素(5)照光得到  $\beta$ -lumithiocolchicine(6)、 $\gamma$ -lumithiocolchicine(7)。

Bussotti[12]在其秋水仙素衍生物 colchicone(B1)、甲硫秋水仙素衍生物 thiocolchicone(C1)光化學行為之研究中，發現化合物(B1)於甲醇中照光可以反應產生化合物(B2)，而化合物(C1)卻不能反應，表示其兩者之間存在著光化學性質的差異。Bussotti 用 Woodward-Hoffmann 的電環反應來解釋化合物(B1)的化學反應機制，卻無法用同樣機制解釋化合物(C1)，並且 Bussotti 認為化合物(C1)於甲醇中照光無法反應 (如 Scheme 4)。



Scheme 4

Nery[13]曾探討化合物(1)在不同溶劑極性對此光照產物“ $\beta$ -form 化合物(2)： $\gamma$ -form 化合物(3)”比率的影響，並探討光轉換機制是否牽涉到叁態能階能量轉移，“ $\beta$ -form/ $\gamma$ -form”比值大小與溶劑是否形成氫鍵有關。因此，它們各別的光化學及光物理性質為如何？SCH<sub>3</sub>在化合物(5)tropone 上光量子效應會與化合物(1)的OCH<sub>3</sub>在 tropone 上光量子效應會有所不同嗎？tropone 在秋水仙素與甲硫秋水仙素化學反應機制所扮演的角色為何？因此希望能從光量子效應的觀點來整理出甲硫秋水仙素化學反應機制是否與秋水仙素相同亦或另有一截然不同的化學反應機制？利用高效能液相層析儀(HPLC)進行化學動力學的分析，以化合物(1)或化合物(5)在不同極性溶劑光量子效應的比較，嘗試瞭解其光化學反應機制，以期對 tropone 光化學研究有進一步之瞭解。

### 三、實驗

#### (一) 化合物(2)~(4)的合成

秋水仙素(colchicine)(1)可以購買取得(SIGMA)，以乙酸乙酯(ethyl acetate)再結晶，並經真空昇華，可得光譜級純度。取 0.5 克化合物(1)溶於 600 mL 甲醇，置於照光玻璃儀器內，以 Hanovia 450W 中壓汞燈內照光反應進行，溶液由淡黃漸成棕色，30 分鐘後，收集溶液濃縮後以 TLC 片分離(乙酸乙酯當沖提液)，刮取 Rf=0.69 及 Rf=0.49 處之矽膠，萃取濃縮後可得淡黃色晶體(化合物(2), Rf=0.69)約 0.325 克(產率 65%)及乳白色晶體(化合物(3), Rf=0.49)約 0.033 克(產率 7%)，分別將化合物(1)、(2)、(3)以乙酸乙酯再結晶，經真空昇華，反覆數次後即可得光譜級之純度，以核磁共振光譜儀(NMR)、紅外線光譜儀(IR)、質譜儀(MS)光譜鑑定之。將 0.2 克的化合物(2)溶於 5 mL 甲醇於石英試管中，以 450W 中壓汞燈照射約 2 小時，可得白色沉澱即化合物(4)，約 0.020 克(產率 10%)，過濾再結晶之，同上述純化步驟後，以 NMR、IR、MS 光譜鑑定之，各光譜數據如下：

##### 1. 化合物(1)

<sup>1</sup>H-NMR: 7.80(s, 1H, N-H), 7.66(s, 1H, H-8), 7.39(d, 1H, J=12Hz, H-11), 6.92(d, 1H, J=12Hz, H-12), 6.53(s, 1H, H-4), 4.67(m, 1H, H-7), 4.01(s, 3H, OCH<sub>3</sub> of C-2), 3.93(s, 3H, OCH<sub>3</sub> of C-3), 3.91(s, 3H, OCH<sub>3</sub> of C-1), 3.60(s, 3H, OCH<sub>3</sub> of C-10), 2.50(m, 4H, H-5 and H-6), 1.98(s, 3H, CH<sub>3</sub>); IR(cm<sup>-1</sup>): 3360, 2923, 2852, 1959, 1633, 1558, 1467, 1253, 1137, 1094, 1019; MS m/z: 399

[M]。

2. 化合物(2)

$^1\text{H-NMR}$  : 6.66(d, 1H, J=8Hz, H-7b), 6.47(s, 1H, H-4), 6.02(d, 1H, J=8Hz, H-10a), 4.80(s, 1H, H-7), 3.95(s, 3H, OCH<sub>3</sub> of C-9), 3.85(s, 3H, OCH<sub>3</sub> of C-2), 3.84(s, 3H, OCH<sub>3</sub> of C-3), 3.69(s, 3H, OCH<sub>3</sub> of C-1), 3.61(d, 1H, H of C-10), 2.70(m, 4H, H-5 and H-6), 2.00(s, 3H, CH<sub>3</sub>); IR(cm<sup>-1</sup>): 3359, 2923, 2852, 1959, 1710, 1659, 1467, 1134; MS m/z 399 [M]。

3. 化合物(3)

$^1\text{H-NMR}$  : 6.66(d, 1H, J=8Hz, H-7b), 6.47(s, 1H, H-4), 6.02(d, 1H, J=8Hz, H-10a), 4.68(s, 1H, H-7), 4.12(s, 3H, OCH<sub>3</sub> of C-2), 3.92(s, 3H, OCH<sub>3</sub> of C-3), 3.89(s, 3H, OCH<sub>3</sub> of C-1), 3.72(s, 3H, OCH<sub>3</sub> of C-9), 3.48(d, 1H, H of C-10), 2.70(m, 4H, H-5 and H-6), 2.00(s, 3H, CH<sub>3</sub>); IR(cm<sup>-1</sup>): 3311, 2921, 2850, 1703, 1632, 1606, 1450, 1401, 1127, 1037, 999, 983; MS m/z 399 [M]。

4. 化合物(4)

$^1\text{H-NMR}$  : 6.66(d, 2H, J=8Hz, H-7b), 6.47(s, 2H, H-4), 6.02(d, 2H, J=8Hz, H-8c), 4.68(s, 2H, H-7), 3.85(s, 6H, OCH<sub>3</sub> of C-2), 3.84(s, 6H, OCH<sub>3</sub> of C-3), 3.69(s, 6H, OCH<sub>3</sub> of C-1), 2.7(m, 8H, H-5 and H-6), 3.95(s, 6H, OCH<sub>3</sub> of C-8a), 2.00(s, 6H, CH<sub>3</sub>), 1.25(d, 2H, H of C-8b); IR(cm<sup>-1</sup>): 3358, 2921, 2850, 1734, 1633, 1369, 1316, 1129; MS m/z 798。

(二) 化合物(5)的合成

取化合物(1)1克以 15 mL 甲醇在溫水浴中溶解，待其冷卻至室溫後，緩緩加入 15% 甲基硫醇鈉(CH<sub>3</sub>SNa)水溶液 6 mL，於室溫下攪拌 6 小時，反應混合液於 40°C 減壓濃縮至約 15mL，濃縮液以氯仿萃取(25 mL×5)，氯仿萃取液經水洗、乾燥(無水硫酸鎂)及過濾後，濾液於 45°C 減壓蒸乾，殘餘物(residue)經製備型管柱分離(沖提液「乙醇：乙酸乙酯=1：4」)，即可得約 0.6 克的甲硫秋水仙素(thiocolchicine)(5)產物(產率 60%)，以乙酸乙酯再結晶，真空昇華反覆數次後即可得光譜級之純度，以 NMR、IR、MS 光譜鑑定之，其光譜數據如下：

1. 化合物(5)

$^1\text{H-NMR}$  : 7.41(s, 1H, H-8), 7.33(d, 1H, J=12Hz, H-11), 7.1(d, 1H, J=4Hz, H-12), 6.53(s, 1H, H-4), 4.67(m, 1H, H-7), 3.94(s, 3H, OCH<sub>3</sub> of C-2), 3.90(s, 3H,



OCH<sub>3</sub> of C-3), 3.66(s, 3H, OCH<sub>3</sub> of C-1), 2.50(m, 4H, H-5 and H-6), 2.40(s, 3H, SCH<sub>3</sub> of C-10), 1.99(s, 3H, CH<sub>3</sub>); IR(cm<sup>-1</sup>): 3359, 2923, 2852, 1659, 1632, 1540, 1467, 1195, 1136, 1023, 786; MS m/z 415 [M]。

### (三) 化合物(6)~(7)的合成

取 0.5 克化合物(5)溶於 600 mL 甲醇，置於照光玻璃儀器內，以 Hanovia 450W 中壓汞燈內照光反應進行，溶液由淡黃漸成棕色，8 小時後，每隔 2 小時以高效能液相層析儀追蹤，當化合物(5)減為原來 1/4 量以下即可停止照光，收集溶液濃縮後以 TLC 片分離(乙酸乙酯當沖提液)，刮取 R<sub>f</sub>=0.55 矽膠，萃取濃縮後可得淡黃色固體(化合物(6)、化合物(7))之混合物。使用製備型 HPLC 管柱，沖提液「乙醇：乙酸乙酯=1：5」，可得化合物(6)約 0.050 克(產率 10%)，化合物(7)約 0.017 克(產率 3.4%)，以乙酸乙酯再結晶，真空昇華，反覆數次後即可得光譜級之純度，以 NMR、IR、MS 光譜鑑定之，各光譜數據如下：

#### 1. 化合物(6)

<sup>1</sup>H-NMR : 7.87(d, 1H, J=8Hz, H-7b), 7.48(d, 1H, J=8Hz, H-10a), 6.34(s, 1H, H-4), 4.62(s, 1H, H-7), 3.90(s, 3H, OCH<sub>3</sub> of C-2), 3.70(s, 3H, OCH<sub>3</sub> of C-3), 3.63(s, 3H, OCH<sub>3</sub> of C-1), 3.61(d, 1H, H of C-10), 2.91(s, 3H, SCH<sub>3</sub> of C-9), 2.50(m, 4H, H-5 and H-6), 2.00(s, 3H, CH<sub>3</sub>); IR(cm<sup>-1</sup>): 3360, 3004, 2924, 2852, 1659, 1632, 1551, 1467, 1410, 1135, 1094, 786, 763; MS m/z 415 [M]。

#### 2. 化合物(7)

<sup>1</sup>H-NMR : 7.95(d, 1H, J=8Hz, H-7b), 7.51(d, 1H, J=8Hz, H-10a), 6.53(s, 1H, H-4), 4.60(s, 1H, H-7), 3.97(s, 3H, OCH<sub>3</sub> of C-2), 3.94(s, 3H, OCH<sub>3</sub> of C-3), 3.73(d, 1H, H of C-10), 3.65(s, 3H, OCH<sub>3</sub> of C-1), 2.91(s, 3H, SCH<sub>3</sub> of C-9), 2.50(m, 4H, H-5 and H-6), 2.0(s, 3H, CH<sub>3</sub>); IR(cm<sup>-1</sup>): 3359, 2922, 2851, 1659, 1632, 1468, 1410, 1135, 701; MS m/z 415 [M]。

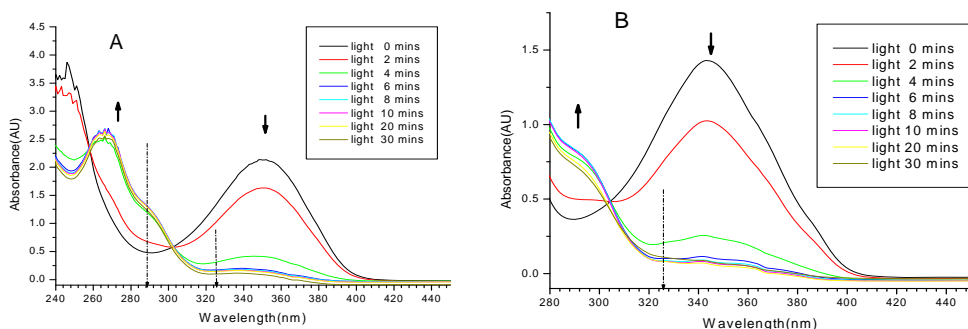
### (四) 照光反應動力學

將化合物(1)與化合物(5)濃度各配成約  $1 \times 10^{-4}$ M 的甲醇與苯溶液，固定體積(6 mL)至每一支 Pyrex 試管，每隔固定照光時間，分別於可見-紫外光光譜儀偵測其吸收光譜，及以高效能液相層析儀 HPLC(254nm)追蹤產物變化，如此可以得知光化學反應物與產物彼此之間互相消長的情形。

## 三、結果與討論

## (一) 秋水仙素(colchicine)系列化合物

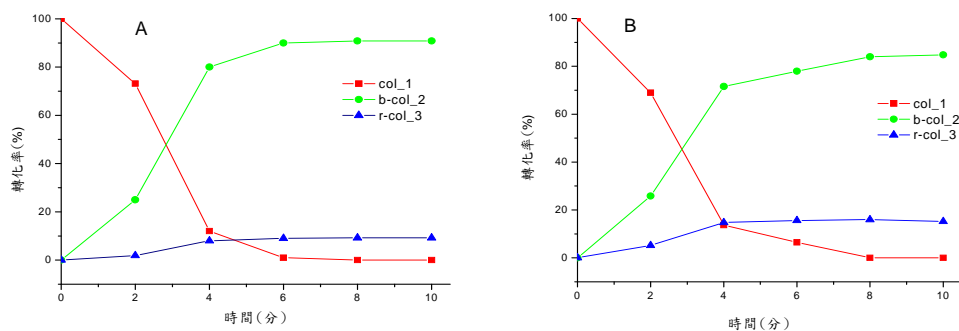
化合物(1)的光照反應，本論文選定二種不同極性溶劑，探討溶劑極性對化合物(1)在照光過程中化學反應的情形。以不同溶劑分別標示清楚，每隔固定照光時間分別拿掉甲醇與苯溶液 Pyrex 試管，最後再將每一支試管分別於可見-紫外光光譜儀偵測其吸收光譜，如圖一所示。



圖一、 $10^{-4}$ M 化合物(1)隨著照光時間 UV-Vis 吸收值變化的情形(A)甲醇、(B)苯

由光譜圖可發現，不管在極性或非極性溶劑中，化合物(1)的光化學反應皆相當迅速；在照光 10 分鐘內即很有效率的反應完成(大於 95% 的轉換率)，以 HPLC 追蹤其產物則幾乎完全形成化合物(2)及化合物(3)產物，顯現秋水仙素在照光下極容易進行環合反應，生成  $\beta$ -lumicolchicine(2)及  $\gamma$ -lumicolchicine(3)。

由反應物及生成物消長比較(如圖二所示)，化合物(1)的光照產物，主產物為化合物(2)並伴隨少量的化合物(3)，在甲醇溶劑中，化合物(3)產率約 9%，在苯溶劑中，化合物(3)產率有相對提高至約 15%，使得化合物(2)對化合物(3)的比值( $\beta/\gamma$ )，在此 2 種溶劑中有顯著差異，其差異約 2 倍如表一所示，顯現不管在極性或非極性溶劑中， $\beta$ -form 之化合物(2)仍然為主要產物，而在非極性溶劑中， $\gamma$ -form 之化合物(3)產率增加，導致  $\beta/\gamma$  比值顯著降低。



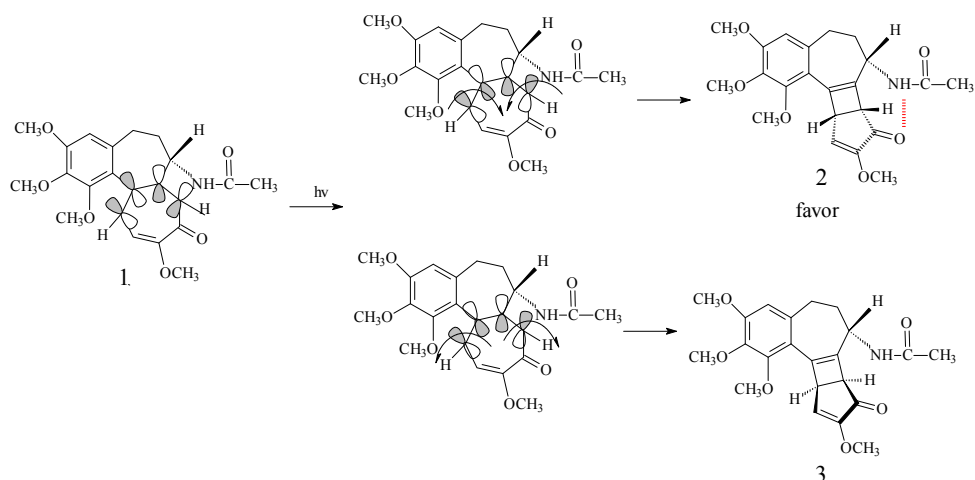
圖二、 $10^{-4}$ M 化合物(1)照光 10 分鐘的反應物與產物消長情形：(A)甲醇、(B)苯

由反應物及生成物消長比較(如圖二所示)，化合物(1)的光照產物，主產物為化合物(2)並伴隨少量的化合物(3)，在甲醇溶劑中，化合物(3)產率約 9%，在苯溶劑中，化合物(3)產率有相對提高至約 15%，使得化合物(2)對化合物(3)的比值( $\beta/\gamma$ )，在此 2 種溶劑中有顯著差異，其差異約 2 倍如表一所示，顯現不管在極性或非極性溶劑中， $\beta$ -form 之化合物(2)仍然為主要產物，而在非極性溶劑中， $\gamma$ -form 之化合物(3)產率增加，導致  $\beta/\gamma$  比值顯著降低。

表一：化合物(2)/化合物(3)隨著照光時間間隔所顯示的比值( $\beta/\gamma$ )

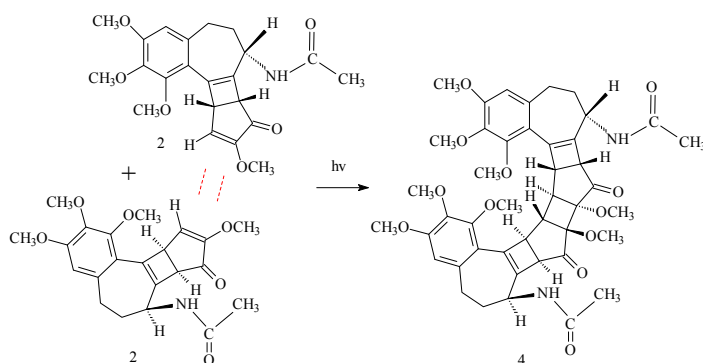
照光時間(分)	2	4	6	8	10
$\beta/\gamma$ (甲醇)	13.1	9.9	10.0	9.9	9.8
$\beta/\gamma$ (苯)	5.0	4.8	5.0	5.2	5.6

推測化合物(1)的光照化學反應，是因為化合物(1)上 tropone 環之兩對  $\pi$  電子進行 Woodward-Hoffmann 的反向旋轉電環反應(disrotatory electrocyclic ring closure reaction)，可以解釋最後的光照產物只有兩種異構化的型式，其反應機制為類似 1,3-丁二烯之光化學環化反應，為以反向旋轉運行模式導致鍵結的協同反應(concerted reaction)，其中  $\pi$  電子往環內旋轉鍵結形成化合物(2)，往環外旋轉鍵結則形成化合物(3)，如 Scheme 5 所示。因為所形成之  $\beta$ -isomer 會有分子內氫鍵(紅色虛線)，有利於化合物(2)形成，此乃因為  $\pi$  電子往環內旋轉鍵結使第 7b、10a 位置上的氫同時朝上，導致第 7b、10a 位置上的氫與第 7 位置氮上的氫在 C 環同一側，第 7 位置上的氮上的氫與 D 環會在 C 環的另一側，造成 D 環羰基上的氧與氮上的氫有分子內氫鍵形成，產生較安定的化合物(2)，而化合物(3)無分子內氫鍵而產率相對比較少，造成如此立體上明顯有選擇性差異的結果。



Scheme 5

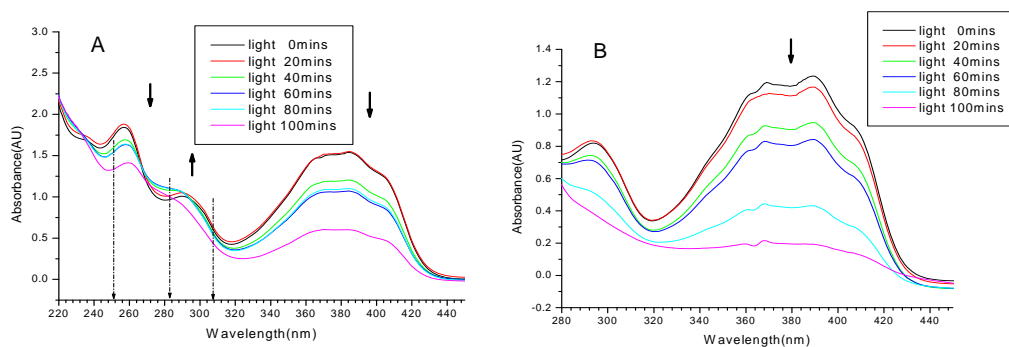
化合物(4)的合成是由化合物(2)進行光照化學反應得到，由化合物(4)的結構判斷，其反應機制為化合物(2)上之 D 環上進行分子間〔2+2〕環化加成(cycloaddition)之頭對頭二聚物(head-to-head dimer)，如 Scheme 6 所示。



Scheme 6

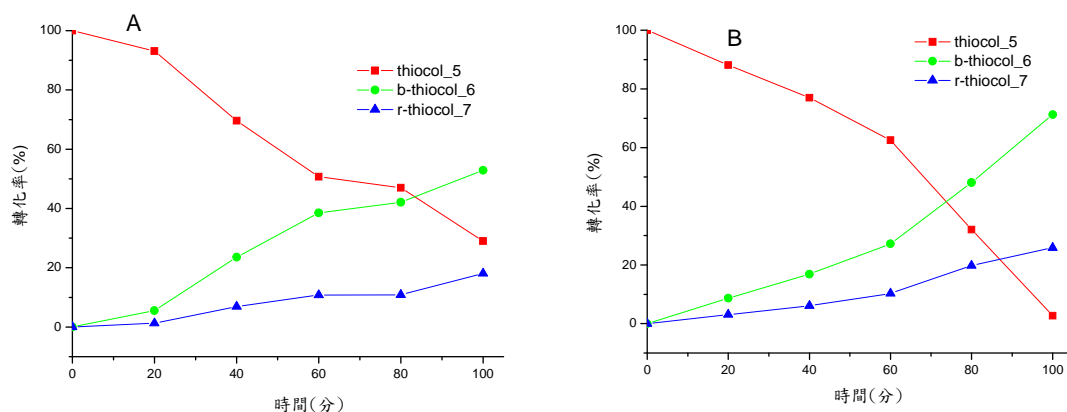
## (二) 甲硫秋水仙素(thiocolchicine)系列化合物

化合物(5)的光照反應之可見-紫外光光譜圖，如圖三所示。



圖三、 $10^{-4}M$  化合物(5)隨著照光時間 UV-Vis 吸收值變化的情形(A)甲醇、(B)苯

由光譜圖可發現，不管在極性或非極性溶劑中，化合物(5)的光化學反應速率相對於化合物(1)緩慢；在苯溶劑中照光 100 分鐘才反應完成(大於 95% 的轉換率)，以 HPLC 追蹤其產物則幾乎完全形成化合物(6)及化合物(7)產物。由於甲硫秋水仙素比秋水仙素在照光下不容易進行環合反應，顯示含甲硫基結構之甲硫秋水仙素相對於含甲氧基結構之秋水仙素具有較高耐光穩定性；此外將化合物(6)進行光照化學反應 20 小時，亦未得到相對應的  $\alpha$ -lumithiocolchicine，有可能是因為反應的量太少以及反應的時間不足，造成  $\alpha$ -lumithiocolchicine 的生成無法被偵測。而化合物(2)進行光照化學反應 2 小時即可得到  $\alpha$ -lumicolchicine。



圖四、 $10^{-4}M$  化合物(5)照光 100 分鐘的反應物與產物消長情形：(A)甲醇、(B)苯

由反應物及生成物消長比較(如圖四所示)，化合物(5)的光照產物，主產物為化合物(6)及次要產物化合物(7)，在甲醇溶劑中，化合物(6)產率約 52%，化合物(7)產率約 18%。在苯溶劑中，化合物(6)產率約 71%，化合物(7)產率約 26%，使得化合物(6)對化合物(7)的比值( $\beta/\gamma$ )，在此 2 種溶劑中並未有顯著差異，如表二所示，顯現不管在極性或非極性溶劑中， $\beta/\gamma$  比值並未如化合物(1)的光照產物比值變動如此明顯。

表二：化合物(6)/化合物(7)隨著照光時間間隔所顯示的比值( $\beta/\gamma$ )

照光時間(分)	20	40	60	80	100
$\beta/\gamma$ (甲醇)	4.3	3.4	3.6	3.9	2.9
$\beta/\gamma$ (苯)	2.8	2.8	2.6	2.4	2.8

化合物(1)經照光後產生  $\beta$ -form 化合物(2)、 $\gamma$ -form 化合物(3)，及化合物(5)經照光後產生  $\beta$ -form 化合物(6)、 $\gamma$ -form 化合物(7)，從結構來看，主要是發生在 tropone 環由七環變成四環并五環，唯一的差別是在化合物(1)tropone 環上有一甲氧基( $OCH_3$ )取代基，而在化合物(5)上則為甲硫基( $SCH_3$ )，而這也影響了溶劑對化合物(1)與化合物(5)之  $\beta/\gamma$  比值，及造成光化學反應速率的差異。

綜合秋水仙素(1)與甲硫秋水仙素(5)分別在甲醇與苯中照光的動力學分析中可以歸納兩點觀察的結果：(一)兩者光化學反應速率不同，不管是在甲醇或苯中照光，化合物(1)皆大於化合物(5)，相差約 10 倍。(二)化合物(1)與化合物(5)之  $\beta/\gamma$  在不同溶劑(MeOH、Benzene)有著截然不同的表現，化合物(1)之  $\beta/\gamma$  比值大於化合物(5)之  $\beta/\gamma$  比值，顯現化合物(1)的光照產物有較高之立體選擇性。

#### 四、結論

分析秋水仙素(1)光化學反應在甲醇(極性溶劑)與苯(非極性溶劑)中各別的  $\beta/\gamma$  值，分別為  $\beta/\gamma(\text{甲醇})=9.8$ ， $\beta/\gamma(\text{苯})=5.6$ ； $\beta/\gamma(\text{甲醇})$ 約為  $\beta/\gamma(\text{苯})$ 的兩倍，這可由  $\beta$ -form 有分子內氫鍵，在極性溶劑中更為穩定而更有利生成，由化合物(2)繼續照光可得化合物(4)，證實是由  $\beta$ -form 頭對頭組合的二聚物。甲硫秋水仙素(5)於照光時， $\beta/\gamma$  遠比秋水仙素(1)低，受溶劑極性的影響也是發生相同情形[ $\beta/\gamma(\text{甲醇})=2.9$ ； $\beta/\gamma(\text{苯})=2.8$ ]，判斷甲硫秋水仙素(5)亦進行 Woodward-Hoffmann 環合反應機制，而動力學控制產物比熱力學控制來得有利。化合物(6)繼續照光 20 小時亦無法形成二聚物，可能是因為甲硫基的低反應性及較大的立體阻礙所造成。

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# The study of the photochemical reaction of colchicine and thiocolchicine

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## Abstract

The photochemical reactions of colchicine and thiocolchicine were studied in this thesis. The difference of structure between colchicine(1) and thiocolchicine(5) is that hydroxyl functional group(-OH) on the tropolone ring is substituted, one is methoxyl functional group(-OCH<sub>3</sub>), and the other is methylthio functional group(-SCH<sub>3</sub>). Because of their similar structure make the physical and chemical properties similarly, and both they are effective treatment for gout drugs. However, the poor resistance to UV light of the tropone which is easily photocyclized by irradiation makes them easy to perish. Irradiation of colchicine(1) gives mainly two photoproducts:  $\beta$ -lumicolchicine(2) and  $\gamma$ -lumicolchicine(3). Further irradiation of  $\beta$ -lumicolchicine(2) gives the photoproduct of  $\alpha$ -lumicolchicine(4). Irradiation of thiocolchicine(5) gives  $\beta$ -lumithiocolchicine(6) and  $\gamma$ -lumithiocolchicine(7). The chemical kinetic of colchicine(1) and thiocolchicine(5) is studied and the ratios ( $\beta/\gamma$ ) of photoproducts is analyzed under different polar solvents and irradiative times. The results show that both colchicine(1) and thiocolchicine(5) favor the Woodward-Hoffmann electrocyclic reaction. Colchicine(1) has higher photochemical reactivity and higher product stereoselectivity with the methoxyl functional group(-OCH<sub>3</sub>) of tropone. Thiocolchicine(5) with the methylthio functional group(-SCH<sub>3</sub>) of tropone has lower photochemical reactivity and lower product stereoselectivity.

Key Words: colchicine, cyclization, stereoselectivity



# 證 明 書

茲證明孫崇文先生、陳順基老師、方泰山老師合著並投稿於本校《慈濟技術學院學報》第二十一期之論文—〈秋水仙素與甲硫秋水仙素光化學反應的研究〉，確實經本組以匿名方式送審外審委員審核，並經外審委員以「推薦刊登」及「修正後推薦刊登」審核通過，爰將於2013年8月刊登於上開期別。今證為屬實，特立此書。

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