Brief Communications

Chemiluminescence during interaction of 1,2,4-trioxolanes and 1,2,4,5-tetraoxanes with iron compounds

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The reaction of triterpene 1,2,4-trioxolanes with the system $FeCl_3$—L-cysteine hydrochloride in the presence of rhodamine G is accompanied by chemiluminescence, whose emitter is an excited dye molecule. Light emission was also found in the biologically important reaction of 1,2,4,5-tetraoxanes with Fe^{2+} ions in aqueous acetonitrile.

Key words: trioxolanes, tetraoxanes, chemiluminescence, triterpenoids.

In the recent decade, 1,2,4-trioxolanes (secondary ozonides) and 1,2,4,5-tetraoxanes attract attention of the world chemical society due to their outstanding biological activity.[1—4] The development of studies in this area occurs *via* two main routes: synthesis of new peroxide derivatives and investigation of their pharmacological properties.[1—5] Some trioxolanes are comparable with and even exceed the known medicine artemisinin and its analogs in antimalarial activity.[6] Presently, one of adamantanone trioxolane, arterolane (OZ277), undergoes the third phase of clinic tests as a drug against malaria. The tests are performed by the Ranbaxy Co.[7]

In the present work, we report the discovery of a new important property of trioxolanes, namely the ability to generate electron-excited states during chemical transformations, and new chemiluminescence appeared upon the interaction with divalent iron. Taking into account the importance of this class of peroxides for medicine, we may consider our results as the first stage in the development of a new approach for studying the properties of pharmacologically promising agents of peroxide nature based on chemiluminescence (CL).

Experimental

(3*R*,5*R*)-19β,28-Epoxy-4,5-seco-18α-olenane-3(5)-ozonide (**1**) and (3*S*,5*S*)-19β,28-epoxy-4,5-seco-18α-olenane-3(5)-ozonide (**2**) were synthesized from allobetulin according to a procedure described earlier.[8] Diperoxides of acetone **3** and 1,1,1-trifluoroacetone **4** were synthesized by known procedures.[9,10]

Photoluminescence spectra of rhodamine G and 1,1,1-trifluoroacetone in a MeCN—H_2O (1 : 1) mixture were recorded on a CM 2203 SOLAR spectrofluorimeter. Chemiluminescence in the visible spectral region was detected on a setup with a photoelectron multiplier (FEU-119) sensitive in the visible spectral region (λ = 330—750 nm) as follows. A solution of rhodamine G and $FeCl_3$ was added to the cell placed above the

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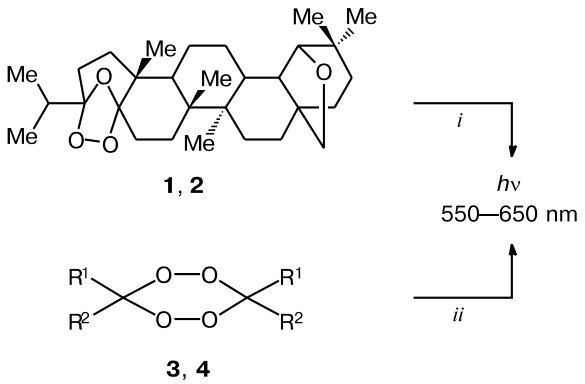
FEU photocathode and containing a solution of trioxolane and L-cysteine hydrochloride in such a way that the final concentrations of peroxides **1** and **2**, rhodamine G, and $FeCl_3$ would be $1.5 \cdot 10^{-3}$ mol L^{-1} and that of L-cysteine hydrochloride would be $3 \cdot 10^{-3}$ mol L^{-1}. In experiments involving tetraoxanes, a solution of $FeSO_4$ and rhodamine G was added to a solution of diperoxides **3** or **4**. The temperature of the experimental cell and the pusher was maintained at 70 °C for the reactions of trioxolanes **1** and **2** and acetone diperoxide **3** and at 30 °C for the reactions of tetraoxane **4**. A MeCN—H_2O (1 : 1) mixture was used as the solvent in all experiments. Depending on tasks of experiments, argon or oxygen was bubbled through a solution during the reaction. The CL spectrum was recorded using boundary light filters arranged in a cassette between the bottom of the temperature-maintained cell with the sample and the FEU photocathode.

Results and Discussion

The reaction of triterpenoid peroxides **1** and **2** with iron chloride in aqueous acetonitrile in the presence of L-cysteine hydrochloride and rhodamine G is accompanied by luminescence in the visible spectral region (Scheme 1).

Scheme 1



1: (3*R*, 5*R*); **2:** (3*S*, 5*S*); **3:** $R^1 = R^2 = Me$; **4:** $R^1 = Me$, $R^2 = CF_3$
i. $FeCl_3$, L-cysteine, rhodamine G, MeCN—H_2O (1 : 1);
ii. $FeSO_4$, rhodamine G, MeCN—H_2O (1 : 1).

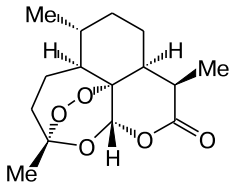
It turned out that for the reaction of **1** and **2** with $FeCl_3$ in an oxygen atmosphere the CL intensity increases considerably compared to the process occurring under argon. The TLC analysis performed after the decay of CL observed in experiments, which were carried out in both O_2 and argon, showed the absence of peroxides **1** and **2** in the reaction mixture.

The region of luminescence appeared upon the interaction of peroxides **1** and **2** with Fe^{3+} ions in the presence of L-cysteine was determined. The emission is concentrated in the range λ = 550—650 nm, which coincides with the luminescence spectrum of rhodamine G in aqueous acetonitrile and, hence, the dye is the emitter of the observed CL.

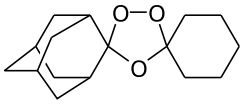
It is known that biologically active peroxides, *viz.*, trioxolanes, tetraoxanes, and trioxanes, react with divalent iron compounds to form free radicals.[2,4,11—14] It was also noticed that in the presence of a reducing agent Fe^{3+} ions cause the decomposition of peroxides (hemin or $FeCl_3$ can act as a source of Fe^{3+} ions and the reducing agent can be L-cysteine or sodium dithionate). Taking into account these facts and the influence of oxygen on the CL intensity, we may suppose that the emission appears according to the mechanism of recombination of peroxy radicals[15] due to the energy transfer from the excited carbonyl compounds to the activator rhodamine G. At the same time, we cannot exclude the generation of CL directly in the course of the reaction of the dye with peroxide or reactive radicals formed upon the reaction of the dye with iron compounds.

We found that the reactions of related 1,2,4,5-tetraoxanes **3** and **4** with the Fe^{2+} ion is also accompanied by the generation of electron-excited states. In particular, CL was observed in the reaction of acetone and trifluoroacetone diperoxides with $FeSO_4$ in the presence of rhodamine G (see Scheme 1). Remarkably, in the case of diperoxide **4**, chemiluminescence (λ = 370—480 nm, trifluoroacetone as an emitter) is detected in the absence of the dye as well.

It should be noted in conclusion that the reactions of biologically active trioxane artemisinin and trioxolane OZ03 (see Refs 1—3) with $FeSO_4$ or a $FeCl_3$—L-cysteine system in the presence of rhodamine G in aqueous acetonitrile is accompanied by CL.



Artemisinin



OZ03

Thus, we discovered chemiluminescence in reactions of the new class of high-energy peroxides, trioxolanes, and for the first time observed CL in the biologically important reaction of tetraoxanes with Fe^{2+} ions. It is shown that CL in the reaction of trioxolanes and tetraoxanes with iron compounds is a general phenomenon. The latter seems very important, because it is assumed that this is the interaction of peroxides with divalent iron of heme which results in the formation of reactive radicals leading to the death of a malaria parasite.[2,4,11—12] The results obtained open new prospects for investigation of the stability and reactivity of these peroxides and the mechanism of biological effect and analytical determination of trioxolanes and tetraoxanes possessing valuable pharmacological activity.

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