CHEMILUMINESCENCE IN THE AUTOXIDATION OF THE PYRUVIC ACID ANALOGUES OF A THYROID HORMONE AND RELATED MOLECULES

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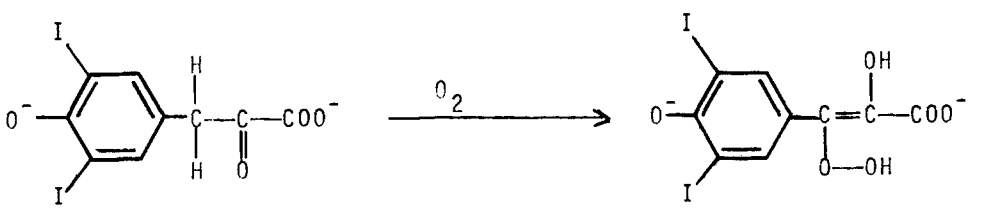
Summary Since the hydroperoxide formed from 4-hydroxy-3,5-diiodopheyl-pyruvic acid and oxygen, a likely precursor of thyroxine, is also known to undergo cleavage to 4-hydroxy-3,5-diiodobenzaldehyde and oxalic acid, that is, to products expected from a dioxetane intermediate, we have investigated the chemiluminescence of aerated solutions of 4-hydroxy-3,5-diiodophenylpyruvic acid. Light was emitted in dimethyl-sulfoxide solution containing potassium t-butoxide. The emitting species is the excited singlet aldehyde. Other pyruvic acid analogues, including that of the hormone 3,5,3'-triiodothyronine, chemiluminesce in similar conditions. Extremely weak emission occurs in aqueous solution.

In many luminescent reactions the products formed are as expected from the cleavage of an intermediate with a dioxetane structure, one of the carbonyl compounds being generated in an excited eletronic state (1). As judged from products formation and other evidence, a dioxetane intermediate is likely to be formed also in several oxidative biochemically important "dark" processes (2).

DIHPPA hydroperoxide, a likely intermediate in thyroxine biosynthesis (3,4), is formed from DIHPPA and O_2:



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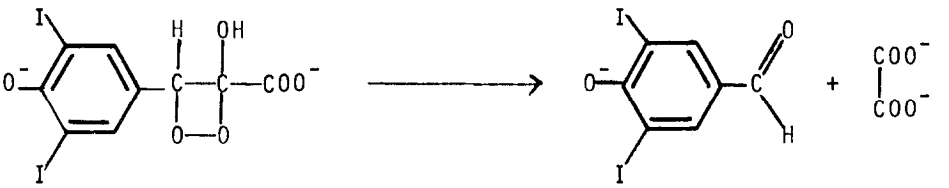
Abbreviations: DIHPPA, 4-hydroxy-3,5-diiodophenylpyruvic acid; DIHBA, 4-hydroxy-3,5-diiodobenzaldehyde; DMSO, dimethylsulfoxide; BuOK, potassium butoxide.

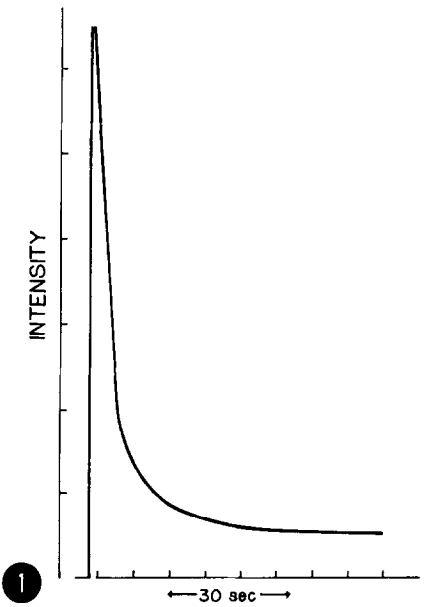
In alkaline solutions this hydroperoxide, even in anaerobiosis, forms DIHBA and oxalate (3), that is, the products expected from the cleavage of the dioxetane tautomer:



DIHBA and oxalate can be formed directly in aerated alkaline solutions of DIHPPA; similarly, p-hydroxyphenylpyruvic acid forms p-hydroxybenzaldehyde and oxalate (3). It was therefore of considerable interest to look for chemiluminescence in the autoxidation of these pyruvic acid derivatives. Furthermore, a dioxetane structure originating from a conjugated hydroperoxide is believed to be involved in some bioluminescent processes (5). The investigation has been extended to the chloro and bromo analogues of DIHPPA and to the pyruvic acid analog of the hormone 3,5,3'-triiodothyronine.

Experimental

The compounds were prepared from their corresponding amino acids by N-chloroacetylation, condensation to the oxazolone and hydrolysis (6). The luminescent reaction was studied by preparing an approximately 6×10^{-4}M solution of the compound in dry aerated DMSO and by adding to 2.0 ml of this solution in a quartz cell, 0.4 ml of a 6×10^{-2}M solution of tert-BuOK in tert-butanol. The reaction temperature was kept at 40°C with a thermostatic cell holder. The intensity and spectral distribution of the chemiluminescence emission were measured on a Hitachi MPF-2A type fluorescence spectrophotometer with the exciting source off. Fluorescence spectra were measured on this same apparatus. No correction was made for the wavelength sensiti-



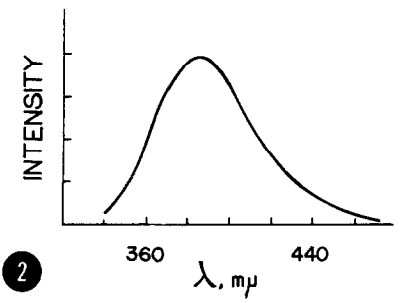


Figure 1. Light emission by 4-hydroxy-3,5-dichlorophenylpyruvic acid in aerated DMSO containing potassium tert-butoxide as a function of time. For details, see text.

Figure 2. Chemiluminescence spectrum of the longtail emission shown in Figure 1.

vity of the phototube or the intensity fluctuation of the exciting source. To detect chemiluminescence in aqueous solution the enhanced single photoelectron counting method was used (7).

Results and Discussion

All compounds studied emit light when dissolved in DMSO in the presence of ter-BuOK. Figure 1 shows the emission obtained with the chloro analog of DIHPPA. The fast decay to a continuous weak emission is probably due to oxygen consumption; the shape of the curve is influenced by the concentration of ter-BuOK. This behavior was general.

The chemiluminescence spectrum of the emission on the long-tail decay stage had a maximum in the region around 400 mμ. Figure 2

TABLE I

Chemiluminescence data for the pyruvic acid analogs of 3,5-diiodotyrosine and of some related molecules in DMSO containing potassium t-butoxide and in aqueous solution

	DMSO		WATER[a]
	Relative Intensity	λmax mμ	Relative Intensity[b]
4-hydroxyphenylpyruvic acid	9	380-390	0.7
4-hydroxy-3,5-dichlorophenylpyruvic acid	40	390	0.94
4-hydroxy-3,5-dibromophenylpyruvic acid	10	390-400	0.88
4-hydroxy-3,5-diiodophenylpyruvic acid	1	400-410	1.0
3,5,3'-triiodothyropyruvic acid	0.5	410	2.0

[a]NaOH /substrate ratio was 12.5. [b]Relative total light emission/1000 sec.

shows the chemiluminescence spectrum obtained with the chloro analog of DIHPPA. The position of the maximum and the relative intensity of the emission for all the compounds investigated are collected in Table 1. In every case the chemiluminescence spectrum and the fluorescence spectrum of the spent reaction mixture peaked at the same position. In turn, as shown in the case of DIHPPA, the fluorescence of the aldehyde also has its maximum at that position. Therefore the emitting species is the excited singlet aldehyde.

All compounds were found to emit very weakly in aqueous solution. The intensity was influenced by the NaOH concentration in a manner which was different for each compound. The relative intensities when the NaOH concentration was 12.5 times that of the substrate are presented in Table I.

Although the quantum yield is certainly low, the primary yield of excited species may have been much higher in view of the expected low efficiency of fluorescence emission. Despite the use of an aprotic solvent -a common procedure to observe chemiluminescence even with luciferins systems- and the fact that the halogenated aldehydes formed in our systems seem to have never been reported "in vivo" (p-hydroxybenzaldehyde is however widely found in plants) the present results are of interest in connection with the generation of electronic energy in dark biochemical processes (2,8).

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