CHEMILUMINESCENCE of INDOLYL PEROXIDES

by

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ABSTRACT

Indolyl hydroperoxides have been discovered to be chemiluminescent in presence of potassium t-butoxide in dimethyl sulphoxide. A number of derivatives of indole and the corresponding peroxide have been prepared in an effort to elucidate the mechanism of chemiluminescence of this class of compound. The chemiluminescent reaction of two of the brightest hydroperoxides (XllA and XVlllA) yielded a major product, the amides (Xlla and XVllla), in about 70% yield. The emitter has been identified as the amide product based on spectroscopic data.

It was found that electron-donating substituents enhance the chemiluminescence intensity. A study of 2-(p-phenyl) substituted indolyl hydroperoxides shows a Hammett correlation with σ for 7 of the compounds.

The chemiluminescence of compounds (XllA and (XVlllA) shows a pseudo first order dependence on the concentration of (XllA and XVlllA) (in excess base).

Energy transfer has been observed to occur in the thermochemiluminescence of 11-tetrahydroperoxyindolenine (XXVA).

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ACKNOWLEDGMENT

I would like to express my sincere gratitude to Dr. Frank McCapra for suggesting this research problem and for guiding it to its completion with inspiration, encouragement and willing advice.

INTRODUCTION

The phenomenon of chemiluminescence has recently become the subject of increased study.[1] One of the first examples of chemiluminescence was reported in 1877 by Radziszewski[2] who observed light emission from the reaction of lophine (2, 4, 5-triphenylimidazole) with strong base and oxygen. Chemiluminescence can also be conveniently demonstrated by adding 30% hydrogen peroxide to a mixture of equal quantities of 0.1M pyrogallol, 1M sodium carbonate and 10M formaldehyde solution. A faint rosy glow is visible in complete darkness.[3] Since the first discovery of chemiluminescence of lophine, brighter compounds have been extensively studied and the work reviewed.[1,4,5,6]

Chemiluminescence is not confined to reactions of organic compounds. Recent reports have shown that chemiluminescence occurs during the formation of $BrCl$[7], and the reaction of SO and oxygen.[8] In addition, chemiluminescence was observed even in Grignard reagents[9] as early as 1907. Reactions in living matter sometimes emit light, a phenomenon known as bioluminescence. This attractive glow is found in luminous bacteria, glow-worms, fire-flies and some fungi.[10,11]

In the chemiluminescence of liquid solutions, the conversion of chemical energy into light energy involves the formation of a reactant, intermediate, or product molecule in an excited electronic state (directly or by energy transfer)

and the emission of a photon by that species. Since excited states are also reached by the absorption of light, it should be possible to correlate the wave length distribution in chemiluminescence with that in phosphorescence, if a triplet state were the emitter, or in fluorescence, if a singlet state were involved.

The study of chemiluminescence of organic compounds in solution is hampered by the lack of a sufficient number of reactions which give a bright emission of light. This problem has been slightly alleviated by the advances in the construction of sensitive equipment[12,13] which would easily detect even weak chemiluminescence. However, the problem of quenching (absorption of the emitted light by colored reaction media) will always remain. Despite these difficulties, some order in the study of chemiluminescence of organic compounds in solution has been established. At the present all organic solution chemiluminescence may be distinquished by three principle types of reaction which produce enough energy (40-80 kcal/mole) to allow emission[14] of visible light. These are: electron-recombination, ion-recombination, and multiple-bond rearrangements with large energy release.
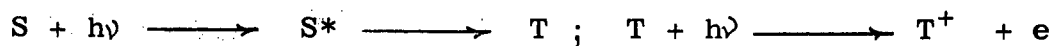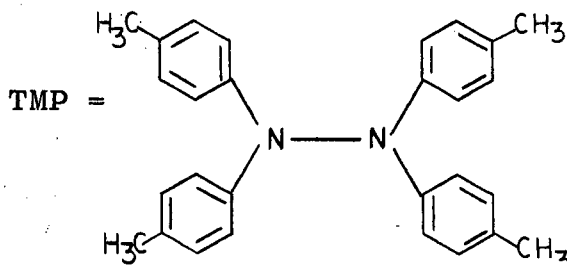
Electron-Recombination.

Such a process is believed to occur when organic amines (such as N, N, N', N'-tetramethylparaphenylenediamine) (TMP) in low-temperature glass are strongly illuminated giving an afterglow. Electrons are liberated by the light (UV), and

become trapped in the glass medium, from which they may return

slowly to the resulting amine cations. The emission is chiefly

phosphorescence and the excitation is a 2-photon process[15]

(Fig. 1):   Fig. 1

TMP =

$$S + h\nu \longrightarrow S^* \longrightarrow T \; ; \; T + h\nu \longrightarrow T^+ + e$$

Possibly the ionized molecule is frozen in its triplet-state

atomic configuration so that the return of the electron reforms

state T rather than S* (* = excited state, S= singlet, T=triplet).

Ion-Recombination.

Chemiluminescence as a result of ion-recombination is

common in ionised aromatic hydrocarbons. Hercules[16] has

discussed possible mechanisms for the chemiluminescence of

electrogenerated species based on qualitative electrochemical

experiments with a platinum electrode in deoxygenated non-

aqueous solvents. Electrochemically generated aromatic

hydrocarbon anions (such as that formed from anthracene) emit

the hydrocarbon fluorescence when oxidized by anodic products.

The lowest excited singlet state of aromatic hydrocarbon has

been suggested as the emitting species. Chandross and

Sonntag[17] have recently shown that chemiluminescence occurs
when 9, 10-diphenylanthracene anion radical (DPA⁻) is
treated with the 9, 10-dichloride of DPA or a variety of
oxidizing agents. They proposed that oxidation of DPA⁻
produces DPA in an excited state (DPA*) which emits light in
returning to the ground state. These results suggest two
possible mechanisms for the chemiluminescent reaction occurring
during electrolysis of aromatic hydrocarbons. The radical
anion R⁻ formed at the cathode is oxidized directly to an
excited state R* at the anode surface, or an oxidant is
produced at the anode which reacts with R⁻ forming R*. Further
studies,[18,19] in fact, have narrowed _down_ to the mechanism which
involves the formation of an oxidant at the anode. Oxidizing
agents such as bromine and chlorine were tested and shown to
be directly involved in the chemiluminescent reaction of DPA[18].
The chemiluminescent reaction of DPA⁻ with DPA dichloride
prompted Sonntag and co-worker[19,20,21] to postulate the
following mechanism to be the most reasonable one (Fig. 2).

Fig. 2.

$$DPA^- + DPACl_2 \longrightarrow DPA + Cl^- + DPACl^{\cdot} =$$

$$DPACl^{\cdot} + DPA^- \longrightarrow DPA + DPA^* \text{ (excited)} + Cl^-$$
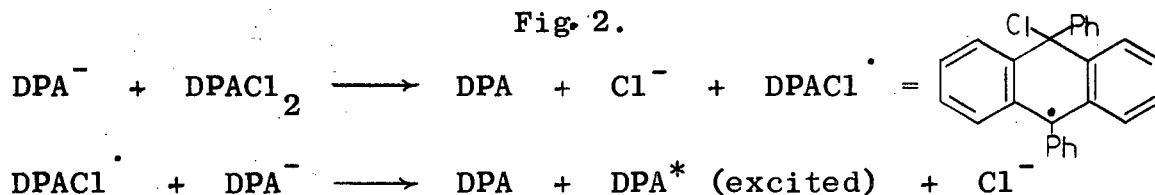
This is probably the simplest chemiluminescent reaction
known. The formation of electronically excited molecules
involves only the transfer of an electron from the radical
anion to another radical which serves as an oxidizing agent

in a process related to recombination chemiluminescence.

Multiple-Bond Rearrangement with Large Energy Release.

These are mostly base-catalysed autoxidations. The best known is the oxidation of luminol (I) (3-amino-phthalhydrazide) with peroxide and sodium hypochlorite in weak alkaline aqueous solution or by oxygen in dimethyl sulphoxide and sodium hydroxide.

The study of the chemiluminescence of luminol[22] is complete in many respects; the emitter has been identified, the order of the chemiluminescent reaction determined and the quantum yield measured. Furthermore the substituent pattern and steric hindrance have a pronounced effect on luminol chemiluminescence.

The chemiluminescence of luminol was first reported by Albrecht.[23] However recent findings have brought more useful knowledge of the chemiluminescence.[24,25,26] The following scheme illustrates the overall course of the reaction (Eq. 1):[24]

$$I + 2NaOH \xrightarrow{O_2} N_2 + 2H_2O + h\nu + II \qquad 1.$$

(I)   (II)
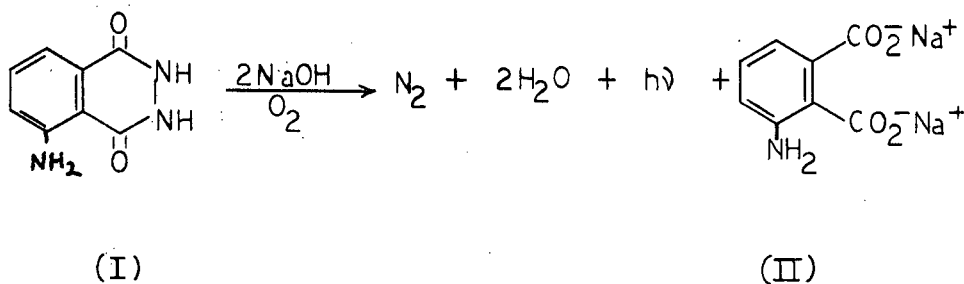
The products of the reaction are nitrogen and sodium
aminophthalate (II). The identity of the chemiluminescence
spectrum of luminol with the fluorescence spectrum of (II)
leaves little doubt as to the identity of the emitting molecule.
The maximum of both chemiluminescence and fluorescence in
water is 424 mμ and in dimethyl sulphoxide, 485 mμ. For
maximum chemiluminescence and fluorescence the presence of
dimethyl sulphoxide solvent is essential. Very high strength
of base apparently was required.

When the chemiluminescent oxidation of luminol was
carried out with O^{18} enriched oxygen gas, it was found that
over 85% of the label of isotope ended up in aminophthalate (II). This
confirms that the origin of the oxygen in (II) is oxygen gas
and not hydroxide ion or the water present in dimethyl
sulphoxide. Further study[25] showed that the emission (Eq. 1)
followed overall first order kinetics. It is interesting that
the quantum yield for the chemiluminescence of luminol in
dimethyl sulphoxide is only about 5%; but this represents one
of the highest yields yet measured for a chemiluminescent
reaction[27].

The precise mechanism of formation of an excited
state species from a ground state molecule in solution at room
temperature (Fig. 3) is unknown for multiple-bond rearrangement
type of chemiluminescent reaction. However since molecular
oxygen is a reactant and has a triplet ground state, White
et al[25] suggested that adducts of oxygen may exist as triplet

state complexes or diradicals.[28] Thus loss of nitrogen from

the adduct could lead to a vibrationally excited triplet

state of the potential emitter. Intersystem crossing to an

isoenergetic level of the excited singlet state and emission

of a photon would then complete the process (Fig. 3).

Fig. 3

paths a, b, c, and d = chemiluminescence;

paths e and d = fluorescence of emitting species.

Ground State Singlet of
Emitting Species
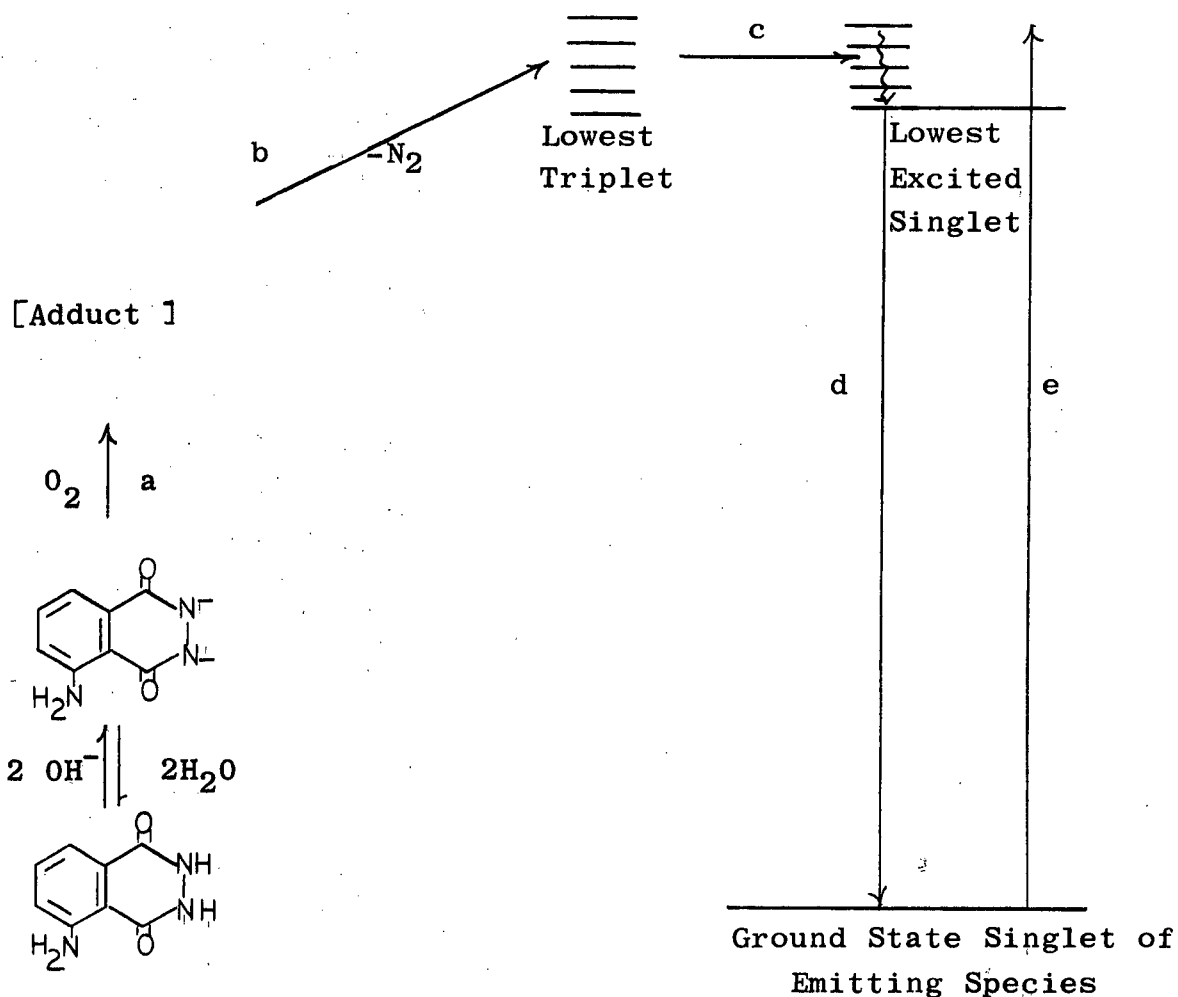
Various substituents in the benzene ring of luminol have a pronounced effect on the chemiluminescence. Study of these substituted luminols showed that the chemiluminescence was enhanced by electron-releasing substituents in the benzene nucleus of the phthalhydrazide system, and weakened by electron-attracting substituents.[29] Such effects are illustrated by the following derivatives of luminol (III) and (IV) in which the quantum yield of the chemiluminescence of (III) and (IV) was found to exceed that of luminol by 13 and 30% respectively.[24]
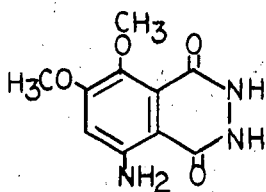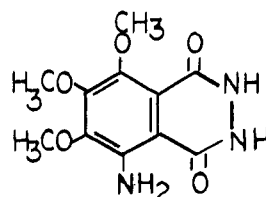
(III)   (IV)

Decreased chemiluminescence of quantum yield has been attributed to steric hindered substituents. Several examples of this effect have been found to occur.[30]

The chemiluminescence of lophines (Va) and lophine peroxides (VIa) is similar in many respects to that of luminol. Like luminol, light yields increased with electron-releasing substituents. In fact, when the relative intensities (log. I/Io, I = the intensity observed for the derivative and Io = intensity for lophine oxidation) emitted by a similar series of substituted lophines were plotted against the corresponding Hammett substituent constants, σ, a linear relationship was obtained.[31]

(V)

(VI)

(VII)

a. $R_1 = R_2 = H$

b. $R_1 = (CH_3)_2N$, $R_2 = H$

c. $R_1 = (CH_3)_2N$, $R_2 = OCH_3$
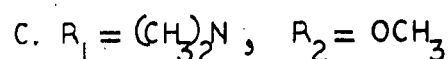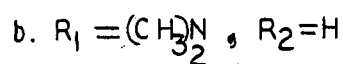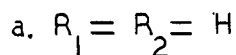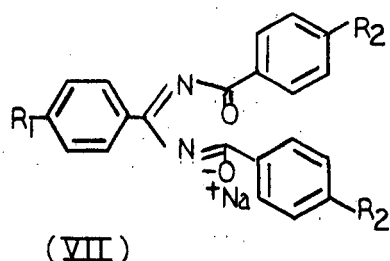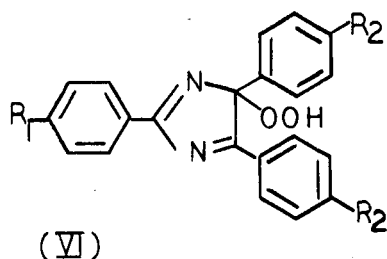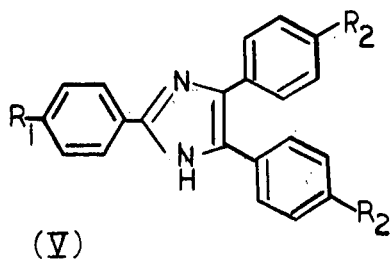
The study of the brighter chemiluminescent derivatives
of lophine (Vb,c) and lophine peroxides (VIb,c) brought much
insight into the identity of the emitter and the mechanism
of the chemical reactions.[32] In each case the reaction of
lophine (Vb,c) in potassium hydroxide solution and in presence
of oxygen, and lophine peroxides (VIb,c) on treatment with
potassium produced a long-lived emission. However the former
reaction gave degraded products, whereas the latter gave
mostly dibenzoylbenzamidine salts (VIIb,c) (ca. 80%).
The almost perfect match of the fluorescence spectra of the
dibenzoylbenzamidine salts (VIIb,c) and the chemiluminescence
spectra of the lophines (Vb,c) and lophine peroxides (VIb,c)
suggested that the excited states of (VII,b,c) were the
light emitters. White[32] proposed that a singlet excited state
would be more likely than a triplet since light emission from
lophines and the peroxides was unaffected by oxygen or by β-
carotene, both of which are efficient triplet state quenchers.

The fact that lophine peroxides (Vlb,c) reacted with base to yield light by first order kinetics, and that the integrated light yields of lophine peroxides (Vlb,c) were proportional to the amount of lophine peroxides (Vlb,c), rules out a pathway in which two lophine peroxide molecules or excited state molecules combine to emit a photon.[32] The suggested mechanism is shown (Fig. 4) as occurring via the four-membered cyclic peroxide intermediate (VllA).[6]

Fig. 4
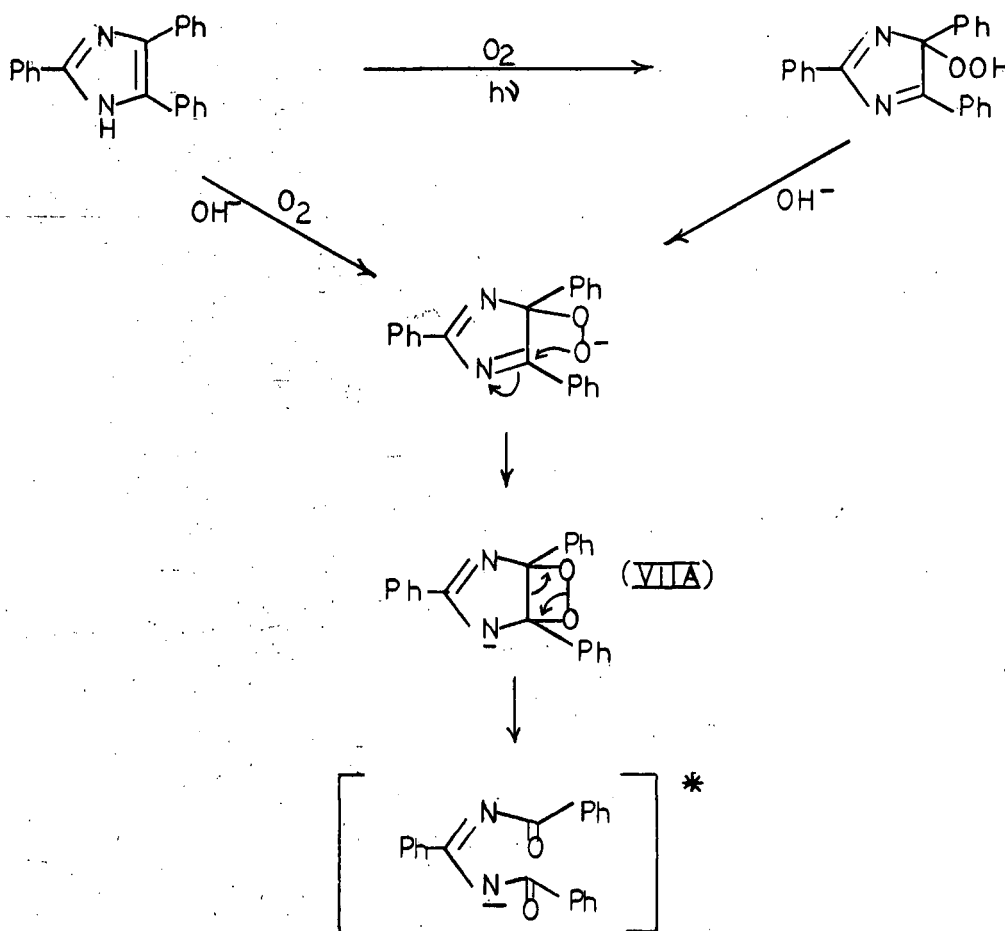
In the reactions of luminol, lophine and lucigenin it was established that in all cases a major product of oxidation is a compound with a newly formed carbonyl group. A further feature in common is the implication of a peroxide intermediate. A series of acridinium salts (Vlll), which are powerfully chemiluminescent on the addition of alkaline hydrogen peroxide have recently been discovered, based on a general theory of these chemiluminescent reactions suggested by McCapra and Richardson.[6] The sole isolated product of the reaction, N-methylacridone (VlllA), was identified as the emitter. Confirmation was based on spectroscopic measurements.[6] It is reasonable to suppose that lucigenin (1X) and these compounds (Vlll) are related in their chemiluminescent reactions, giving rise to the mechanism shown.[6]

The discovery was

(VIII)

$X = NO_3^-$, $CH_3SO_4^-$

$R = COCl$, $COPh$, CN, CO_2Ph, CO_2CH_3, $CONH_2$, $COOH$.
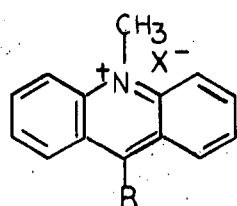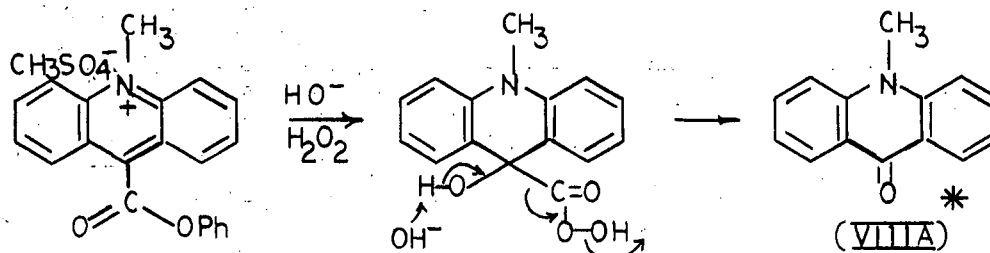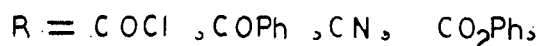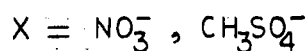
(VIIIA)
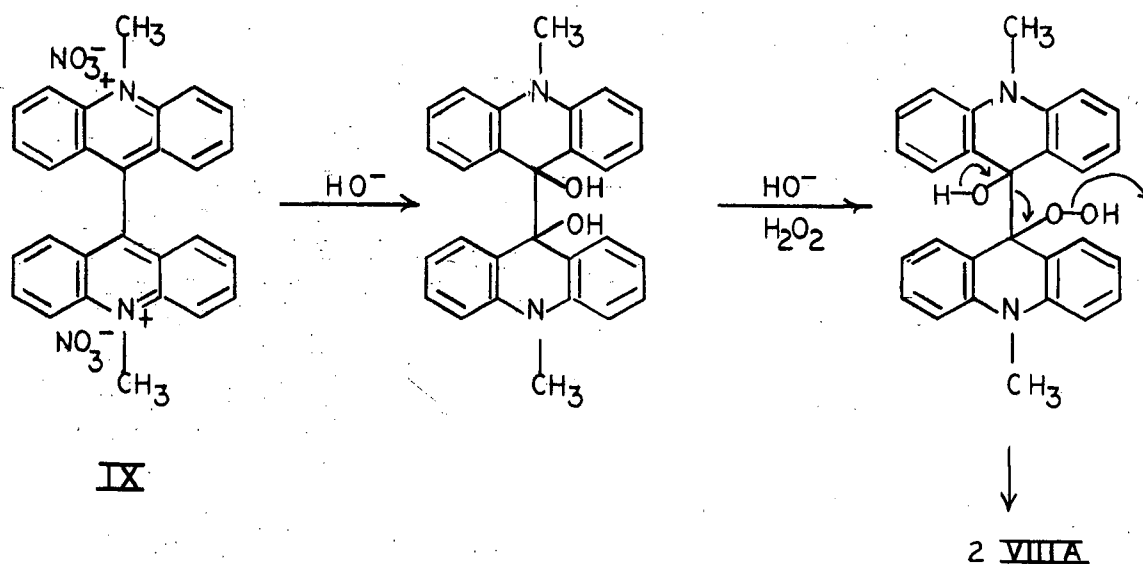
Unlike most of the well known chemiluminescent compounds such as luminol, lucigenin and lophine which apparently play no role in bioluminescence, the indole nucleus is definitely involved in such systems.[33] A careful survey of the literature failed to show any report of light emission during the reaction of indolyl hydroperoxide and base. In our laboratory a series of indole hydroperoxides similar to lophine hydroperoxide has been studied in extensive detail. A series of 20 indolyl hydroperoxides has been prepared. The possible emitter has been identified and the mechanism of the chemiluminescent reaction has been studied. It is hoped that this effort will bring about a better understanding of the mechanism involved in bioluminescent reactions.

PART I: SYNTHESIS AND DECOMPOSITION OF

INDOLYL HYDROPEROXIDES

EXPERIMENTAL

All melting points were determined using a Reichert hot stage melting point apparatus, and are uncorrected.

The microanalyses were performed by Dr. A. Bernhardt and his associates, Mikroanalytisches Laboratorium im Max-Planck-Institute fur Kohlenforschung, 433 Mulheim, West Germany, and by Mr. P. Borda, Chemistry Department, University of British Columbia.

The ultraviolet spectra were recorded on a Cary-11 or Cary-14 recording spectrophotometer in ethanol (95%) solution.

The infrared spectra were recorded on a Perkin Elmer-21 spectrophotometer calibrated with reference to the 1601 cm^{-1} peak of polystyrene.

The nuclear magnetic resonance (NMR) spectra were recorded and integrated by Mrs. A. Brewster on a Varian A-60 NMR spectrometer. Chemical shifts are given in taus (τ) downfield from tetramethyl silane. The solvent used was deuteriochloroform.

The mass spectrometric analyses were perfomed by Mr. G. D. Gunn using a MS9 and an Atlas high resolution mass spectrometer.

Thin layer chromatography (TLC) was carried out on silica gel G.

The fluorescent and chemiluminescent spectra were measured on an Aminco-Bowman spectrofluorometer.

The efficiency of the chemiluminescent reactions was measured on a type-564 storage oscilloscope, in which a voltage of 700 volts was applied to the photomultiplier tube.

The indoles (X to XVlll) were synthesized from phenylhydrazine and the corresponding ketone by the method of Witkop and co-worker.[34]

3-Methyl-2-phenylindole (X).

To a mixture of propiophenone (6.7 g, 0.05 mole), prepared from dried benzene and propionyl chloride by the method of Read,[35] and phenylhydrazine (5 ml, 0.05 mole) was added approximately 20g of polyphosphoric acid. The mixture was stirred by a mechanical stirrer for 15 minutes. The hot viscous mixture was poured into ice-water (100 ml) and a white precipitate appeared. The precipitate was filtered off and washed several times with water. Yield: 6g, (58%). This precipitate was recrystallized from petroleum ether (P.E. 60-80°) as prisms, m.p. 91 -92° (lit.[36] m.p. 93°).

(XA): Hydroperoxide of (X).

<u>All</u> the indolylhydroperoxides were prepared by dissolving the indole in sufficient hot petroleum ether (60-80°) to prevent rapid crystallization of the compound on cooling and a trace of one of the indoleninyl hydroperoxides was added to the solution. Stirring was continued until

separation of the crude crystalline hydroperoxide appeared to be complete, the product was collected and recrystallized as quickly as possible from ethyl acetate / petroleum ether.

Indole (X) (1.0 g) dissolved in petroleum ether (100ml) was stirred by a magnetic stirring bar for one day in air. The yellow precipitate which came down was recrystallized from ethyl acetate / petroleum ether (1:3), m.p. 144-145° (lit.[36a] m.p. 157°). Yield: 0.9 g, (86%). λmax 314 mμ (ϵ12,850), 245(16,000), 239(17,500), 230(15,500).

2-(p-Hydroxphenyl)-3-methylindole (X1).

p-Hydroxypropiophenone (7.5 g, 0.5 mole) and phenylhydrazine (5 ml) condensed in the usual way afforded 5.5 g, (50%) yellow solid. It was recrystallized from petroleum ether as yellow fibrous needles, m.p. 102-103°. Anal. Calcd. for $C_{15}H_{13}NO$: C, 80.69; H, 5.87; N, 6.27. Found: C, 80.54; H, 5.67; N, 6.07.

(X1A): Hydroperoxide of (X1).

Indole (X1) (0.3 g) in petroleum ether (200 ml) was stirred for 2 days. Yield of crude product 0.29 g, (87%). Recrystallization from ethyl acetate / petroleum ether gave m.p. 207-208°. λmax 330 mμ (ϵ18,600), 250 (sh., 9,500), 240(sh., 15,700), 235(16,300). Anal. Calcd. for $C_{15}H_{13}NO_3$: C, 70.58; H, 5.13; N, 5.49. Found: C, 70.85; H, 5.36; N, 5.69.

2-(p-Methoxyphenyl)-3-methylindole (Xll).

p-Methoxypropiophenone (8.2 g, 0.5 mole) and phenyl-
hydrazine (5 ml) in polyphosphoric acid gave 9.5 g, (80%)
red solids. After extracting the crude product with petroleum
ether (60-80°), subsequent recrystallization from ethyl acetate /
petroleum ether gave yellow crystals m.p. 123-124° (lit.[37]
m.p. 124°).

(XllA): Hydroperoxide of (Xll).

Indole (Xll) (1.0 g) in P.E. (100 ml) was stirred for
5 hours. Final yield of powdery peroxide 0.96 g, (85%).
Recrystallization from ethyl acetate / P.E. gave m.p. 135-136°.
λmax (KBr disk) 3050 cm^{-1}(s), 2780(s), 1602(s), 1920(w),
1650(m), 1505(s), 1450(m), 1425(s), 1370(w), 1306(m), 1260(s),
1180(s), 1100(m), 1030(m), 840(m), 765(m). λmax 328 mμ
(ϵ15,600), 250(8,900), 240(14,400), 234(15,600). Anal. Calcd.
for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.27;
H, 5.55; N, 5.30.

2-(p-Dimethylaminophenyl)-3-methylindole (Xlll).

p-Dimethylaminopropiophenone (3g, 0.017 mole),
prepared from distilled dimethylaniline and propionyl chloride
by the method of Nineham,[38] was dissolved in benzene (7 ml).
This mixture was stirred with phenylhydrazine (5 ml) in the
usual way and condition. Recrystallization from P.E. gave
3.5 g, (83%), m.p. 128-129°. Anal. Calcd. for $C_{17}H_{18}N_2$:
C, 81.56; H, 7.25. Found: C, 81.54; H, 7.20.

(Xlll A): Hydroperoxide of (Xlll).

Indole (Xlll) (0.3 g) in P.E. (175 ml) was stirred to give a peroxide with m.p. 150-151° after recrystallization from ethyl acetate / P.E. λmax 370 mμ (ϵ 20,000), 260(sh., 8,500), 247(11,300), 230(12,000). Anal. Calcd. for $C_{17}H_{18}N_2O_2$: C, 72.32; H, 6.43; N, 9.92. Found: C, 72.17; H, 6.44; N, 9.89.

2-(3', 4', 5'-trimethoxyphenyl)-3-methylindole (XlV).

3,4,5-Trimethoxypropiophenone (2.5, 0.013 mole), prepared from diethylcadmium and 3, 4, 5-trimethoxybenzoyl chloride by the method of Gutsche et al[39], was condensed with phenylhydrazine (7 ml) in polyphosphoric acid. When 2.5 g, (68%) crude product was recrystallized from benzene / P.E. m.p. 160-161° was obtained. NMR absorption at 7.55 τ (3H, singlet), 6.14 (6H, singlet), 6.08 (3H, singlet), 3.20 (2H, singlet), 2.30-2.80 (4H, multiplet), 1.80(1H, singlet). Anal. Calcd. for $C_{18}H_{19}NO_3$: C, 72.70; H, 6.44. Found: C, 72.60; H, 6.40.

(XlVA): Hydroperoxide of (XlV).

Due to incomplete conversion from this indole into its respective hydroperoxide, no representative analytical sample was obtained.

2-(p-Bromophenyl)-3-methylindole (XV).

p-Bromopropiophenone (5 g, 0.175 mole) and phenyl-hydrazine (7 ml) reacted in the usual way giving 6.3 g, (85%) crystalline prisms with m.p. 168-169°. Anal Calcd. for $C_{15}H_{12}NBr$: C, 62.90; H, 4.23; Br, 27.92. Found: C, 63.26;

H, 4.45; Br, 28.20.

(XVA): Hydroperoxide of (XV).

Indole (XV) (1 g) in P.E. (100 ml) stirred in the usual way gave 0.9 g prisms, (82%) with m.p. 166-167°. λmax 317 mμ (ϵ 16,000), 250(15,000), 241(15,800), 234(14,700). Anal. Calcd. for $C_{15}H_{12}NO_2Br$: C, 56.62; H, 3.80; N, 4.40. Found: C, 56.50; H, 3.95; N, 4.42.

2-(p-Chlorophenyl)-3-methylindole (XVl).

Chloropropiophenone (5 g, 0.03 mole) and phenylhydrazine (7 ml) reacted to give 5 g, (69%) white crystals with m.p. 172-173°. NMR, 7.6τ (3H, singlet), 2.3-3.0 (9H, multiplet) Anal. Calcd. for $C_{15}H_{12}NCl$: C, 74.53; H, 5.01; N, 5.80. Found: C, 74.69; H, 5.39; N, 5.76.

(XVlA): Hydroperoxide of (XVl).

Indole (XVl) (1 g) in P.E. (100 ml) stirred in the usual way gave 0.88 g white prisms, (78%). When recrystallized from ethylacetate / P.E. m.p. 135-136°. λmax 317 mμ (ϵ 16,500), 248(18, 000), 241(19,000), 234(sh., 17,700). Anal. Calcd. for $C_{15}H_{12}NO_2Cl$: C, 65.82; H, 4.42; N, 5.12. Found: C, 65.81; H, 4.51; N, 5.22.

2-(p-Fluorophenyl)-3-methylindole (XVll).

p-Fluoropropiophenone (5 g, 0.033 mole), and phenylhydrazine (7 ml) condensed in the usual way gave 7.0 g, (93%) white solids. When recrystallized from ethyl acetate / P.E. white prisms had m.p. 142-143°. Anal. Calcd. for $C_{15}H_{12}NF$:

C, 79.97; H, 5.37; N, 6.22. Found: C, 80.25; H, 5.19; N, 6.17.

(XVllA): Hydroperoxide of (XVll).

Indole (XVll) (1 g) in P.E. (100 ml) gave 0.95 g, (84%)
peroxide. Recrystallization from ethyl acetate / P.E., m.p.
137-138°. λmax, 314 mμ (ϵ 11,000), 250(sh., 12,300),
238(14,500), 230(sh., 13,300). Anal. Calcd. for $C_{15}H_{12}NO_2F$:
C, 70.03; H, 4.70; N, 5.45. Found: C, 70.03; H, 4.69;
N, 5.23.

2,3-Dimethylindole (XVlll).

2-Butanone (5 g, 0.07 mole) and phenylhydrazine (10 ml)
were stirred in polyphosphoric acid. Work up in the usual
way gave 9.5 g, (92%) of white solids. Recrystallization
from methanol / water afforded white prisms with m.p. 103-104°
(lit.[40] m.p. 106-107°) NMR 7.8 , 7.75 τ (6H, doublet), 2.3-3.0
(5H, multiplet).

(XVlllA): Hydroperoxide of (XVlll).

Indole (XVlll) (1 g) dissolved in P.E. (100 ml) was
stirred in the usual way. The reaction mixture was kept cooled
by ice bath and a small stream of oxygen gas was bubbled into
it so that decomposition of the peroxide formed was nil.
Yield: 1.10 g, (90%) of white prisms with m.p. 118-119°
(lit.[41] m.p. 113°; yield 25%). λmax 280 mμ (sh.,ϵ 3,160),
262(3,860), 225(sh., 14,000), 218(21,500), 215(19,500).

The indoles (XlX to XXIV) were synthesized from
α-bromoketone and aniline by the method of Clerc-Bory.[35b]

5-Methoxy-3-methyl-2-phenylindole (X1X).

A mixture of p-anisidine (5 g, 0.04 mole) and α-bromopropiophenone (2 g, 0.009 mole), prepared from propiophenone by the method of Aldous et al,[42] were stirred and heated in a silicone oil-bath maintained at a temperature 180°. After 10 min. the reaction mixture was poured into 1NHCl (50 ml). The resulting granular product was filtered off and recrystallized from glacial acetic acid. Yield: 1.8 g, (81%) yellow crystalline flakes, with m.p. 115-116° (lit.[35b] m.p. 115°).

(X1XA): Hydroperoxide of (X1X).

Indole (X1X) (0.5 g) dissolved in P.E. (175 ml) formed 0.51 g, (90%) of white peroxide with m.p. 148-150° (lit.[41] m.p. 148-150°).

6-Methoxy-3-methyl-2-phenylindole (XX).

m-Anisidine (5 g, 0.04 mole) and α-bromopropiophenone (2 g, 0.009 mole) were stirred and heated to give 1.6 g, (72%) indole with m.p. 164-165° (lit.[35b] m.p. 164°).

(XXA): Hydroperoxide of (XX).

Indole (XX) (0.5 g) in P.E. (175 ml) was stirred until white peroxide came out. The crude peroxide recrystallized from ethyl acetate / P.E., 0.49 g, (86%) had m.p. 156-157°. Anal. Calcd. for $C_{16}H_{15}NO$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.29; H, 5.76; N, 5.32.

7-Methoxy-3-methyl-2-phenylindole (XX1).

o-Anisidine (3 g, 0.024 mole) and α-bromopropiophenone

(4 g, 0.019 mole) reacted in the usual way to give 3.5 g, (79%) white solids. This recrystallized from glacial acetic acid as square crystals with m.p. 90-91°. NMR 7.6 τ (3H, singlet), 6.1(3.H, singlet), 2.3-3.4(8H, multiplet), 1.7(1H, broad singlet). Anal. Calcd. for $C_{16}H_{15}NO$: C, 80.98; H, 6.37; N, 6.90. Found: C, 80.83; H, 6.22; N, 5.72.

(XX1A): Hydroperoxide of (XX1).

Due to difficulty in forming the peroxide, no representative analytical sample was obtained.

p-Methoxy-α-bromopropiophenone.

p-Methoxypropiophenone (63 g, 0.390 mole) was brominated according to the procedure of Heizelman[43]. The ketone in chloroform (100 ml) was stirred with liquid bromine 63.5 g, 0.397 mole) also in chloroform (30 ml). Stirring was continued for 1 1/2 hr. during which time the reaction mixture was kept cool in an ice-bath. The reaction mixture was washed several times with water and upon removal of chloroform, the remaining oil crystallized out as yellow prisms. This was recrystallized from ethanol, affording 90 g, (94%) yellow prisms with m.p. 66-67°. Anal. Calcd. for $C_{10}H_{11}O_2Br$: C, 49.41; H, 4.56; Br, 32.87. Found: C, 49.58; H, 4.76; Br, 32.72.

5-Methoxy-2-(p-methoxyphenyl-3-methylindole (XX11).

p-Anisidine (5 g, 0.04 mole) and p-methyl-α-bromopropiophenone (2 g, 0.008 mole) heated in the usual way

gave 1.8 g, (81%) white solids. Recrystallization from glacial acetic acid afforded white needles with m.p. 139-140$^{\text{O}}$ (lit.^{44} m.p. 139$^{\text{O}}$).

(XX11A): Hydroperoxide of (XX11).

Indole (XX11) (1 g) in P.E. (100 ml) formed the corresponding peroxide in 0.80 g, (71%) yield. Recrystallization from ethyl acetate / P.E. afforded prisms with m.p. 129-130$^{\text{O}}$. Anal. Calcd. for $C_{17}H_{17}NO_4$: C, 68.21; H, 5.73; N, 4.68. Found: C, 68.06; H, 5.94; N, 4.95.

7-Methoxy-2-(p-methoxypehnyl)-3-methylindole (XX111).

Reaction of o-anisidine (10 g, 0.08 mole) and p-methoxy-α-bromopropiophenone (4 g, 0.016 mole) afforded 2.5 g, (56%) white crystals from glacial acetic acid, m.p. 114-115$^{\text{O}}$. NMR 7.7 τ (3H, singlet), 6.3(3H, singlet), 6.2(3H, singlet), 2.5-3.5(7H, multiplet), 1.9(1H, broad singlet). Anal. Calcd. for $C_{17}H_{17}NO_2$: C, 76.38; H, 6.41; N, 5.24. Found: C, 76.67; H, 6.31; N, 5.42.

(XX111A): Hydroperoxide of (XX111).

Indole (XX111) (1 g) in P.E. (100 ml) formed the corresponding peroxide in 0.82 g, (71%) yield. Recrystallization from ethyl acetate / P.E. afforded prisms with m.p. 132-133$^{\text{O}}$. Anal. Calcd. for $C_{17}H_{17}NO_4$: C, 68.21; H, 5.73; N, 4.68. Found: C, 68.50; H, 5.73; N, 4.39.

2-Phenyl-3-methyl-5,6-benzaindole (XXlV).

β-Naphthylamine (2 g, 0.014 mole) and α-bromopropiophenone
(1 g, 0.005 mole) heated in the usual way produced a crystalline
solid from glacial acetic acid, m.p. 152-153°. NMR 7.2 τ
(3H, singlet), 2-2.7(11H, multiplet), 1.4, 1.5(1H, doublet).
Anal. Calcd. for $C_{19}H_{15}N$: C, 88.68; H, 5.88; N, 5.44. Found:
C, 88.48; H, 5.53; N, 5.15. Hydroperoxide formation was
unsuccessful, and the indole remained unchanged.

Tetrahydrocarbazole (XXV).

This compound, prepared from phenylhydrazine and
cyclohexanone had m.p. 120-121° (lit.[45] m.p. 116-117°).

(XXVA): 11-Hydroperoxtetrahydrocarbazolenine, hydroperoxide of (XXV).

The most satisfactory preparation of this compound
involved the stirring of a saturated solution of tetrahydro-
carbazole (XXV) in P.E. (60-80°) at room temperature in
good contact with the atmosphere. Almost quantitative
conversion occurred in 2 hours. Recrystallization from ethyl
acetate / P.E. gave white prisms, m.p. 134-135° (lit.[46]
m.p. 134°).

6-Aminotetrahydrocarbazole (XXVl).

This compound, prepared from tetrahydrocarbazole by
nitration and reduction with Pd/C in ethyl acetate had m.p.
149-151° (lit.[47] m.p. 152°).

(XXVlA): 11-Hydroperoxy-6-aminotetrahydrocarbazolenine, hydroperoxide of (XXVl).

6-Aminotetrahydrocarbazole (0.50 g) was dissolved in benzene (100 ml) and stirred in contact with air for 5 hours. The peroxide was obtained by filtration as brown microcrystalline needles, m.p. 133-135° (with decomposition) (0.23 g). λmax 332(sh., ϵ 2,700), 295(6,000), 230(10,300). νmax(nujol) 3400 cm^{-1} (NH, -OOH). Anal. Calcd. for $C_{12}H_{12}N_2O_2$: C, 66.02; H, 6.47; N, 12.84. Found: C, 66.20; H, 6.60; N, 12.72.

Lophine Hydroperoxide (Vla).

Lophine (0.35 g) was dissolved in chloroform (300 ml) in a quartz flask and haematoporphyrin (0.03 g) added. The flask was irradiated at 20° (air cooling) for 5 hr. with four G.E. 20W fluorescent tubes, passing oxygen through the solution in a slow stream. Removal of the solvent in vacuo, treatment with charcoal and recrystallization from ether / P.E. at low temperature gave white prisms (0.10 g), m.p. 160-165° (varies with rate of heating) (lit.[48] m.p. 170°).

3,4,5-Triphenylpyrazole (XXVll).

ω-Nitrostyrene (10.7 g, 0.18 mole), prepared from benzaldehyde and nitromethane by the method of Worrall,[49] and diphenyldiazomethane (15 g, 0.096 mole), prepared by oxidation[50] of benzophenone hydrazone with HgO were allowed to react according to the procedure of Parham et al.[51] Total yield: 9 g, (50%) white needles with m.p. 164-165°

(lit.[51] m.p. 165°).

Attempt synthesis of 3, 4, 5-triphenylpyrazole Hydroperoxide.

Attempts to form a hydroperoxide from pyrazole, using the same conditions and quantities as in the lophine hydroperoxide, was unsuccessful. The pyrazole remained unchanged.

Attempted synthesis of N-methyltryptamine Hydroperoxide.

Attempts to form the hydroperoxide of N-methyltryptamine[52], using the same conditions and quantities as for lophine hydroperoxide, were unsuccessful. The N-methyltryptamine (XXVlll) decomposed slowly.

1, 1'-Bi(2, 4, 5-triphenylimidazole) (XXlX).

This compound, prepared from lophine by oxidation with potassium ferricyanide / potassium hydroxide solution had m.p. 195-196,° yellow prisms (lit.[53] m.p. 199-201°). λmax 268 mμ (ϵ 22,400). M.W. (osmometric) 605, mass spectral major peaks at 590 and 295. Anal. Calcd. for $C_{21}H_{15}N_2$: C, 85.40; H, 5.12; N, 9.49. Found: C, 85.42; H, 5.09; N, 9.49. This compound is unstable in 5% HCl, H_2/PdC. 5% KOH and t-BuOOH; forming lophine in all cases.

Potassium t-butoxide.

This strong base was prepared by the method of Vogel.[54]

Products of Chemiluminescent Reaction.

11-Hydroperoxytetrahydrocarbazolenine (XXVA) (3.8 g)
in xylene (75 ml) was heated at 110° and held at this temperature
until luminescence had ceased (10 min). Removal of the xylene
under reduced pressure and trituration with ether gave the
lactam (XXX) 6.80 g) as prisms m.p. 156-157° (lit.[46] m.p.
156-157°). Chromatography of the residue on grade III alumina
(100 g) gave eight crystalline compounds, not all of which
were investigated. The principle products (see Fig. 7) were
identical in properties to those described by Witkop.[46]

Base treatment of 2-phenyl-3-methyl-3-hydroperoxyindolenine (X) and products of chemiluminescent reaction.

The hydroperoxide (XA) (0.239 g) in dimethyl sulphoxide
(DMSO) (100 ml) was added excess 0.1M potassium t-butoxide in
DMSO (15 ml), and stirred until luminescence had ceased. (10
min). At once the reaction mixture was quenched with water,
and extracted several times with diethyl ether. Removal of
the ether gave a brown viscous oil which was chromatographed
on grade III alumina (100 g). Elution with benzene / P.E.
(2:8) gave three fractions indicating 3 compounds, by T.L.C.

2-Benzamidoacetophenone (Xa) (first fraction).

A white solid, recrystallized from P.E. as needles,
m.p. 88-90° (lit.[55] m.p. 98°). Total yield: 0.32 g,
(69% of the 3 fractions). νmax (chloroform): 3300 cm^{-1} (-NH),
1600 cm^{-1} (C=O). NMR 7.2τ (3H, singlet), 2-3 (9H, multiplet).
Anal. Calcd. for $C_{15}H_{13}NO_2$: C, 75.30; H, 5.48; N, 5.85. Found:

C, 75.52; H, 5.64; N, 5.97.

2-Methyl-2-phenyl-ψ-indoxyl (Xb) (second fraction).

A yellow solid, recrystallized from benzene / P.E. as yellow needles with m.p. 110-111°. Total yield: 0.052 g, (11%). Anal. Calcd. for $C_{15}H_{13}NO$: C, 80.69; H, 5.87; N, 6.27. Found: C, 80.61; H, 6.01; N, 6.50.

Rearrangement of 2-phenyl-3-methyl-3-hydroperoxyindolenine (XA).

2-Methyl-2-phenyl-ψ-indoxyl (Xb) was also synthesized in 80% yield when hydroperoxide (X) (0.19 g) in methanol (10 ml) and 20% sodium hydroxide (5 ml) was refluxed for 3 days, followed by ether extraction. The spectral data of these two compounds were identical. However using a similar procedure (in 2N sodium hydroxide) Witkop[36] did not obtain any product. NMR 8.3 τ (3H, singlet), 4.9 (1H, broad singlet), 2.3-3.3 (9H, multiplet).

2-Phenyl-3-methyl-3-hydroxyindolenine (Xc) (third fraction).

Alcohol (Xc) was also synthesized using the following procedure: hydroperoxide (XA) (0.5 g) dissolved in ethyl acetate (75 ml) was reduced with Pd/C and 53 cc. hydrogen. Yield: 0.3 g, (67%). Physical and spectroscopic data of these two compounds were identical.

Base treatment of 2-(p-methoxyphenyl)-3-methyl-3-hydroperoxyindolenine (XllA) and products of chemiluminescent reaction.

To the hydroperoxide (XllA) (0.269 g) in dimethyl sulphoxide (100 ml) was added excess 0.1M potassium

t-butoxide / DMSO (18 ml) and stirred until luminescence
had ceased (10 min.). Immediately the reaction was quenched
with water, and ethereal extractions and removal of ether
gave an amorphous solid which was chromatographed on grade III
alumina (150 g). Elution with benzene / P.E. (2:8) gave two
major fractions, and a trace of yellow compound.

2-(p-methoxybenzamido)acetophenone (Xlla) (first fraction).

The white solid crystallized from P.E. as needles
m.p. 128-129°. Total yield: 0.100 g, (60% of the 2 fraction)
λmax 336 mμ ($\in$8,370), 280(17,510), 251(25,500), 218(22,600).
γmax (KBr disk) 3200 cm^{-1} (m), 1672(m), 1646(s), 1604(m),
1585(m), 1530(m), 1506(m), 1450(m), 1365(m), 1306(m), 1250(s),
1190(m), 1106(w), 1025(m), 960(w), 900(w), 850(w), 760(m),
680(w). Anal. Calcd. for $C_{16}H_{15}NO_2$: C, 75.87; H, 5.97;
N, 5.53. Found: C, 76.00; H, 6.15; N, 5.64.

2-(p-Methoxyphenyl)-3-methyl-3-hydroxyindolenine (Xllb) (second fraction).

Total yield of 70 mg, (40%) was recrystallized from
ethyl acetate / P.E., m.p. 152-153°. λmax 330 mμ ($\in$18,600),
250(6,700), 240(12,800), 235(13,750). Anal. Calcd. for
$C_{16}H_{15}NO_2$: C, 75.87; H, 5.97; N, 5.53. Found: C, 76.00;
H, 6.15; N, 5.64.

Hydrogenation of 2-(p-methoxyphenyl)-3-methyl-3-hydroperoxy-indolenine (XllA).

Alcohol (Xllb) was also synthesized by hydrogenation
of hydroperoxide (XllA). (0.5 g) was added to presaturated

10% Pt/C in ethyl acetate and 46 cc. of hydrogen was taken up.
The solution was filtered through celite and the solvent
was removed to give a gum. Recrystallization from ethyl
acetate / P.E. afforded 0.30 g, (64%) prisms. Spectral
data of these two compounds were identical.

Rearrangement of 2-(p-methoxyphenyl)-3-methyl-3-hydroperoxy-indolenine (XllA).

Hydroperoxide (XllA) (0.10 g) dissolved in glyme
(5 ml) was refluxed with 0.1NHCl (5 ml) for 1 hr. (Xlla)
(0.075 g) crystallized out as needles. The spectral data of
this synthetic sample were identical to the one from the column.

Base treatment of 2, 3-dimethyl-3-hydroperoxyindolenine (XVlllA) and products of the chemiluminescent reaction.

The hydroperoxide (XVlllA) (0.177 g) dissolved in
dimethyl sulphoxide (100 ml) was added to excess 0.1N potassium
t-butoxide (15 ml) and stirred until luminescence had ceased
(5 min.). Extraction and work up in the usual way afforded a
brown oil, which was chromatographed on neutral grade III
alumina (100 g). Elution with benzene / P.E. (1:4) gave a
fraction of white solids (0.120 g, 67.5% of total isolated from
the column). The remaining was a brown tar. Recrystallization
of the solids from ethyl acetate / P.E. afforded crystalline
needles, m.p. 72-73° which is 2-acetamidoacetophenone (XVllla).
Anal. Calcd. for $C_{10}H_{11}NO_2$: C, 67.78; H, 6.26. Found:
C, 67.71; H, 6.42.

Synthesis of 2-acetamidoacetophenone (XVlla).

o- Aminoacetophenone (0.10 g) in excess acetic anhydride (5ml) was warmed gently for 15 min. on a steam bath. Addition of ice-water to the reaction mixture caused precipitation. The white precipitate was filtered off and recrystallized from ethanol / water. Yield: 0.110 g white needles, (85%). The spectral data of the synthetic sample was identical to the sample isolated from the column. Mixed m.p. gave no change.

Hydrogenation of 2,3-dimethyl-3-hydroperoxyindolenine (XVlllA).

The hydroperoxide (XVlllA) (0.5 g) was added to pre-saturated 10% Pd/C in ethyl acetate and 72 cc. hydrogen was taken up upon stirring. The solution was filtered through celite and the solvent was removed to give an oil. Recrystallization of the oil from ethyl acetate / P.E. afforded white prisms. 0.30 g, (57%), m.p. 130-131° (lit.[41] m.p. 136-139°). This compound is 2,3-dimethyl-3-hydroxyindolenine (XVlllb).

DISCUSSION

The light emitted from excited molecules formed by
chemical reaction has previously been defined as
chemiluminescence. This is a particularly challenging field
because of its numerous difficulties. The reactions have to
be very energetic, so that electronically excited molecules
(40-80 kcal/mole) may be formed. Difficulties can also be
encountered in identifying the emitter; the lack of detail in
fluorescence bands is here a limitation. Emission may come
from excited reactants, products or some other substance
present. Since overall light yields (quanta per molecule
reacting) are usually low, and sometimes exceedingly low,
while the reaction itself may take more than one course
yielding several products, the precise chemistry of the
emitting process may be very elusive. Few, if any,
chemiluminescent reactions can be said to be fully understood.

The study of indolyl peroxides was particularly
attractive since decomposition of peroxides to a dicarbonyl
compound is well known, largely due to the investigation of
Witkop.[36] Recent reports of tryptamine (indole) residues in
bioluminescent systems, such as Cyprindina hilgendrofii[60]
and Renillo reniformis[33] make the discovery of chemiluminescence[6,61,62]
in indolyl peroxides particularly interesting. The investigation
of indolyl peroxide chemiluminescence was divided into three
stages: the first objective was to synthesize these indoles
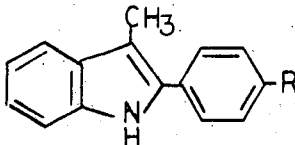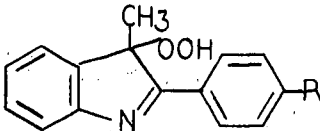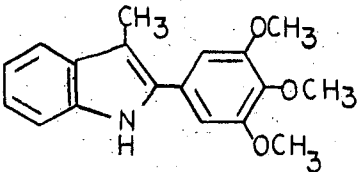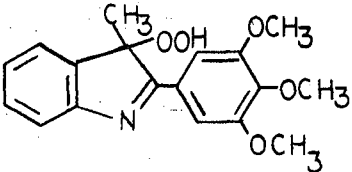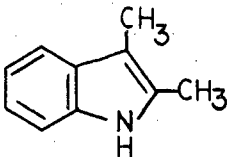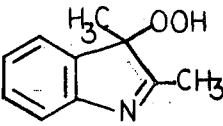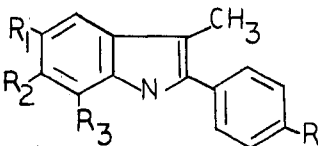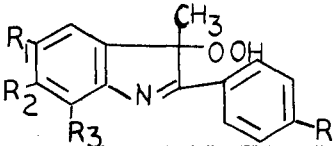and their peroxides (Part I); then the chemiluminescence and

fluorescence measurements were made to determine the emitter
and the regions of emission (Part II); finally the
mechanism of the chemiluminescent reaction was studied by
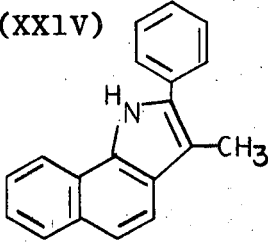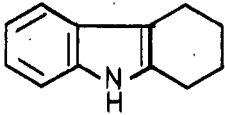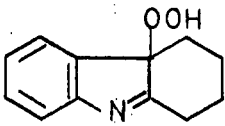kinetic methods (Part III).
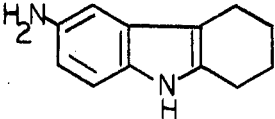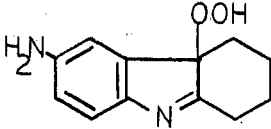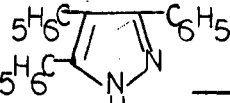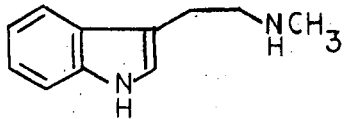
A) Synthesis of Indoles.

Before the chemiluminescence of indolyl peroxides
can be properly studied in any great detail, a very bright
chemiluminescent reaction is required. A bright reaction
not only minimizes the problem of self quenching, but also
increases the accuracy of the chemiluminescence emission
determination. Consequently a series of 20 indoles and their
corresponding peroxides was prepared. Thirteen of these
indoles and their peroxides are apparently unreported in the
literature. All of these compounds gave satisfactory elemental
analyses, infrared, ultraviolet and nuclear magnetic resonance
absorption spectra, as well as satisfactory chemiluminescence
(Table 1, color and intensity of chemiluminescence in potassium
t-butoxide / DMSO).

Table 1.

indole	indolyl peroxide	chemiluminescence	
		color	I

Continued

(X), R=H	(XA), R=H;	yellow	weak
(X1), R=OH	(X1A), R=OH	yellow	medium
(X11), R=OCH$_3$	(X11A), R=OCH$_3$	yellowish green	strong
(X111), R=N(CH$_3$)$_2$	(X111A), R=N(CH$_3$)$_2$	yellow	strong
(X1V) *structure: 3-methyl-2-(3,4,5-trimethoxyphenyl)indole*	(X1VA) *structure: 3-methyl-3-hydroperoxy-2-(3,4,5-trimethoxyphenyl)indolenine*	yellow	weak
(XV), R=Br	(XVA), R=Br	"	"
(XV1), R=Cl	(XV1A) R=Cl	"	"
(XV11), R=F	(XV11A), R=F	"	"
(XV111) *structure: 2,3-dimethylindole*	(XV111A) *structure: 3-methyl-3-hydroperoxy-2-methylindolenine*	yellowish green	strongest
(X1X), R$_1$=OCH$_3$, R$_2$=R$_3$=R=H *structure: indole with R$_1$, R$_2$, R$_3$ substituents, 3-CH$_3$, 2-(4-R-phenyl)*	(X1XA), R$_1$=OCH$_3$ R$_2$=R$_3$=R=H *structure: corresponding hydroperoxy indolenine*	yellow	weak
			Continued

(XX), R_2=OCH$_3$, $R=R_1=R_3$=H	(XXA), R_2=OCH$_3$ $R=R_1=R_3$H	yellow	weak
(XX1), R_3=OCH$_3$, $R=R_1=R_2$=H	(XX1A), R_3=OCH$_3$, $R=R_1=R_2$=H	"	"
(XX11), $R=R_1$=OCH$_3$, $R_2=R_3$=H	(XX11A), $R=R_1$=OCH$_3$, $R_2=R_3$=H	"	"
(XX111), $R=R_3$=OCH$_3$, $R_1=R_2$=H	(X111A), $R=R_3$OCH$_3$, $R_1=R_2$=H	" Not a pure sample	"
(XX1V)	(XX1V) not formed -inert		
(XXV)	(XXVA)	blue on heating	weak in xylene
(XXV1)	(XXV1A)	"	"
(XXVII)	(XXV11A), not formed -inert		
(XXVIII)	(XXV111A), not formed -inert		Continued

(Va) C_6H_5C—N=, C_6H_5, N-H, C_6H_5	(VIa) C_6H_5C, C_6H_5, OOH, N=, C_6H_5	yellow	weak
(XXIX) C_6H_5C, N=, C_6H_5, N, C_6H_5, C_6H_5C, N, C_6H_5, N=, C_6H_5	(XXIXA) as above not isolatable	"	"

Two established procedures[34,36b] were employed to synthesize the indoles. Firstly, using the method of Witkop et al,[34] (condensation of a hydrazine and ketone mixture using polyphosphoric acid) indoles (X to XVlll) were produced. This cyclodehydration was a vigorous exothermic reaction, and a color change from orange to dark brown took place during the course of the reaction. This method was especially suitable for producing indoles with substitution in the 2-phenyl ring. Surprisingly, polymerization was very low, consequently an average of 70% yield was obtained. The overall course of the reaction is as follows:

$$\text{[phenylhydrazine, } N\text{-}NH_2] + [O=C\text{-}R, CH_3] \xrightarrow{H^+} \text{[3-methyl-2-R-indole, } CH_3, N\text{-}H, R]$$
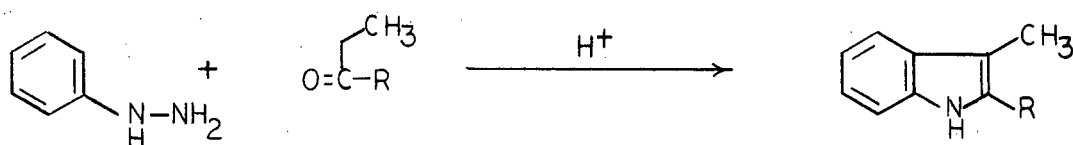
The other procedure applied for synthesizing the remainder of the indoles (XlX to XXVl) was that of Bory.[36]

This procedure requires the heating of an aniline and
bromoketone mixture. It proved most convenient for producing
indoles with substituents in the benzenoid ring of the indole
position of the molecule, since substituted anilines are more
easily available than substituted phenylhydrazines. By the
use of these two procedures various substituted indoles were made
available.

B) Synthesis of Indole Peroxides.

The synthesis of indolyl peroxides has been studied
by Witkop[36,46] and Beer[41] independently. The formation
of peroxides (Eq. 2) was envisaged as arising from a radical
chain reaction[63,64] (Fig. 5).

Fig. 5
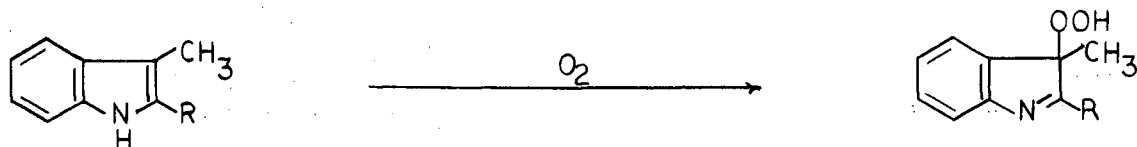
Most of the peroxides were formed simply by stirring a clear
solution of indole in petroleum ether (60-80°) in contact
with air. Due to slow evaporation and progressive formation
of peroxide, the powdery peroxide precipitates out.[64] Other
methods such as standing a petroleum ether solution of indole
in air,[63] catalytic oxidation using platinum catalyst,[63] and
irradiation[48] in presence of oxygen, were unsatisfactory due
to low yield and extensive decomposition.

Attempts to prepare peroxides from indoles (XX1V) and (XXV111) by stirring in the open air have not been successful; these indoles appear to be relatively resistant to the action of oxygen.
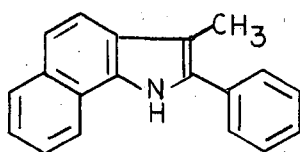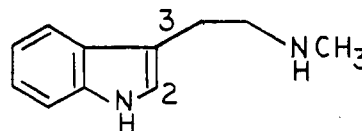
However the anion of (XXV111)[52] gave a fairly strong emission from oxidation with oxygen, using the now almost standard dimethyl sulphoxide - potassium t-butoxide system.[1] We feel this compound serves as a particularly good model for luciferins[33] containing the indole nucleus since tryptophan is almost certainly a precursor of these. It is known that uptake of oxygen by proteins can be ascribed to the tryptophan moiety,[65,66,67] and we suggest that the generally observed luminescence of proteins with oxidizing agent is due to the phenomenon described here.

It is interesting and significant that structural features which inhibit or prevent peroxide formation in the indole series, viz; a) N-substitution, b) the presence of strongly electron-attracting groups in the benzene ring, and c) the presence of a phenyl group in the 3-position, have been found to be necessary or desirable for successful oxidation of indoles to o-acylamino-ketone with chromic acid.[68] It has

been suggested that such oxidation depends on the
stabilization of the imino-tautomer (XXX1) in the imino-
ketemine system (XXX1 ⇌ XXX11).
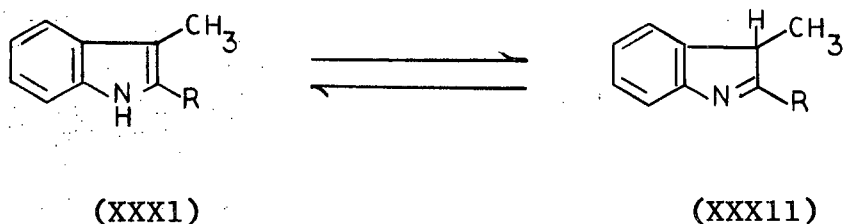
In addition, reports of indolyl peroxides without substituents
at the 2-position are rare, whereas peroxides with substituents
varying from methyl[41] to phenyl[63] and benzyl[69] are common.
Difficulty in converting 3, 4, 5-triphenylpyrazole (XXV11)
to its peroxide was also encountered. Despite the
similarities in structure and conjugation to lophine (Va)
peroxidation was not initiated upon irradiation in presence
of oxygen.

Fig. 6
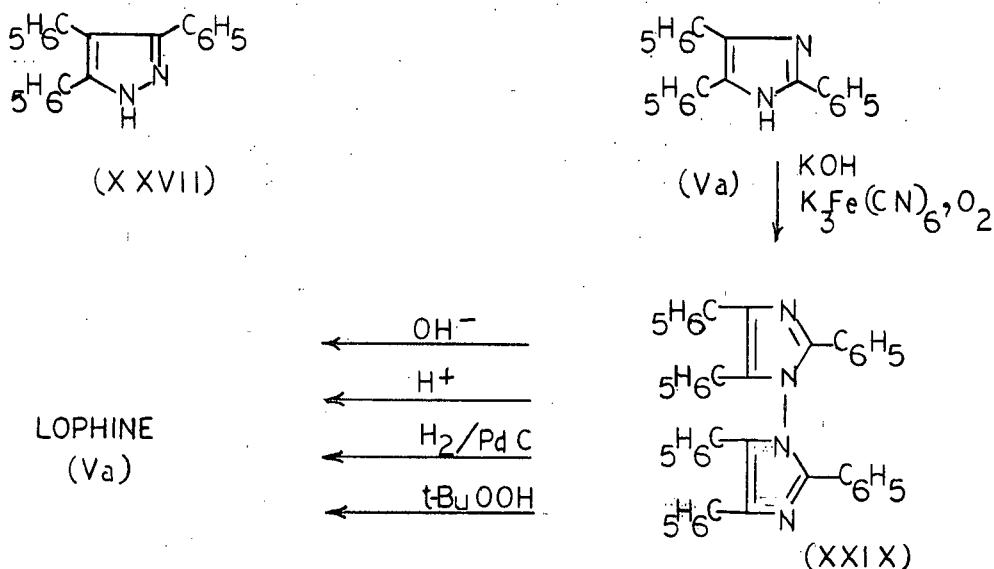
Further chemical studies showed that (XXVll) is completely

inert to oxidation in $KOH/K_3Fe(CN)_6$ and oxygen, whereas

lophine[70] under such conditions formed a dimer (XX1X)

(Fig. 6), which is unstable in acid, base, heat, catalytic

hydrogenation and peroxide (t-BuOOH) forming lophine in all

cases.

It must be pointed out that if favourable conditions

are found for the formation of peroxide, the yield can be

increased and decomposition can be minimized. Such was the

case with the peroxide of 2, 3-dimethylindole, in which the

reaction mixture was kept cool, therefore increasing the

yield from 25%[41] to 90%.

c)   Chemiluminescence and Decomposition of Indole Peroxides.

The decomposition of indolyl peroxides in aqueous

medium has been studied independently by Witkop[46] and Beer[41]

and co-workers. Beer et al[64] showed that tetrahydrocarbazolyl

hydroperoxide, and certain of its derivatives emitted a blue

flash at the melting point. This and the recent report[33]

that bioluminescent systems such as cypridina luciferin are

thought to contain an indole nucleus, encouraged us to

reinvestigate a similar series of indolyl peroxides. Our

primary concern was to find the best possible conditions for

chemiluminescence, and consequently the decomposition of

indolyl peroxides was investigated by pyrolysis and in

aqueous and nonaqueous conditions.

Tetrahydrocarbazolyl hydroperoxide (XXVA) on heating in xylene at 120° emits light at 500 mμ (cf. Part II). The resulting product mixture is extremely complex. However the major components (Fig. 7) are the lactam (XXX), tetrahydro-carbazole (XXV), the hydroxyquinolines (XXXa), and (XXXb), the spiroketone (XXXc) and the dimer (XXXd).
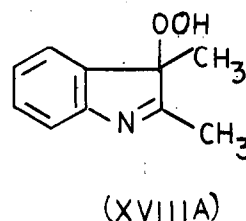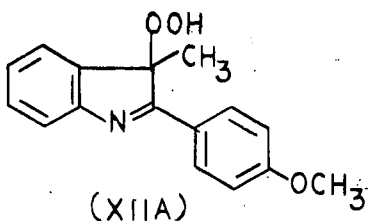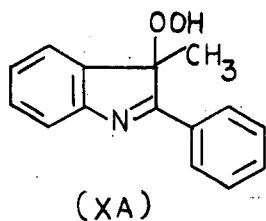
Fig. 7
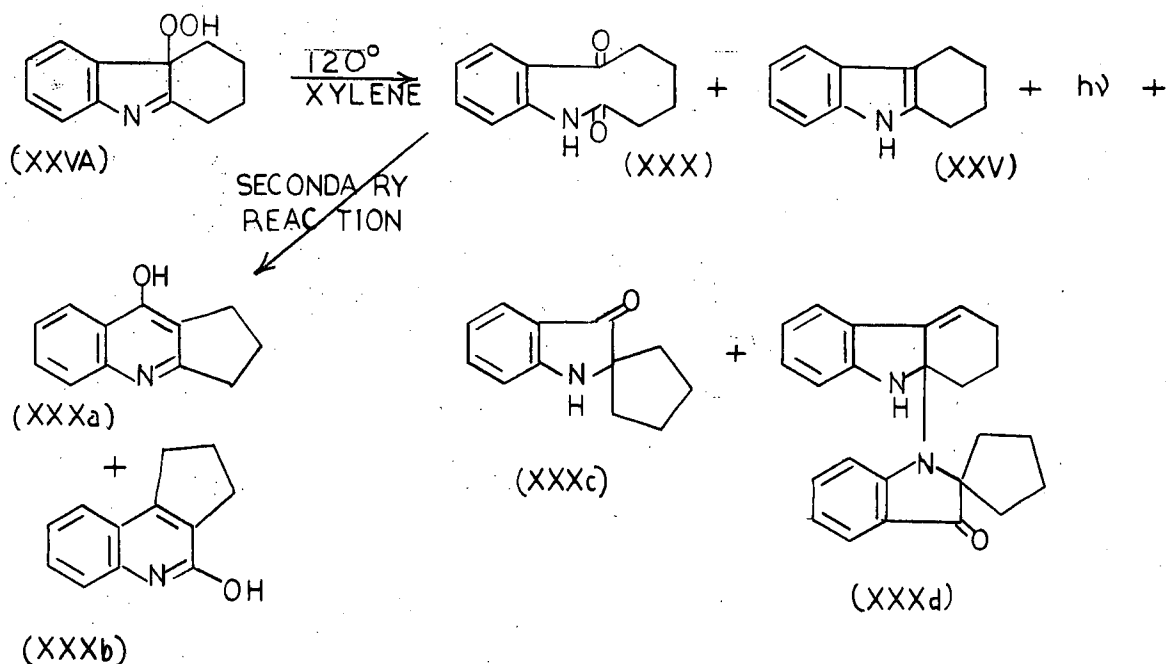
The distribution of products is fairly similar to that of acid catalyzed decomposition as reported by Witkop et al[46]. In acid, where the yield of lactam (XXX) is high no light was emitted. Examination of various solvents for the thermochemiluminescent decomposition of this hydroperoxide showed that pyridine, chlorobenzene and diglyme were all

satisfactory.   Considerably less luminescence was observed
in hot dimethyl sulphoxide and dimethyl formamide.
Treatment of the tetrahydrocarbazolyl hydroperoxide with
ethanolic base gave no light, but treatment with carefully
dried potassium t-butoxide in DMSO gave a bright short-lived
flash.

The indolenyl 3-hydroperoxides (XA, XllA, XVlllA and
those in Table 1) have been shown to emit light on heating,
or on treatment with potassium t-butoxide in dimethyl
sulphoxide (DMSO).   No light is emitted in basic hydroxylic
solvents, although the products are the same.

(XA)   (XIIA)   (XVIIIA)

Hydroperoxide (XA) in potassium t-butoxide in DMSO
gave a yellow emission and three reaction products which are
similar to those isolated from the decomposition of tetra-
hydrocarbazolyl hydroperoxide (XXVA) (Fig.7).   However only
three components, the dicarbonyl compound (Xa), the
spiroketone (Xb) and the alcohol (Xc) were produced, thus
the process of identifying the possible emitter is very
much facilitated.   It is interesting that the dicarbonyl
(Xa) was isolated in 69% yield.

Fig. 8
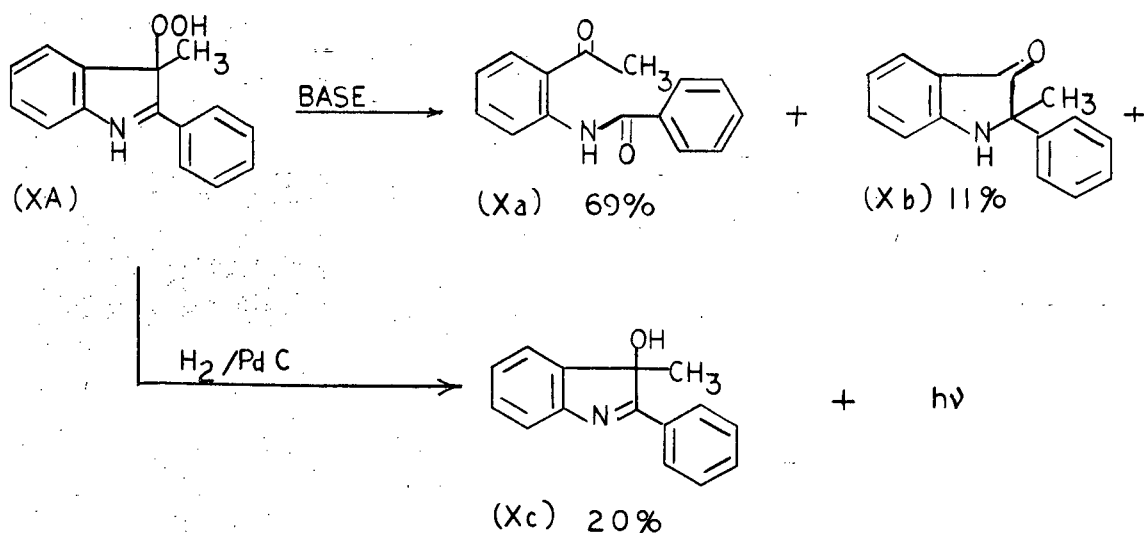
Secondary Reaction:
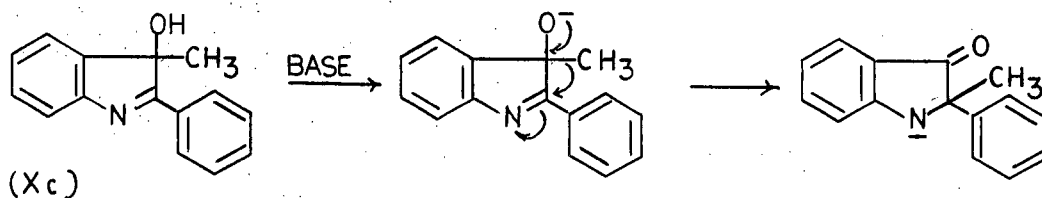
T.L.C. analysis instantly after the decomposition of (XA)

showed no spiroketone (Xb). It is likely that the

spiroketone is formed as a result of a secondary reaction

under prolonged basic conditions. In fact the spiroketone

(Xb) was synthesized by treatment of the alcohol (Xc)

with base. The alcohol (Xc) was obtained by hydrogenation

of the hydroperoxide (XA). Therefore, it is unlikely that

the spiroketone (Xb) could be the emitter. Furthermore it

is not fluorescent in strong base.

Similar treatment of the hydroperoxide (XllA) gave
a green emission and two reaction products (XXlla) and
(XXllb) in which the structures were confirmed by synthesis
and by the usual spectroscopic procedures (Fig. 9).

Fig. 9
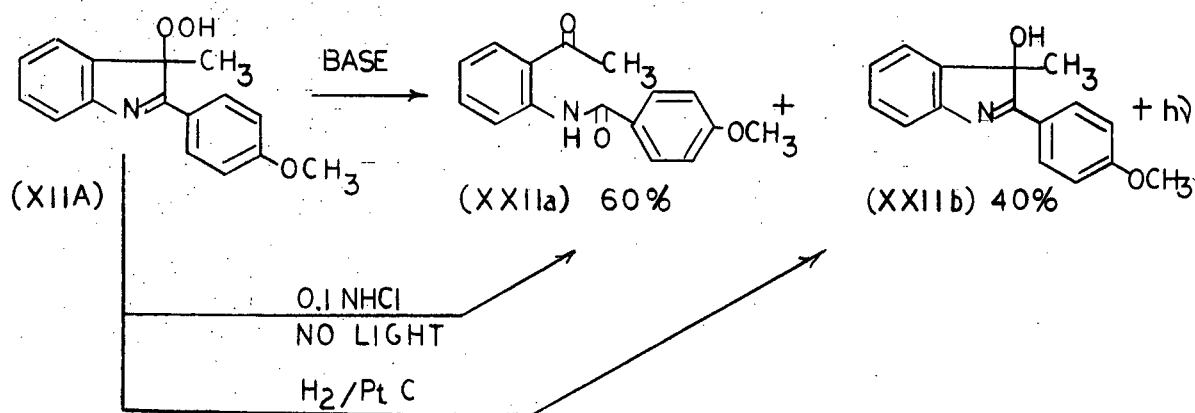
It must be pointed out that the hydroperoxide (XllA) in
acid gave the same two reaction products but no light was
emitted. However similar base treatment of the
hydroperoxide (XVlllA), giving the brightest green emission
of all the indolyl peroxides studied, produced only one
reaction product, dicarbonyl product (XXVllla), in 68%
yield, although small amounts of degradation products from the
corresponding alcohol were also present (Fig. 10).

Fig. 10

(XVIIIA)   (XVIIIa) 68 %
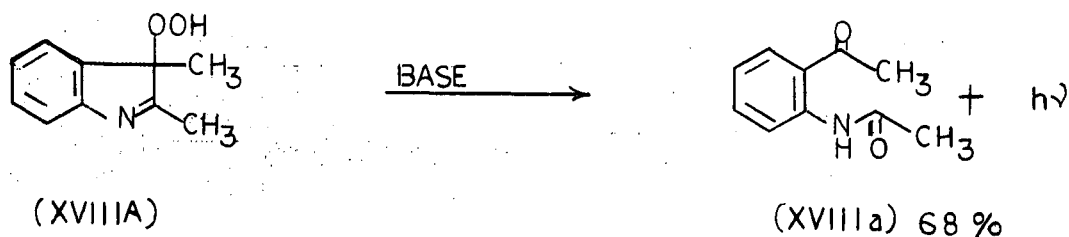
There is striking similarity to the behaviour of lophine
hydroperoxide (VIb,c) with ethanolic base, in which the
dibenzoylamidine (VIIb,c) is produced in high yield and
which has been identified as the emitter.[32] Based on analogy
alone, one can predict that the dicarbonyl products (Xa, XIIa,
and XVIIIa) are the likely emitters.

PART II: CHEMILUMINESCENCE AND FLUORESCENCE
STUDY

EXPERIMENTAL

Measurement of Chemiluminescence.

The chemiluminescence from the indole peroxide-base reaction was measured with an Aminco-Bowman spectrophoto-fluorometer equipped with a 1P28 photomultiplier tube. In order to achieve high sensitivity the reaction was carried out in a quartz optical cell placed in the photomultiplier shutter compartment directly in front of the photomultiplier tube.

Thermochemiluminescence of 11-hydroperoxytetra-
hydrocarbazolenine (XXVA) and sensitizer.[56]

Hydroperoxide (XXVA) (20 mg) was added to xylene (15 ml) at 120°. Blue light emitted was measured at 500 mμ.

Hydroperoxide (XXVA) (20 mg) and 9, 10-diphenylanthracene (20 mg) added to xylene (15 ml) at 120° gave emission peaks at 442 mμ and 500 mμ.

Chemiluminescent spectrum of 2-(p-methoxyphenyl)
-3-methylhydroperoxyindolenine (X11A).

To hydroperoxide (X11A) (3 ml of 10^{-2}M in DMSO) in a quartz cell was added potassium t-butoxide in DMSO (0.6 ml of 4×10^{-3}M solution). The emission peak was observed at 495 mμ.

Fluorescent spectrum of 2-(p-methoxybenzamido)aceto-phenone (X11a).

The diketone (X11a) (3 ml of 10^{-3}M/DMSO) in a quartz cell was added potassium t-butoxide/DMSO (0.6 ml of

4 x 10^{-3}M/DMSO). The emission peak was observed at 495 mμ.
This fluorescent spectrum was superimposable on the chemi-
luminescent spectrum of the hydroperoxide (X11A) in equal
concentration and sensitivity.

Fluorescent spectrum of 2-(p-methoxyphenyl)-3-methy-
3-hydroxyindolenine (X11b).

With similar concentration and conditions as previous
fluorescent spectrum, an emission peak was observed at 485 mμ.

Chemiluminescent spectrum of 2, 3-dimethyl-3-hydroper-
oxyindolenine (XV111A).

With similar concentration and conditions as previous
chemiluminescent spectrum, an emission peak was observed at
518 mμ. superimposable upon the fluorescent spectrum of
2-(acetamido)acetophenone (XV111a).

The chemiluminescence intensity of (XV111A)
(10^{-3}M, 0.001 volt) equalled the chemiluminescence intensity
of X11A) (10^{-2}M, 0.001 volt). Therefore the efficiency of
the latter is approximately ten times the former.

The fluorescent spectrum of quinine sulfate
(10^{-5}M in 1NH$_2$SO$_4$) was measured at 451 mμ. When the integrated
intensity of quinine sulfate and diketone (XV111a) was compared,
a quantum yield of 0.016 was obtained for (XV111a) as
calculated by the method of Calvert et al.[57] The reference
quantum yield of 0.510 for quinine sulfate was taken from
the paper by Melhuish.[58]

Comparative chemiluminescence efficiency determination of indolenyl hydroperoxides.

The chemiluminescence efficiency of indolenyl peroxide was measured on a Tektronix type-564 storage oscilloscope, in which a net voltage of 700 volts was applied to the photomultiplier tube. The chemiluminescent reaction unit, placed 10 cm away from the photomultiplier tube, consisted of a beaker (10 ml) with a magnetic stirring bar. Each time potassium t-butoxide/DMSO 3 ml, 10^{-2}M) was poured all at once into the beaker containing the stirred hydroperoxide in DMSO (2 ml, 10^{-3}M), and simultaneously the oscilloscope was triggered. Scanning time was 0.1 sec/cm. The area traced out by the chemiluminescence emission of the indolyl hydroperoxide was cut out and weighed. The weight of 2-(p-dimethylamino phenyl)-3-methyl-3-hydroperoxyindolenine (XlllA) was arbitrarily set at 100 units.

| Hydroperoxide	R	Wt.(g)	Sens.(volt)	Unit	σ^{59}
X111A	$N(CH_3)_2$	0.3452	2	100	-.600
X11A	OCH_3	0.0835	10	82.5	-.268
X1A	OH	0.1110	5	66.5	-.357
XV11A	F	0.1120	0.2	3.5	-.062
XA	H	0.0300	0.05	1.8	0
XVA	Br	0.2642	0.05	0.5	+.227
XV1A	Cl	0.2640	0.05	0.5	+.232
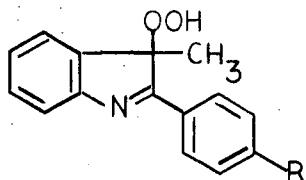
DISCUSSION

A) <u>Energy Transfer</u>

One possible explanation[61] of the chemiluminescence

of organic compounds is that an intermediate peroxide

molecule decomposes exothermically to form a carbonyl group

in an excited state. Emission of light from this state if

the carbonyl product is fluorescent, or transfer of the

energy to another fluorescent molecule can also occur.

The thermochemiluminescence of tetrahydrocarbazolyl

hydroperoxide (XXVA) was measured at 500 mμ . The major

product of this reaction was the lactam (XXX) which in fact

is not fluorescent. We find that the intensity of light

is proportional to the concentration of the spiroketone (XXXc)

which is formed in the reaction and which was also added.

The addition of 9, 10-diphenyl anthracene increases the

intensity (emission 450 mμ) one hundred-fold, and it is

possible to observe the gradual increase of the 500 mμ peak

as the reaction proceeds. This supports the suggestion

that the emitter is formed during the reaction and that a

transfer of energy from some other source is occurring.

Self-absorption by the yellow solution can account for the

shift to long wavelength of both the spiroketone (XXXc) and

the diphenyl-anthracene (fluorescing normally at 465 mμ and

415 mμ respectively). Thus rigorous identification of the

emitting molecule is made difficult. However based on the

analogy of the light-producing step of lophine and indolyl

peroxides (Ref. p.52), in which the carbonyl product is the

emitter, we can suggest that the excited state of the

lactam (XXX) is the emitter in which the energy is transferred

to neighbouring molecules such as spiroketone (XXXc) or

diphenylanthracene which are present. This process can be

represented diagramatically (Fig. 11):

Fig. 11
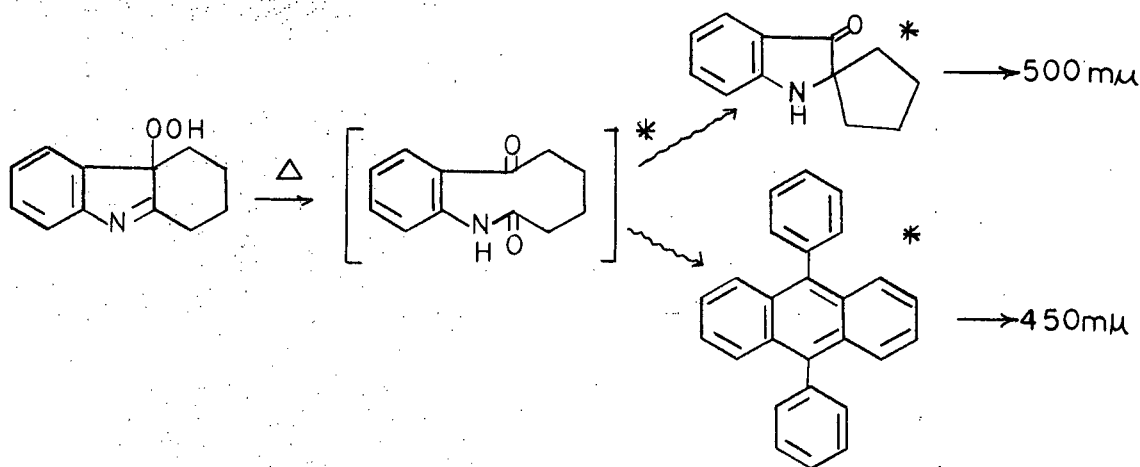

Although the mechanism of the "light" and "dark"

reactions has not yet been proven, the present experimental

data suggest two possible working mechanisms. The fact

that chemiluminescence occurs only either by heating or

by the action of dry potassium t-butoxide/DMSO suggests that

the resulting decomposition could occur via an energetic

four-centered transition state as follows:[61]
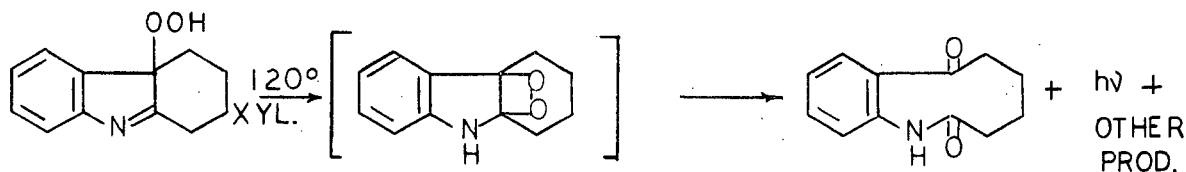
Since no light is observed in solvents with water present or in ethanol, this suggests an internal base catalysed decomposition. This may be a route which is not energetic enough to elevate the carbonyl product to an electronically excited state (Eq. 3).
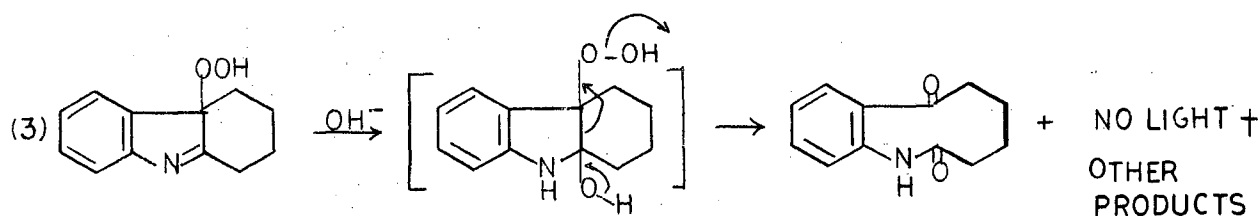
B) Emitter Identification.

The chemiluminescence study of two of the brightest light producing indolyl peroxides has identified the emitting species in this series of indolyl peroxides as the dicarbonyl product. A bright green light is observed on treatment of a solution (10^{-4}M) of either (X11A) or (XV111A) in DMSO with potassium t-butoxide also in DMSO (10^{-3}M) (Fig. 12). Stronger solutions (10^{-1}M) were easily visible in daylight. As mentioned previously (Part I) the only products isolated were shown to be (XXa), (XXb) and (X11c) from the reaction of (X1 A) and (XV111a) from the reaction of (XV111A). The compound (X11b) is slowly formed from (X11c) under the influence of base and therefore is probably not a product of the light reaction.

Fig. 12

(XIIc)

(XIIb)

(XIIA & XVIIIA)

(XIIa & XVIIIa) , For (XIIA,a) R= <benzene>—OCH₃ · For (XVIIIA,a) R = CH₃
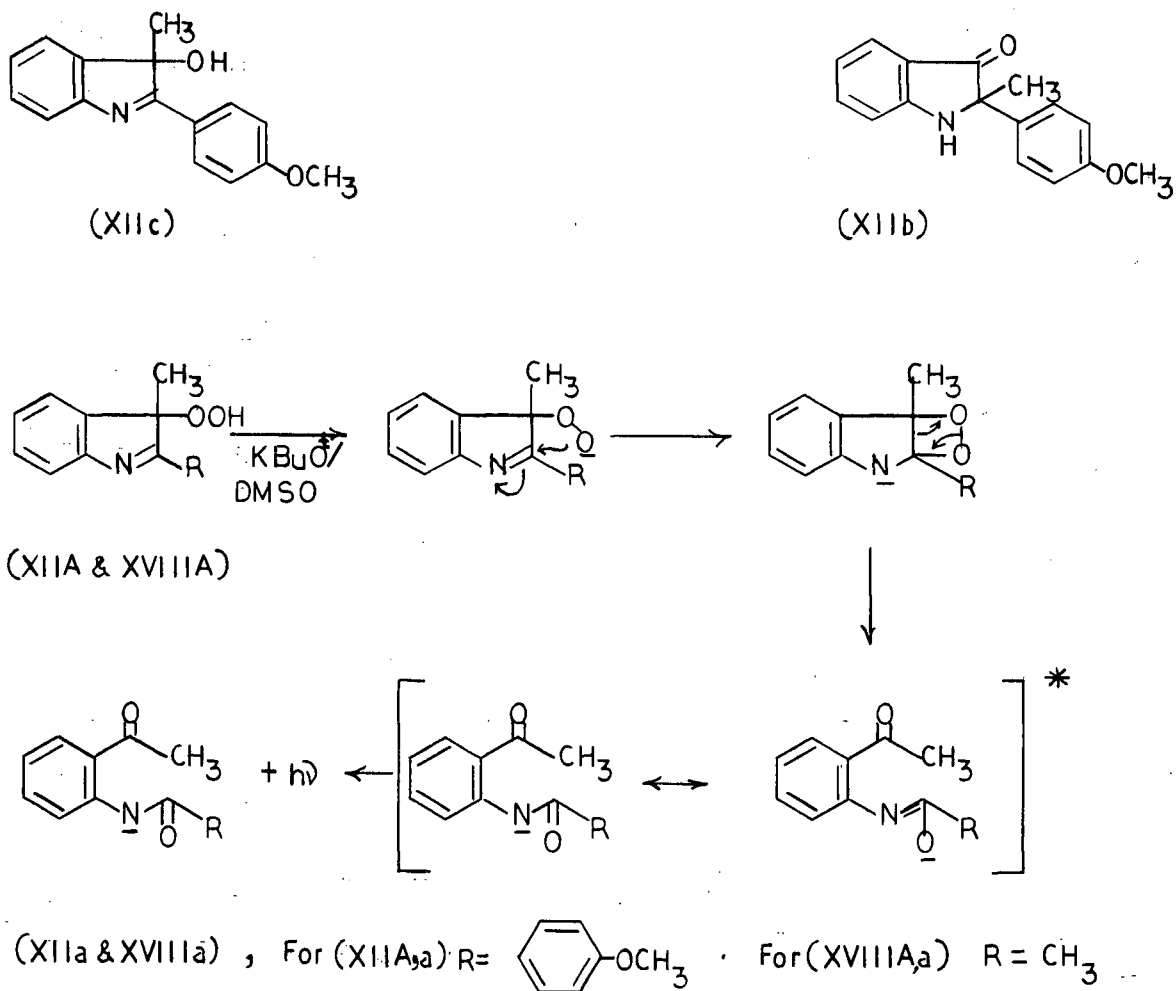
The major product of the reaction is the dicarbonyl compound
(60-70%) in both cases. The fluorescence spectrum of
(XVIIIa) has a maximum at 518 mμ , superimposable upon the
chemiluminescence spectrum of XVIIIA) (Fig. 13).

Fig. 13
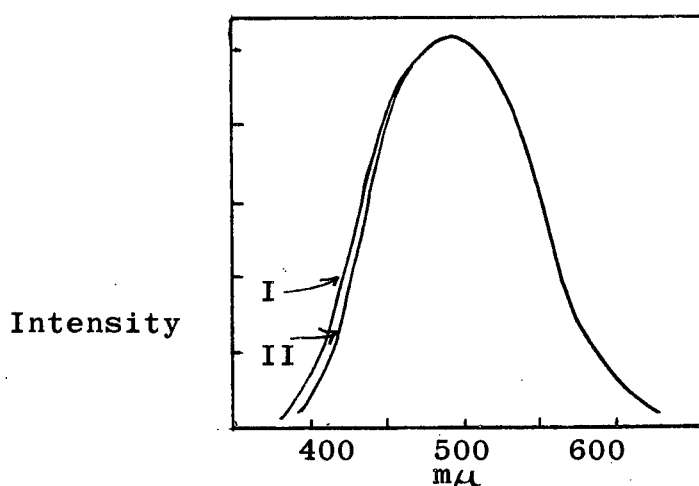
(I) Fluorescence of o-acetamidoacetophenone (XVllla)
(II)Chemiluminescence of 2, 3-dimethyl-3-hydroperoxy-
     indolenine (XVlllA), both in Kt-BuO/DMSO.

Fig. 14
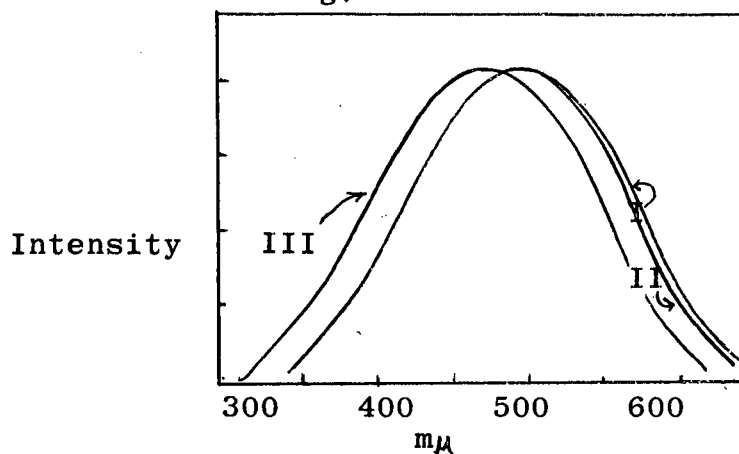
(I)   Fluorescence of 2-(p-methoxybenzamido)acetophenone
      (Xlla).
(II) Chemiluminescence of 2-(p-methoxyphenyl-3-methyl-
      3-hydroperoxyindolenine (XllA).
(III) Fluorescence of 2-(p-methoxyphenyl)-3-methyl-3- hy-
      droxyindolenine (Xllc) all in Kt-BuO/DMSO.

Similarly, the chemiluminescence of (XIIA) is identical to

the fluorescence of (XIIa), the maximum of both occurring

at 495 mμ(Fig. 14). This correspondence between the fluores-

cence and chemiluminescence spectra leaves little doubt as

to the identity of the emitter. Furthermore, the possibility
of the alcohol (Xllc) being an emitter is eliminated since its
fluorescence maximum comes at 485 mμ. Although the chemi-
luminescence spectra of the remaining indolyl peroxides were
not measured due to their low chemiluminescent intensity
(self-absorption would be acute) it is confidently suggested
that the excited state of the corresponding dicarbonyl anions
would be the emitting species of this series of indolyl per-
oxides.

C) Substituent Effect.

Within the group of para-substituted lophines,[25,31]
the light yields increased with the electron-releasing
ability of the substituents. A similar effect has been shown
to occur in the chemiluminescence of indolyl peroxides under
study.

Fig. 15
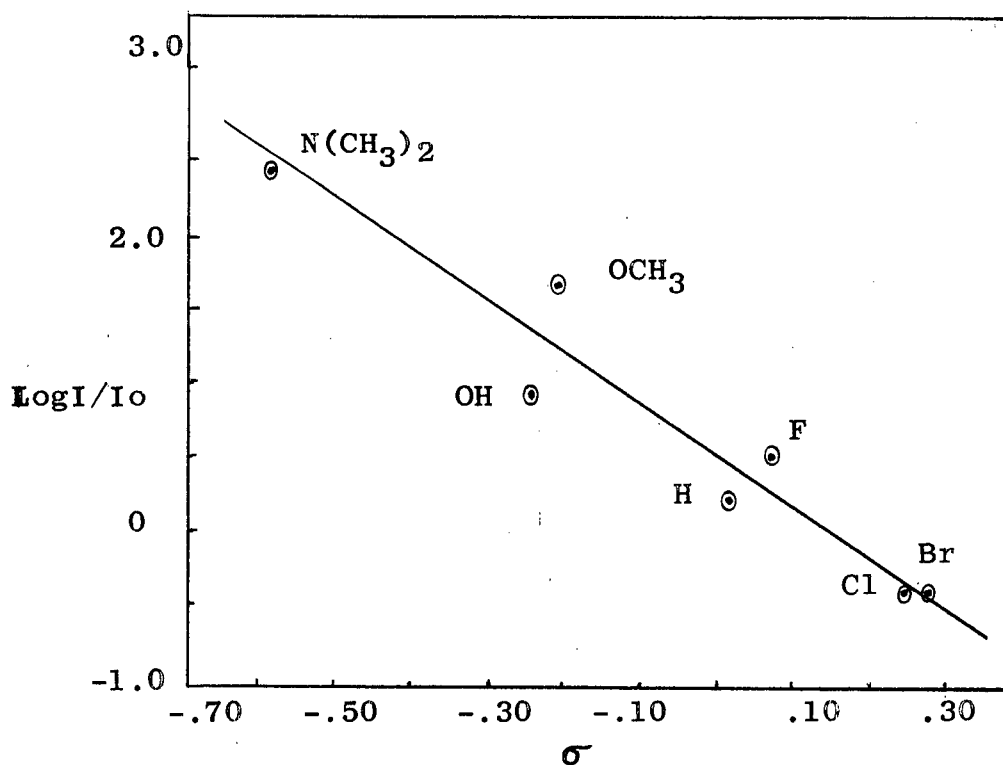
plot of log I/Io vs σ for indole peroxide derivatives with **para**-substituents in the 2-phenyl group.

Figure (15) presents a Hammett plot of log (I/Io) against values of σ taken from Jaffe.[71] I is the intensity observed for the derivative (substituted at the para-position of the 2-phenyl). Io is the intensity for the unsubstituted indolyl peroxide (XA). At the present it is not clear whether the relationship is due to rate (cf. Part III) or enhanced fluorescence efficiencies, as the latter have not been measured.

It is interesting that the ratio of the chemiluminescence

of (XllA): (XVlllA) is equal to the ratio of fluorescence intensities of the corresponding excited products (Xlla): (XVllla) (about 10:1 in each case). It is of significance to point out that we are working with relatively efficient chemiluminescent compounds, as the chemiluminescent intensity of (XVlllA) is estimated to be hundred times more efficient than lophine peroxide. The fluorescent quantum yield of (XVllla) is 0.016 (1.6%) using quinine sulfate as reference.[58] This value represents only one-tenth the magnitude of the fluorescence quantum yield of the aminophthalate ion (II) (10%) recently measured.[27] With such a low fluorescence quantum yield for (XVllla) it is likely that this quantity could be increased by suitable substitution.

PART III: KINETICS OF CHEMILUMINESCENCE

EXPERIMENTAL

Kinetic Method.

The apportus used was the same as that for measuring chemiluminescence efficiencies. However the light emitting source was 35 cm away from the photomultiplier tube for the 2,2-dimethyl-3-hydroperoxyindolenine (XVIIIA) and 2-(p-methoxyphenyl)-3-methyl-3-hydroperoxyindolenine (XIIA). The peroxide (XIIA) and the hydroperoxides containing halogen substituents were 10 cm away since these are much lower in intensity.

A typical run was carried out as follows: a curve traced from the chemiluminescent reaction of (XVIIIA) $(2ml, 10^{-3}M)$ and excess (10 fold) potassium t-butoxide $(3 ml, 10^{-2}M)$ all in DMSO was plotted as log I (I= intensity $\leftrightarrows$ cm) versus time (sec.). From the graph the pseudo-first order constant is calculated. All the reactions were carried out at room temperature.

DISCUSSION

The chemiluminescence of compound (XXA) and (XV11A) showed a first order dependence on the concentration of (X11A) and (XV111A) (in the presence of an excess potassium t-butoxide/DMSO.) Also, on diluting reaction mixtures of these peroxides, the light emission under the same conditions was proportional to the amount of the peroxide used.

Kinetic measurements support our view of the mechanism of the light emission step (Fig. 12. Part II). Peroxide (X11A) and (XV111A) react with base to yield light by first order kinetics. The rates were followed by measuring the light emission from 10^{-3}M to 10^{-5}M solutions of the peroxides treated with an excess of potassium t-butoxide in dimethyl sulphoxide (10^{-2}M) at room temperature; the data are given in Table 2. Plots of log light intensity (a measure of concentration) vs. time for both compounds were straight over 80% of the reaction time (Fig. 16).

Table 2

Molar Concn. (in DMSO)		First Order	Relative
Comp.	KBuO^t	rate constant(sec^{-1})	Intensity
XV111A	0.4x10^{-3}M	-10.37	1000
	0.4x10^{-4}M	-10.37	100
	0.4x10^{-5}M	- 7.35	10
X11A	0.4x10^{-3}M	- 3.22	100
	0.4x10^{-4}M	- 3.40	10
	0.4x10^{-5}M	- 3.22	1
XV11A	0.4x10^{-3}M	- 4.79 (σ= -.062)	0.029
XV1A	0.4x10^{-3}M	- 4.14 (σ= +.227)	0.004
XVA	0.4x10^{-3}M	- 3.86 (σ= +.230)	0.004

Fig. 16

Log (I) vs. time (sec.), I= intensity, of (XllA)

All in DMSO at room temperature.

1. conc. 0.4×10^{-3} M (0.2 volt, 35 cm. distant from phototube)

2. conc. 0.4×10^{-4} M (0.2 volt, 10 cm. distant from phototube)

3. conc. 0.4×10^{-5} M (0.05 volt, 10 cm. distant from phototube)

Fig. 17

$\frac{1}{\sqrt{I}}$ vs. time (sec), I = intensity of (X11A)

All in DMSO at room temperature
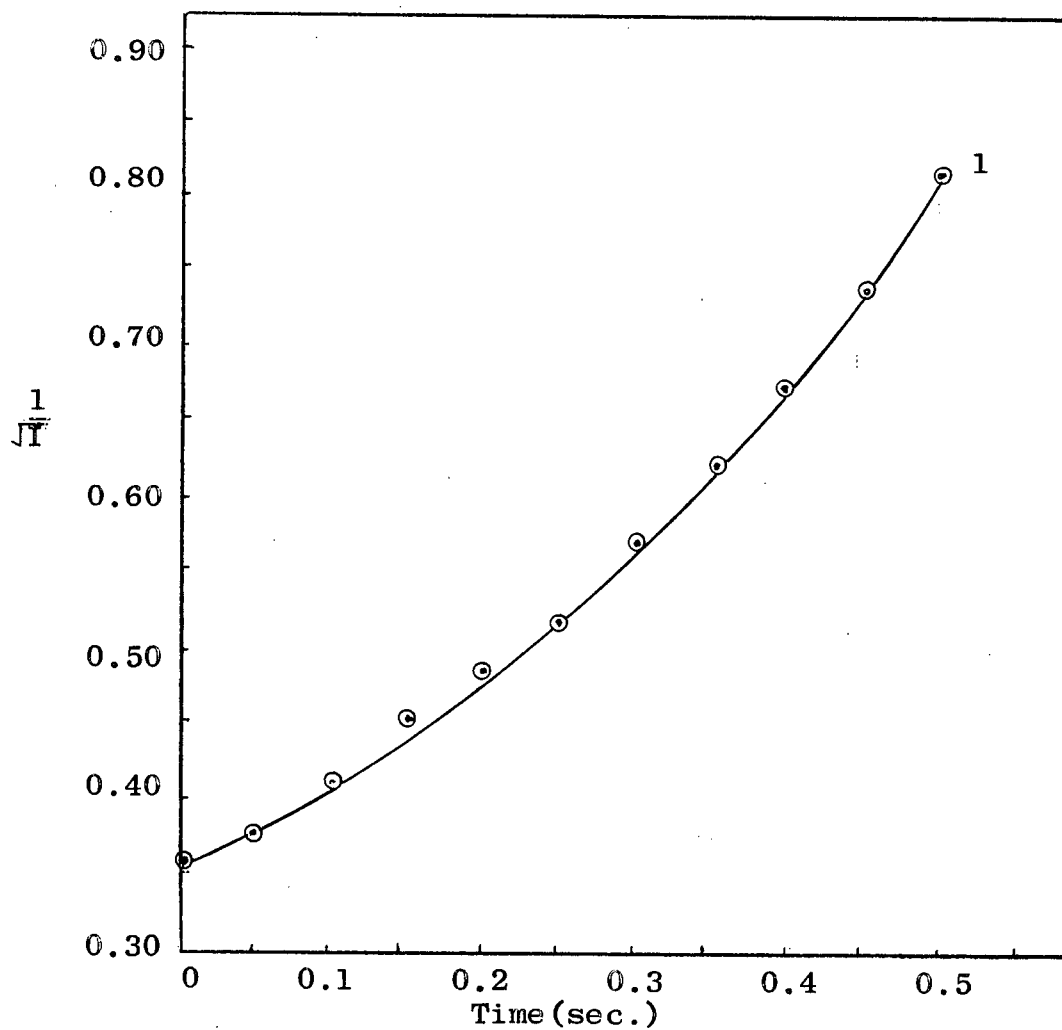
1. conc. 0.4×10^{-3}M (0.2 volt, 35 cm from phototube)

In other checks on the mechanism of the reaction, we have measured the integrated light yields of 2-(p-methoxy-phenyl)-3-methyl-3-hydroperoxyindolenine (XIIA) as a function of dilution. The results shown on Table 3 demonstrate the proportionality of total light yield to the concentration of (XIIA). This fact eliminates a mechanism involving the collision of two peroxide derived molecules in the production of light.

Table 3.

Chemiluminescence System	Total Light Yields (arbitrary units)		
	$0.4x10^{-3}M$	$0.4x10^{-4}M$	$0.4x10^{-5}M$
Comp. (XIIA) in Kt-BuO/DMSO	1.00	0.10	0.001

As a further check, the same data, which show a first order relationship when plotted log (I) vs. time, showed a curve when plotted $1/\sqrt{I}$ vs. time (Fig. 17). Thus this provided further proof that light emission is not a second order function.

Our work has demonstrated that the totally new discovery of the chemiluminescence of indolyl peroxide is similar to other well known chemiluminescent systems.[25,32] In effect, the emitter has been identified as the anion of the dicarbonyl reaction product which is produced in considerably high yield. Here also, electron donating groups enhance the chemiluminescence intensity. The chemiluminescence of indolyl peroxides showed a first order dependence on the concentration. In view of the data available the mechanism (Fig. 12) is suggested. Further isotopic labelling work is continuing in our laboratory in order to establish the proposed mechanism and to link the chemiluminescence of organic compounds with bioluminescence in living matter.

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APPENDIX

Derivation of the rate expression for the kinetics in excess base. Assume a reaction of order "n" in reagent "R" (where $[R]$ = indolyl concentration , $[P]$ = dicarbonyl , and * = excited state) described by the following steps (where solvent quenching is also included).

$$nR \xrightarrow{\quad k_1 \quad} P^* \qquad (1)$$

$$P^* \xrightarrow{\quad k_2 \quad} P + h \qquad (2)$$

$$P^* \xrightarrow{\quad k_Q \quad} P \qquad (3)$$

If reaction (1) is rate controlling, under steady state conditions $(k_2 \gg k_1)$

$$\frac{dp^*}{dt} = 0 = k_1 R^n - (k_2 + k_Q)P^* \qquad (4)$$

$$P^* = \frac{k_1}{k_2 + k_Q} R^n \qquad (5)$$

since $\quad \dfrac{dP}{dt} = (k_2 + k_Q)P^*$ from (2 and 3) $\qquad (6)$

substitute (5) into (6) and obtain

$$\frac{dP}{dt} = k_1 R^n \qquad (7)$$

Furthermore since

$$I = \frac{dh\nu}{dt} = k_2 P^* \quad \text{from (2)} \qquad (8)$$

$$\text{or } I = \frac{k_2 k_1}{k_2 + k_Q} R^n \quad \text{from (5)} \qquad (9)$$

$$R^n = \frac{k_2 + k_Q}{k_1 k_2} I$$

$$\frac{-dR}{dt} = k_1 R^n \quad \text{from (1)} \qquad (10)$$

if n=1, First order

(9) becomes $R = \dfrac{k_2 + k_Q}{k_1 k_2} I$ and differentiating

with respect to (t)

$$\frac{dR}{dt} = \frac{k_2 + k_Q}{k_1 k_2} \frac{dI}{dt} \tag{11}$$

substitute (9) into (10) and equate to (11) to get

$$-\frac{dR}{dt} = k_1 \frac{k_2 + k_Q}{k_1 k_2} I = \frac{k_2 + k_Q}{k_2 k_1} \frac{dI}{dt}$$

$$\frac{dI}{dt} = k_1 I \quad \text{integrating}$$

$$\ln I = k_1 t + c$$

if n=2, Second order

(10) becomes $\dfrac{-dR}{dt} = k_1 R^2$ integrating and get

$$\frac{1}{R} = k_1 t + C \tag{12}$$

then (9) becomes $R^2 = \dfrac{k_2 + k_Q}{k_1^2 k_2} I$ substituting

into (12) and get

$$\frac{1}{\sqrt{I}} = k't + C \quad \text{where } k' = k_1 \sqrt{\frac{k_2 + k_Q}{k_1 k_2}}$$