Just Add Luminol to Turn the Spotlight on Radziszewski Amidation

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ABSTRACT: Radziszewski amidation, namely the peroxide-mediated conversion of nitriles into amides, is a chemiluminescent reaction involving singlet oxygen. Its increasing relevance in green chemistry and analytical chemistry makes it an important subject to be discussed in advanced chemistry courses. To turn the attention of lecturers, teachers, and students toward this reaction, the history of its discovery as well as its practical applications is discussed. Moreover, a simple and effective experiment to demonstrate the chemiluminescent properties of Radziszewski amidation is provided.

1. INTRODUCTION

The amide functional group is widespread in nature and of fundamental importance in chemistry, as it determines the physicochemical properties of countless biological as well as artificial molecules. The formation of amide bonds is one of the most important and studied reactions in organic chemistry. One major family of compounds that contain amide bonds is proteins. Poly(amide)s are also a fundamental class of polymers with countless applications (think, e.g., of nylon-66). It is estimated that more than 25% of all drugs contain at least one amide bond: amidation plays a crucial role in the synthesis of important drugs such as the analgesic acetaminophen, the local anesthetic lidocaine, the antihypotensive agent midodrine, the antibiotics loracarbef and cefpiramide, the antiarrhythmic agent procainamide, and so on.

Because of the tremendous significance of amide formation in synthetic chemistry, methodological research has focused on efficiency and selectivity, and these have reached impressive levels. However, the unacceptable amounts of waste produced have led the ACS GCI Roundtable to label "amide bond formation avoiding poor atom economy reagents" as the most pressing target for sustainable synthetic method development, urging the search for alternative synthetic protocols.

Here, a promising amidation reaction will be discussed: it is based on hydrogen peroxide, requires very mild conditions, generates no waste (water and oxygen being the only byproducts), and it was already discovered in the 19th century. In 1885, Radziszewski reported that the synthesis of amides could be afforded in good yields through the base-catalyzed hydrolysis of aromatic nitriles in the presence of alkaline hydrogen peroxide at 40 °C.3 Since this seminal report, the reaction, which was called "Radziszewski amidation", has been studied, extended, and improved. The basic mechanism of the reaction was initially formulated as in eq 1.

\[
R-C\equiv N + 2 \text{H}_2\text{O}_2 \rightarrow R-\text{CONH}_2 + \text{H}_2\text{O} + \text{O}_2
\]

Wiberg, in the 1950s, as he was studying the reaction of benzonitrile with mildly alkaline hydrogen peroxide, found that the rate-determining step was the reaction of hydroperoxide \(\text{HOO}^-\) with the carbon atom of the nitrile group to give a reactive intermediate, peroxycarboximidic acid (eq 2). The latter would oxidize another molecule of hydrogen peroxide by a successive fast reaction, producing the amide (eq 3).

\[
R-C\equiv N + \text{H}_2\text{O}_2 \rightarrow R-\text{CONH}_2
\]

It was postulated that the reaction of hydroperoxide with nitriles under controlled pH conditions (pH ~ 8) generated short-lived peroxyimidate anions from peroxycarboximidic acids. As the species are too reactive to be isolated, their involvement in the reaction was demonstrated only in 1996 by means of infrared spectroscopy.6

The excellent oxidizing power of peroxycarboximidic acid was soon recognized, and in 1960, Payne demonstrated7,8 that benzeneperoxycarboximidic acid, obtained from benzonitrile under the conditions for Radziszewski amidation, can be useful for the epoxidation of alkenes (Payne epoxidation), another key research area in green chemistry.2

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Noteworthily, Radziszewski himself described one of the first chemiluminescent organic reactions, that is, the oxidation of 2,4,5-triphenylimidazole (lophine)\(^9\) in alcoholic potash when shaken in the presence of air and the consequent emission of green light.\(^{10}\) However, the emission of light arising from the nitrile—hydrogen peroxide system went unnoticed, because it was too weak to be observed by the naked eye. Chemiluminescence is defined as the emission of light from chemical reactions at ordinary room temperatures.\(^{11}\) The oxidation of 3-aminophthalhydrazide (luminol), giving a characteristic blue light, is one of the most common examples of these kinds of reactions.\(^{12}\) The relevance of chemiluminescent reactions is broad and attracts interest in many different fields, including (bio)analytics,\(^{33–16}\) photochemistry,\(^{14}\) and organic chemistry; finally, these reactions have a special role in chemical education as “exocharmic”, i.e., visually exciting demonstrations.\(^9\)

In 1964, McKeown and Waters were able to observe, using a photomultiplier, an emission of light from the acetonitrile–\(\text{H}_2\text{O}_2\) system and to relate it to the generation of singlet oxygen \(^1\text{O}_2\), an excited state of molecular oxygen.\(^{20}\) The intensity, duration, and spectral components of the chemiluminescence were found to be strongly dependent on the nature of the nitrile used as a substrate.

The relaxation of singlet oxygen to the ground state can occur with the emission of photons at 633 and 703 nm, the so-called “dimol chemiluminescence”. This was recently observed and reported as spectroscopic evidence for singlet oxygen generation during the reaction of acetonitrile with alkaline hydrogen peroxide.\(^{31}\) However, singlet oxygen can be produced in several other ways, such as physical (e.g., by photosensitization\(^ {22}\)), chemical (e.g., by the chlorine–hydrogen peroxide system\(^ {23}\) or by the Trautz–Schorigin reaction\(^ ^{24}\)), and biochemical (e.g., by myeloperoxidase in leukocytes\(^ {25}\)).

The reaction of acetonitrile with hydrogen peroxide is a typical example of ultraweak peroxide-induced chemiluminescent reactions, which are now under study for the development of new analytical assays.\(^{26}\) These are based on the ability of singlet oxygen to transfer its energy to other species, which, in turn, can emit light. Tetracycline antibiotics,\(^ {27}\) melatonin,\(^ {28}\) and other indole-containing molecules\(^ {29}\) are known to increase the intensity of the light emitted during the peroxide-mediated oxidation of acetonitrile, which, however, can only be measured using a photomultiplier. A brighter light, clearly visible by the naked eye, can be obtained by the addition of luminol.

2. RESULTS AND DISCUSSION

Furfuryl alcohol is a renowned specific scavenger of singlet oxygen, with which it can react generating a mixture of oxidation products, whose nature and relative yields vary with respect to the solvent employed: Scheme 1 shows the structures of the products that, according to the literature, are expected to form in aqueous solution.\(^ {33}\)

Scheme 1. Structures of Products Obtained by the Reaction of Furfuryl Alcohol with Singlet Oxygen in Aqueous Solution

Interestingly, the addition of up to 0.1 M furfuryl alcohol to the chemiluminescent solution only reduces, instead of quenching, the emission of light. This suggests the involvement of another reactive species. On the other hand, immediate and complete quenching of chemiluminescence can be obtained by the addition of 10 mM tiron, an efficient quencher of the superoxide anion radical \(\text{O}_2^{-}\).\(^ {34–35}\) It is known that superoxide can originate from singlet oxygen because of quenching mechanisms,\(^ {56}\) and the role of the former to promote the chemiluminescence of luminol has already been reported.\(^ {37}\) The overall mechanism can be represented according to Scheme 2:

Scheme 2. Schematic Mechanism of the Luminol-Enhanced Chemiluminescent Emission from Radziszewski Amidation

3. CONCLUSIONS

Radziszewski amidation recently started to gain importance both as a synthetic tool for green chemistry and for the development of chemiluminescent assays. Despite its relevance, however, this subject is not usually covered even in advanced chemistry courses. To turn the interest of researchers, lecturers, and students toward this topic, an account of Radziszewski amidation from its discovery back in the 19th century to its latest applications was given here, together with an easy-to-perform chemiluminescent demonstration.

Rather than as a stand-alone subject of an advanced-level analytical or organic chemistry course/laboratory, the Radziszewski amidation could be used as a starting point to introduce topics ranging from green synthetic approaches in organic chemistry to the development of chemiluminescence-
based methods in analytical chemistry and to singlet oxygen in physical chemistry.

The simple procedure described can be used as a starting point for the development of students’ laboratory activities as well as research projects. A systematic study of how different variables can affect the light-emission step, such as solvent (singlet oxygen’s lifetime is known to be longer in deuterium oxide compared to water\(^{38}\), alkali (e.g., bicarbonate could be used instead of carbonate\(^{39}\)), temperature, and the nature and concentration of the nitrile substrate, could be the subject of an organic/physical chemistry laboratory. The remarkable effect that ketones have been reported to have on this kind of reaction might also be an interesting subject of investigation.\(^{40}\) The possibility to record the luminescence spectra would be significantly advantageous to better compare the results. The chemiluminescence of luminol has a spectrum that extends from roughly 300–500 nm, with a maximum at approximately 445 nm.\(^{37}\) A simple yet effective method to demonstrate it, without the need of dedicated instrumentation, is to use a blue filter. This can be easily realized with methyl orange, an azo dye of common use as a pH indicator. On observing the reaction through an aqueous solution of methyl orange, no chemiluminescent emission should be detected because it absorbs light in the same wavelength range.\(^{41}\) A control experiment could be done using an aqueous solution of methylene blue, which has a negligible absorption between 350 and 500 nm: in that case, the blue light would not be blocked and thus would be visible.\(^{32}\)

4. EXPERIMENTAL SECTION

4.1. Hazards. Concentrated (30 wt %) hydrogen peroxide is toxic and corrosive, causes severe skin burns and eye damage, is harmful if swallowed, and may be harmful if inhaled. It is a strong oxidizer, which may cause fire or explosion. Commercial diluted (3 wt %) hydrogen peroxide is less hazardous and can be used as well. Sodium carbonate and sodium hydroxide are corrosive. Luminol is an irritant. Acetonitrile is toxic. Furfuryl alcohol is an irritant and a permeator. Tiron (disodium 4,5-dihydroxy-1,3-benzenedisulfonate) is an irritant. Protective gloves and goggles are mandatory. Reaction products should be properly disposed.

4.2. Materials. Concentrated hydrogen peroxide, sodium carbonate, sodium hydroxide, luminol (3-aminophthalhydrazide), acetonitrile, ascorbic acid, tiron, and furfuryl alcohol were purchased from Sigma-Aldrich. The following stock solutions should be prepared in advance and used within a day for best results: 0.1 M H\(_2\)O\(_2\) (1 mL of 30 wt % H\(_2\)O\(_2\) diluted to 100 mL with water or 10 mL of 3 wt % H\(_2\)O\(_2\) diluted to 100 mL with water), 0.1 M Na\(_2\)CO\(_3\) (10.6 g of anhydrous Na\(_2\)CO\(_3\) dissolved in 1 L of water), and 10 mM luminol (1.8 g of luminol dissolved in 1 L of 0.1 M NaOH).

4.3. Demonstrating the Luminol-Enhanced Chemiluminescence Emission from Radziszewski Amidation. In a 50 mL flask, 2.5 mL of 0.1 M H\(_2\)O\(_2\) is mixed with 12.5 mL of 0.1 M Na\(_2\)CO\(_3\) and 0.75 mL of the 10 mM luminol solution. The room is darkened, and then 10 mL of acetonitrile is added at once: immediately, an intense blue chemiluminescence develops and remains visible for more than 10 min. In the absence of luminol, no light emission can be observed by the naked eye.

Different scavengers are added in the reaction mixture either immediately before or after the onset of light emission to demonstrate their action. The following amounts are to be added to the reaction mixture to obtain a final 10 mM scavenger concentration: 45 mg of ascorbic acid, 81 mg of tiron, or 23 \(\mu\)L of furfuryl alcohol.

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