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Indigo Chemiluminescence

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Indigotin in basic solutions of polar aprotic solvents reacts with atmospheric oxygen yielding isatin. The reaction is accompanied by emission of light with a quantum yield equal to 2.6×10^{-4} Einstein/mol. The spectral distribution of the indigotin chemiluminescence is that of the isatin fluorescence. Addition of equimolar amounts of isatin to the reaction mixture raises the chemiluminescence quantum yield to 5.3×10^{-4} Einstein/mol. A mechanism involving the intermediate formation of indigotin dioxetan is proposed.

(Keywords: Autoxidation; Chemiluminescence; Indigo; Isatin; Quantum yields)

Indigo-Chemilumineszenz

Indigotin reagiert in basischen Lösungen von polaren aprotischen Lösungsmitteln mit atmosphärischem Sauerstoff zu Isatin. Die Reaktion wird von Lichtemission mit einer Quantenausbeute von $2,6 \times 10^{-4}$ Einstein/mol begleitet. Die spektrale Verteilung der Indigo-Chemilumineszenz ist die der Isatin-Fluoreszenz. Zugabe äquimolarer Isatinmengen zur Reaktionsmischung hebt die Quantenausbeute der Chemilumineszenz auf $5,3 \times 10^{-4}$ Einstein/mol. Es wird ein Mechanismus mit intermediärer Bildung von Indigotin-dioxetan vorgeschlagen.

Introduction

Following the elucidation of the structure of the cypridina luciferin and the luciferins of other marine organisms, interest was re-focused on the chemiluminescence of indole derivatives[1–5] as such luciferins were shown to contain the indole moiety. Thus, a large number of substituted indoles were studied and shown to be moderately chemiluminescent, the energy generating reaction being the cleavage of the 2,3-bond, thought to proceed via an intermediate dioxetan at this site.

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If indigotin (name proposed for pure indigo[6]) is considered an indole derivative, the present authors reached it as a potentially chemiluminescent compound from a different angle. Indeed, the chemiluminescence of indigotin was investigated as part of a general study of chemiluminescent oxidations of compounds having the general formula $F = A$ (F, fluorescent moiety) leading to fluorescent carbonyl products[7–9].

The following sections are therefore concerned with showing that oxidation of indigotin is a chemiluminescent reaction, establishing the optimum conditions for chemiluminescence, identifying the primary excited product and proposing a reaction mechanism.

Results and Discussion

The chemiluminescence of indigotin (I) was studied in polar aprotic solvents such as dimethylsulfoxide (*DMSO*), *N*,*N*-dimethylformamide (*DMF*) and in the solvent reagent *DMSO*—*t*-butanol—potassium-*t*-butoxide. Bases employed were alkali hydroxides and potassium-*t*-butoxide while the oxidants were air, ozone, hydrogen peroxide and potassium persulfate. Maximum chemiluminescence efficiencies were obtained on air-oxidation of indigotin in *DMF* rendered alkaline with sodium hydroxide. Similar results were obtained in *DMSO*, but in this case the light intensity time diagrams were not always reproducible probably due to more strict temperature dependence.

Ozone oxidation of indigotin (I) in *DMF* and *DMSO* solutions was a practically dark reaction although isatin (generally accepted name for indole-2,3-dione) (V), the expected product[6] was identified in the reaction mixture. Ozonation, however, of solutions rendered alkaline by the addition of aqueous sodium hydroxide was moderately chemiluminescent with a quantum yield of 4×10^{-5} Einstein/mol, the excited product being isatin (V) as established by comparison of the reaction's chemiluminescence spectrum, with the fluorescence spectrum of isatin under similar conditions and that of the spent mixture.

More efficiently chemiluminescent was the oxidation of indigotin by air in alkaline *DMF* solutions, the chemiluminescence quantum yield being in this case 2.6×10^{-4} Einstein/mol. It should be noted, however, that the reaction mixture's temperature is critical and should be maintained in the region 9–12 °C. The light intensity-time diagram of such a reaction is shown in Fig. 1, the asterisks (*) indicating additions of sodium hydroxide. It can be seen that addition of alkali to an indigotin solution in *DMF* results in light emission characterised by low intensity and short duration. Then, after an induction period of a few minutes, light emission starts again, the intensity and duration of which is maximum when the temperature does not exceed 12 °C. Further addition of alkali then gives rise to the major light sum obtainable from the reaction, roughly proportional to the light sum of the delayed emission.

The intensity-time diagram and especially the delayed emission of ca. 5 minutes is most intriguing. As it will be seen later, there is no doubt that isatin (V) is the reaction's excited product and this is fairly stable under the experimental conditions employed. Although replacement of sodium hydroxide by potassium *t*-butoxide, a stronger base in polar aprotic solvents, did not increase the quantum yields (in fact they were lower by 1-2 orders of magnitude), it was thought at this point that perhaps the isatin produced as the reaction proceeds is a stronger base than sodium hydroxide in *DMF*, thus facilitating the reaction's first step (Scheme). Our results, however, were inconclusive when the experiment shown in Fig. 1 was repeated in the presence of an added equimolar amount of isatin. Although the chemiluminescence quantum yield was increased by 100%, reaching the figure of 5.3×10^{-4} Einstein/mol, the interval for the delayed emission was only little shortened. Most probably, here, some decomposition slowly occurs to isatin producing a species that catalyses the light reaction; a similar only less pronounced effect has been reported for indole chemiluminescence[2].

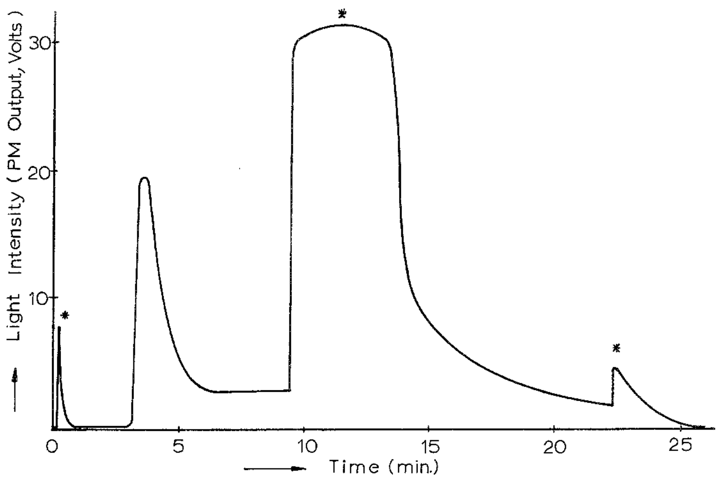


Fig. 1. Light intensity-time diagram of the reaction of indigotin (20 ml, $10^{-4} M$) in *DMF* with air (flow rate 340 ml min^{-1}); asterisks (*) represent additions of 0.1 ml, 2*N*-sodium hydroxide

Regarding the reaction's emitting species, there is strong evidence that this is the isatin anion (V) and this evidence is shown in Fig. 2, where the reaction's chemiluminescence spectrum (C) is compared with the fluorescence spectrum of isatin under conditions similar to those of the light reaction (F_i) and that of the reaction mixture at the end of the chemiluminescence emission (F_{rm}). Indeed, the chemiluminescence spectrum of a reaction should be identical with the fluorescence spectrum of the excited product as both emissions result from the same electronic transition of the same molecule. Furthermore, here, monitoring of the fluorescence associated with the isatin anion as the light reaction proceeds, indicates a continuous increase of isatin concentration.

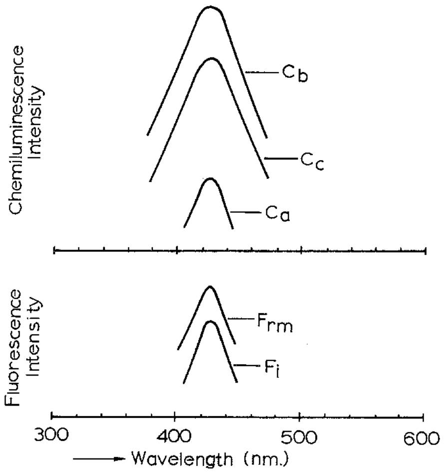
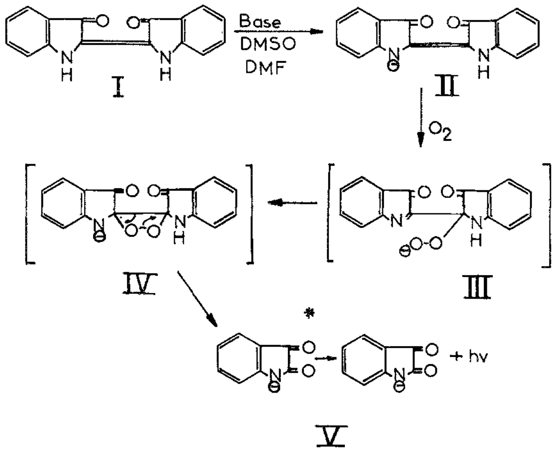


Fig. 2. Luminescence spectra. *C* Chemiluminescence spectra of the reaction after 5 min *a*, 16 min *b*, 24 min *c*, sensitivity × 3 for C_c. F_{rm} fluorescence spectrum of the reaction mixture; F_i fluorescence spectrum of isatin under conditions similar to those of the light reaction

Regarding the mechanism of the air-oxidation light reaction, it should be noted that oxygen is indeed a reactant; replacement of the air-stream by a nitrogen-stream results in a dramatic drop of the light-intensity. An autoxidation mechanism involving the indigotin dioxetan (IV), a most attractive intermediate in chemiluminescence reaction mechanisms is proposed in the Scheme below. A similar mechanism involving cleavage of the 2,3-bond of indole and substituted indoles via an intermediate dioxetan has been proposed for the chemiluminescence of indoles[3, 5]. Here, however, only cleavage of the indigotin 2,2'-bond can account for the formation of isatin (V) and a similar cleavage via a primary ozonide[7,8] can account for the chemiluminescence of the indigotin ozonation reaction described above, where the isatin anion is again the emitting species.

Scheme 1



Experimental

Ozone: A self-constructed Siemens-type ozonator was employed giving an ozone-air stream 0.26% *v/v* at a flow rate of 340 ml min^{-1}.

Chemiluminescence intensity-time diagrams: Air, or ozone-air mixture was bubbled through the reaction mixtures at a flow rate of 340 ml min^{-1}. The reaction vessel (pyrex glass) was securely positioned in front of an EMI 9514 B photomultiplier tube supplied with 1000 V and connected to a BRUKER B-R70D recorder and a TIME ELECTRONICS TS 100A integrator, the reaction vessel and the photomultiplier being housed in a specially constructed dark chamber with suitable inlets for gasses and liquids. The reactions were carried out with 20 ml, $10^{-4} M$ indigotin (EASTMAN) solutions in *DMSO* or *DMF* by squirting in, the appropriate reagents through the dark chamber inlet, several times if necessary, to complete the reactions.

Chemiluminescence quantum yields: The integrals of the chemiluminescence intensity-time diagrams recorded, were compared with the integral of the Luminol Standard[10,11] employing the same optical geometry and the quantum yields were thus calculated based on reactant employed. Corrections due to the photomultiplier's spectral response were unnecessary as the chemiluminescence of the reactions under study as well as that of the Luminol Standard fall in the same spectral region of the photomultiplier's maximum efficiency. Corrections, however, due to self absorption were necessary and were calculated taking into account the mean transmittance of the reaction mixture at the beginning and the end of the reactions at the chemiluminescence emission maximum, as well as the mean light-path in the reaction vessel. The correction factors thus calculated were equal to 2.0 for reactions with alkali-air and alkali-ozone and 2.5 for the same reactions with added isatin.

Spectra: Excitation and fluorescence spectra were run on an AMINCO-BOWMAN spectrophotofluorometer calibrated with a quartz "pen-ray" lamp and are uncorrected. Chemiluminescence spectra were recorded on the same instrument employing fast scanning rates and wide slits, with the excitation source off, while present transmittance was determined with the aid of a CARY 14 spectrophotometer.

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