INDOLE-3-PYRUVIC ACID AS A POTENTIAL LUCIFERIN

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Summary. Indole-3-pyruvic acid luminesces in aerated dimethyl-sulfoxide solutions in the presence of potassium tert-butoxide. The chemiluminescence spectrum indicates the occurrence of multiple bands whose relative intensities change with time. This behaviour is connected with the presence of two forms of indole-pyruvic acid and with two different reactions, namely at the side chain giving indole-3-carboxaldehyde and at the indole nucleus giving ultimately a product of the N-formyl-kynurenine type.

The results suggest that indole-pyruvate is a potential luciferin. This inference is strengthened by the fact that it can originate "in vivo" both indole-aldehyde and oxalate.

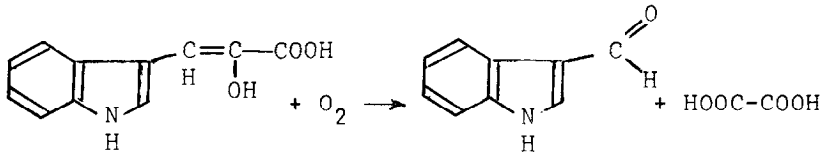
The reaction at the indole nucleus is tentatively considered a model for tryptophan dioxygenase and related systems.

Earlier work (1) indicated that several biochemical systems, besides the luminescent ones, are likely to produce an electronically excited product through the cleavage of an intermediate 1,2-dioxetane On this context, the first system to be experimentally investigated, the cleavage of the hydroperoxide formed from 4-hydroxy-3,5-diiodo-phenylpyruvate and oxygen, produced indeed 4-hydroxy-3,5-diiodo-benzaldehyde in the excited singlet state (2).

A compound which has the potentiality of a luciferin is IPA. It has an activated $-CH_2-$ group and reacts through the enol form with oxygen originating the products expected from a dioxetane

Abbreviations: IPA, Indole-3-pyruvic acid; IA, Indole-3-carboxaldehyde; DMSO, dimethyl-sulfoxide; tert-BuOK, potassium tert-butoxide.

intermediate, namely IA and oxalate (3,4):



IPA IA

Of considerable importance is the fact that IPA can be formed from tryptophan by the amino acid oxidases and that a specific tautomerase which catalyses the formation of the reactive enol tautomer is present in rat liver (5). Most important, tryptophan gives rise "in vivo" to oxalate and there is evidence that the pyruvic analog is an intermediate (6); furthermore in the case of D-tryptophan administration to rats, IA has been isolated from the urine (7).

Of additional interest is the fact that the reaction of IPA and oxygen is expected to give excited products also by reaction of the indole nucleus (8-11).

In bioluminescent systems a luciferin luminesces in the proper system in the presence of a luciferase. However in mechanistic model studies the luciferin is commonly studied in an aprotic solvent in the presence of a strong base.

We report in this paper the luminescence of IPA in aerated DMSO in the presence of tert-BuOK, and we describe the implications of the results in connection with the generation of excited electronic states in biological systems.

Experimental

IPA was from K an K Laboratories. IA was prepared from

indole, dimethylformamide and $POCl_3$ (12). The luminescent reactions were studied by adding 0.4 ml of an approximately 6 x 10^{-2}M solution of tert-BuOK in tert-butanol to 2.0 ml of an approximately 6 x 10^{-4}M solution of the compound in aerated or oxygenated DMSO at room temperature.

The intensity and spectral distribution of the chemiluminescence were measured on an Aminco-Bowman Spectrophotofluorometer with the exciting source off. Fluorescence spectra were measured on this same apparatus without corrections for wavelength sensitivity of the phototube or intensity fluctuation of the excitation source. Absorption spectra were measured in a Zeiss DMR-21 Recording Spectrophotometer.

To prove oxalate formation, water was added to the spent reaction mixture which was then evaporated and the residue taken up in dilute acetic acid and the solution filtered. Addition

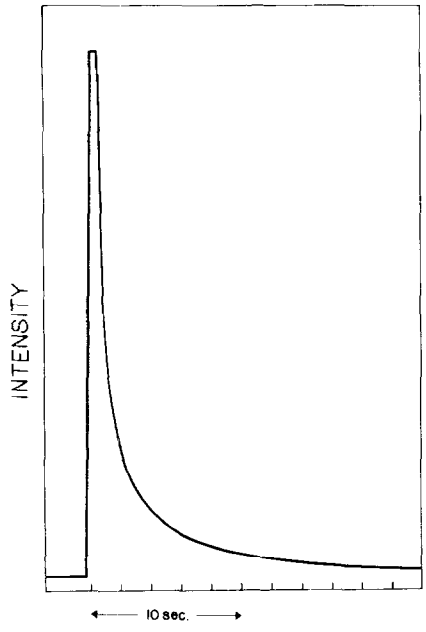
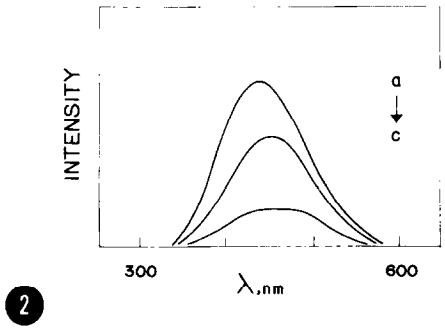


Fig. 1. Light emission by IPA in aerated DMSO containing tert-BuOK, as a function of time.



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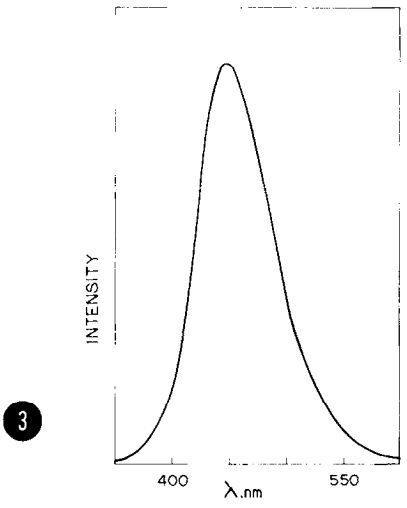

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Fig. 2. Chemiluminescence spectrum of IPA in DMSO taken 5 sec. (a), 10 sec. (b) and 45 sec. (c) after adding tert-BuOK. λ_{max} of curve (a) is at 434 nm; of curve (b) is at 452 nm.

Fig. 3. Fluorescence spectrum of the spent reaction mixture of IPA in DMSO containing tert-BuOK.

of calcium ions at this pH (5.0) or to a NH_4OH alkalinized solution resulted in slow formation of a precipitate identified as calcium oxalate by the glyoxalic acid test (13). No precipitation occurred in the absence of Ca^{++} ions.

RESULTS AND DISCUSSION

The addition of tert-BuOK to a solution of IPA in DMSO results in production of light. A representative example is shown in Fig. 1. The initial rise of curves measured at 435, 450 and 485 nm followed the order $I_{435} < I_{450} \sim I_{485}$ nm. A faint blue emission can be observed even visually. Aeration of the solution in the long decay stage increase the light emission.

The chemiluminescence spectrum taken at the beginning of the decay stage shows a maximum near 435 nm, the exact position

TABLE I

Position of fluorescence and chemiluminescence bands in systems of IPA and IA

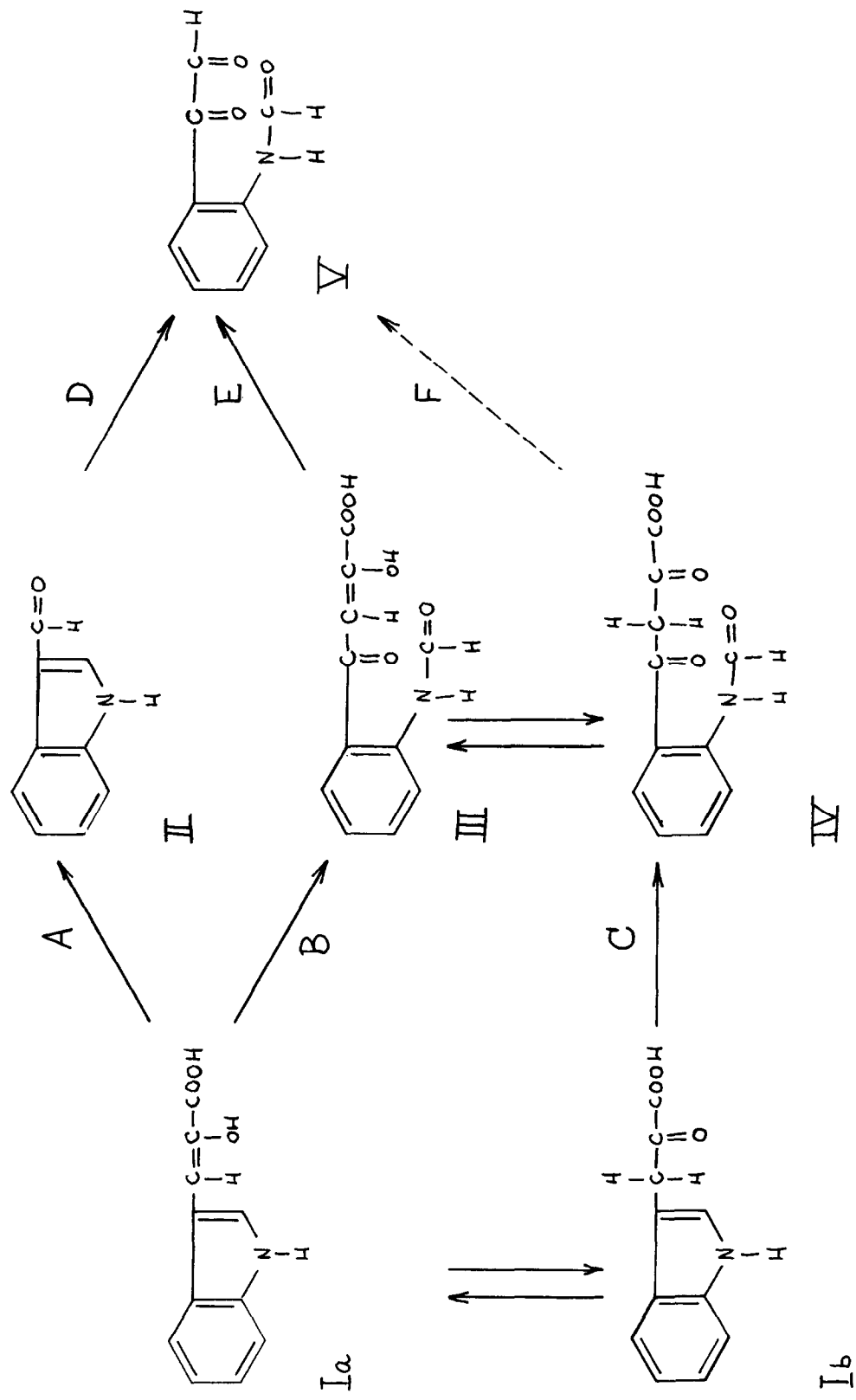
	Fluorescence (nm)	Chemiluminescence (nm)
IPA reaction	-	435; 450; 485
IPA (under N_2)	470	-
IA (under N_2)	430	-
IPA (spent reaction mixture)	450	-
IA reaction	-	445
IA (spent reaction mixture)	445	-

being somewhat, but not considerably affected by the decay. At a later time the chemiluminescence spectrum shows the maximum near 450 nm; this maximum then becomes very flat, clearly indicating multiple chemiluminescent emission (Fig. 2). This behavior was confirmed by several experiments. Occasionally a shoulder near 485 nm could be observed.

Very interestingly, the fluorescence spectrum of the spent reaction mixture showed only one well-defined band peaking near 450 nm (Fig. 3). Also the chemiluminescence spectrum of IA showed a maximum near 450 nm albeit of low intensity; this spent reaction mixture of the aldehyde had a fluorescence spectrum similar to that of the spent reaction mixture from IPA (TABLE I).

We shall discuss our results according to SCHEME I. It must

SCHEME I



however be pointed out that the emitter in the chemiluminescent reaction at the indole nucleus has never been properly identified(8), although, most likely, it should be formed in a dioxygenase type reaction.

Initially at t = o, reactions A, B and C are expected to occur. By analogy with 3-methylindole (8) reaction C is expected to give a relatively strong chemiluminescence emission peaking at 485 nm, the intensity depending upon the relative concentration of the keto form of IPA. It is likely that this reaction is very important at the begining of the reaction because the emission at 485 nm is stronger than at 435 nm.

Reaction A may be responsible for the peak initially seen near 435 nm. If so anionic IA is presumably the emitter because the neutral aldehyde fluoresces maximally near 345 nm (14), whereas the fluorescence emission of oxygen- depleted DMSO solution has a maximum in the 430 nm region (Fig. 4). The further reaction of IA (reaction D) produces only a very weak chemiluminescence confirming an earlier observation (8). This means that the 450 nm

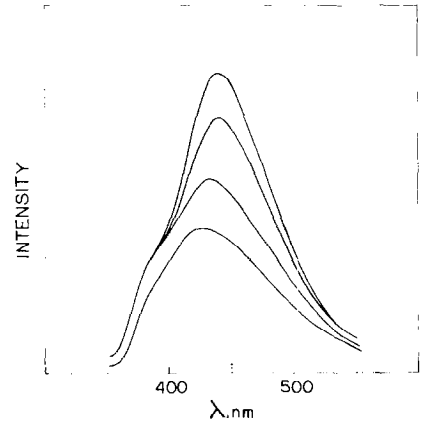


Fig. 4. Fluorescence spectrum of IA in DMSO containing tert-BuOK. The lower curve is the spectrum taken in anaerobiosis. The other curves were taken at various intervals after letting air into the system. λ_{max} for the aldehyde is at 431 nm.

emission observed in the latter stage of the reaction receives only a very small contribution from reaction D and therefore that the conversion of III and IV into V is the main source of that emission.

On this regard specially pertinent is the observation that despite multiple chemiluminescent emissions (Fig. 2) the spent reaction mixture shows only one well-defined maximum (Fig. 3). This means that ultimately V or a closely related species is the main product of the reaction. This inference is also supported by the similarity of the absorption spectrum of the spent reaction mixture of IPA and IA (Fig. 5) despite the expected presence of by-products (8). The absorption and fluorescence spectra of these spent reactions are, as expected, similar to those of N-formyl--kynurenine (15).

The question whether reaction B contributes to the chemiluminescence emission cannot be properly answered with the data

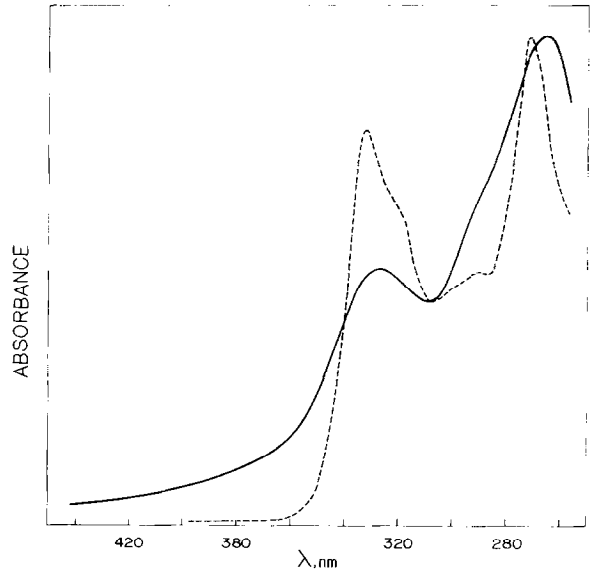


Fig. 5. Normalyzed absorption spectra of the spent reaction mixtures of IPA (——) and IA (---) in DMSO containing tert-BuOK.

available; we presume that in analogy with the IA behavior, Ia having a conjugated side chain should not be strongly chemiluminescent through path B.

Biological implications. The likely formation of electronically excited IA from IPA is of genuine interest in view of the reasons which inspired the present investigation. Moreover IA has also been detected in tissues, including tumors (16).

Bioluminescent processes may be investigated in aprotic solvents containing a strong base. Turning the argument around, the following question arises: if a biochemical system investigated under those conditions and displaying chemiluminescence, would produce under natural conditions an electronically excited product, though not necessarily emissive. The answer to this question is of paramount importance in connection with the generation and use of electronic energy in dark biological processes (1, 17). In our opinion a positive answer is potentially correct. According to this view, in the present case, the chemiluminescent reaction at the indole nucleus, while of interest in connection with the bioluminescence of indolic systems, may also be considered a potential model for tryptophan dioxygenase and similar systems. IPA, would then be a two-fold luciferin.

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