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A Coloured Spin Trap which works as a pH Sensor

Petre Ionita*

Institute of Physical Chemistry, 202 Spl. Independentei, Bucharest 060021, Romania.

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ABSTRACT

Nitration of 4-hydroxybenzaldehyde afforded 2,6-dinitro-4-hydroxybenzaldehyde 2, which in the presence of thionyl chloride is converted to 4-chloro-2,6-dinitrobenzaldehyde 3. This compound is very reactive towards nucleophiles, and reacts easily with methoxyamine, affording the intermediate 4-aminomethoxy-2,6-dinitrobenzaldehyde 4. Reaction of 4 with *N*-*t*-butyl-hydroxylamine led to a new spin trap of the nitrone type, namely 4-aminomethoxy-3,5-dinitrophenyl-1-*t*-butylnitrone 6. The spin-trapping capabilities of the new compound have been tested in a classical system, in which short-lived radicals were generated by irradiation. The new compound 6 contains an acidic proton with a pK_a value of 7.8, and removal of this by a base induces a colour change, from yellow to green-blue. In a similar way, a new stable nitronyl-nitroxide radical 8 has been obtained from the intermediate 4.

KEYWORDS

Synthesis; free radical; spin-trapping; pH sensor; nitrone.

1. Introduction

There is a contemporary interest in the chemistry and biology of short-lived free radicals, since it is well known that they are generated in living systems. Detection and quantification of such reactive species is not a routine task, and there is also no known general system that can be used for all such transient radical species.[1–5]

Nitrones, such as phenyl-*t*-butylnitrone (PBN), have been widely used for the detection and identification of oxygen, carbon, and other reactive species; literature data is abundant on such information.[6–8] After their initial use *in vitro,* nitrones were used *in vivo,* in attempts to visualize directly the formation and the concentration of short-lived radicals in living beings. In the last decade, it has been shown that nitrones may have therapeutic properties, mainly in ageing processes; thus, their administration in neurodegenerative diseases, or even after stroke or ischaemia, increases the life expectancy of the subjects.[9,10]

Previous work showed that such nitrone moieties linked to a stable free radical may lead to hybrid molecules, which can be easily used as sensors and markers in free radical chemistry.[11] On the other hand, a coloured spin trap may provide the opportunity to monitor the radical processes by different complementary means, such as electron paramagnetic resonance (EPR) and UV-visible spectroscopy.[11,12]

In this work we synthesized a new spin trap, which contains in the same molecule a nitrone moiety and an acidic (and chromophoric) group.

2. Experimental

All chemicals, solvents and materials used were purchased from Aldrich or Chimopar (Bucharest, Romania), and used as supplied. UV-visible spectra were recorded on a Hitachi U-3000 (London, UK) UV-visible spectrometer; pH values were measured with a Hanna pHep instrument (Bucharest, Romania); NMR spectra were recorded on a Bruker 300 MHz instrument (Coventry, UK), while the EPR spectra were recorded on a Jeol X-band apparatus (Croissy-sur-Seine, France). Good yields at each of the synthesis steps were obtained, usually between 40 and 80%. Most of the compounds were sufficiently pure to be used without further purification, even for analytical purposes; when a purification step was required, preparative TLC or column chromatography on silica gel was performed.

1: To a solution of 1.2 g *p*-hydroxybenzaldehyde dissolved in the minimum amount (~30 mL) of glacial acetic acid was added slowly (with cooling in an ice bath) 5 g of nitric acid (70%), and the mixture left overnight; the next day the precipitate was filtered off, washed with a small amount of acetic acid and then water, and dried. The yield was ~90%. ^{1}H-NMR (MeOH-d4): δ 9.88 (s, 1H, CHO), 8.58 (s, 1H, OH), 8.08 (d, 1H, CH) and 7.27 ppm (d, 1H, CH). EI *m/z:* 167 ($C_7H_5NO_4$ (M=167).

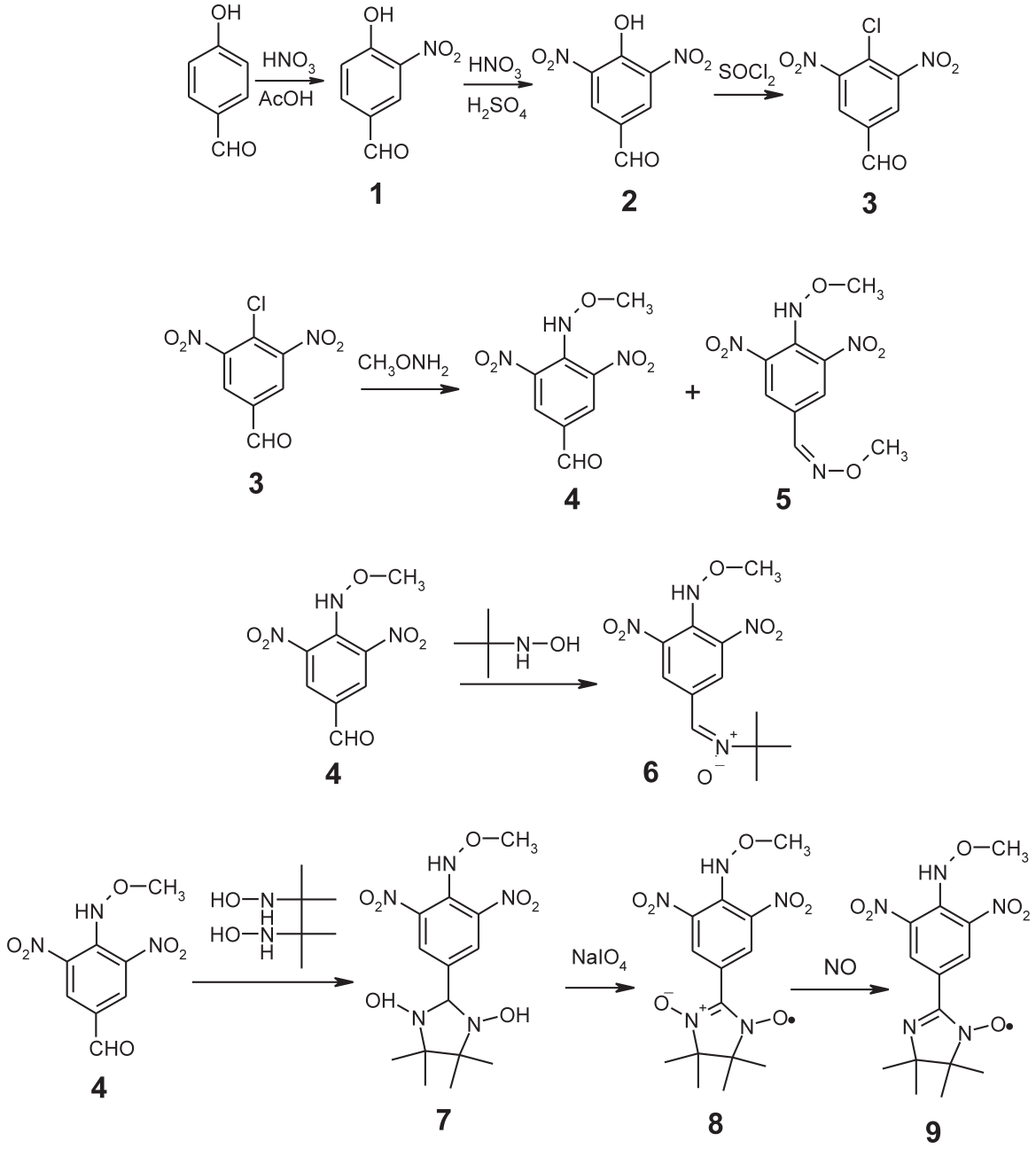
2: 1 g of **1** was treated with a 10 mL mixture (1/3, v/v) of nitric acid (70%) and sulphuric acid (96%), with cooling in an ice bath. The next day 100 mL of cold water was added, and the precipitate formed filtered off, washed with cold water, and dried. The yield was ~80%. ^{1}H-NMR ($CHCl_3$-d): δ 11.90 (s, 1H, OH), 10.00 (s, 1H, CHO) and 8.82 ppm (s, 2H, CH). EI *m/z:* 212 ($C_7H_4N_2O_6$ M=212).

3: 1 g of **2** was added to a mixture of 25 mL toluene and 5 mL thionyl chloride; 0.5 mL of DMF was also added, and the mixture refluxed for about 1 h; after cooling, a large amount of hexane was added (~300 mL), and the precipitate filtered off and washed with cold hexane. The yield was ~90%. ^{1}H-NMR ($CHCl_3$-d): δ 10.07 (s, 1H, CHO) and 8.44 ppm (s, 2H, CH). EI *m/z*: 230 ($C_7H_3ClN_2O_5$ M=230).

4: To 230 mg of **3** dissolved in a 25 mL mixture of DCM/MeOH (1/1, v/v) was added 5 mL triethylamine and 240 mg of *N*-methoxyamine hydrochloride, and the mixture refluxed for 1 h; removal of the solvent and preparative chromatography with DCM as eluent afforded the desired compound. The yield was ~50%. ^{1}H-NMR ($CHCl_3$-d): δ 10.34 (s, 1H, CHO), 9.88 (s, 1H, NH), 8.50 (s, 2H, CH) and 3.80 ppm (s, 3H, CH_3O). CI *m/z*: 241 ($C_8H_7N_3O_6$ M=241). UV-visible: λ_{max} (DCM) 392 nm, under basic conditions (DCM + 5% triethylamine) 543 nm.

5: This derivative was obtained as a by-product in the previous synthesis. The yield was ~30%. ^{1}H-NMR ($CHCl_3$-d): δ 9.89 (s, 1H,

* E-mail: pionita@icf.ro



Scheme 1

NH), 8.67 (s, 1H, CH), 8.11 (s, 1H, CH), 7.90 (s, 1H, CH-N), 3.92 (s, 3H, CH_3O) and 3.69 ppm (s, 3H, CH_3O). CI *m/z*: 270 ($C_9H_{10}N_4O_6$ M=270).

6: To 120 mg of **4** in a 50 mL mixture of DCM/MeOH (1/1, v/v) was added 10 mL pyridine and 240 mg of *N-t*-butyl-hydroxylamine hydrochloride, and the mixture refluxed for two days; removal of the solvent and preparative chromatography with DCM as eluent afforded the desired compound. The yield was ~40%. ^{1}H-NMR ($CHCl_3$-d): δ 9.66 (s, 1H, NH), 9.06 (s, 2H, CH), 7.52 (s, 1H, CH-N), 3.75 (s, 3H, CH_3O) and 1.62 ppm (s, 9H, CH_3). ESI *m/z*: 312 ($C_{12}H_{16}N_4O_6$ M=312). Elemental analysis: found: C, 46.15; H, 5.00; N, 17.71. Calc. for $C_{12}H_{16}N_4O_6$: C, 46.15; H, 5.16; N, 17.94. UV-visible: λ_{max} (DCM) 430 nm, (water) 610 nm.

7: To 120 mg of **4** in a 50 mL mixture of DCM/MeOH (1/1, v/v) was added 5 g sodium hydrogen carbonate and 500 mg of *bis*-hydroxylamine hydrochloride, and the mixture stirred for one day. After filtration, 150 mL DCM was added to the solution, and the resulting mixture was washed with water; the organic layer was separated and dried with anhydrous sodium sulphate, and the solvent removed. No identification steps were performed, and the product was used directly in the next synthesis.

8: To the previously obtained product, 50 mL of DCM was added, together with 5 g of sodium periodate. The mixture was stirred for 1 h, the solid was filtered off, and the solvent removed. The yield was ~20%. EPR (water): a_{N_1} = 8.29 G; a_{N_2} = 8.29 G. Elemental analysis: found: C, 45.45; H, 4.70; N, 18.71. Calc. for $C_{14}H_{18}N_5O_7$: C, 45.65; H, 4.93; N, 19.01.

9: Our attempt to obtain this compound by bubbling nitrogen oxide through a solution of **7** dissolved in DCM failed.

3. Results and Discussion

3.1. Synthesis

Scheme 1 shows the general steps followed in the synthesis of the desired compounds. Thus, starting from the commercially available 4-hydroxybenzaldehyde, nitration was performed in

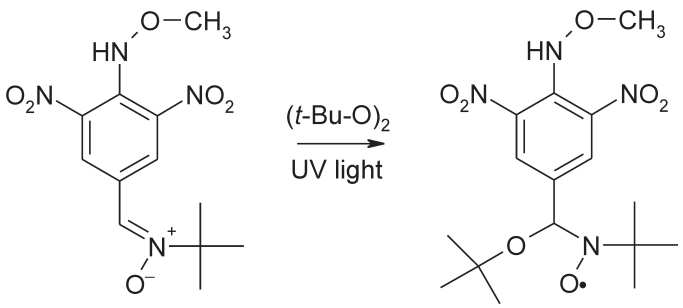


Figure 1 Formation of the spin-adduct.

two steps, in the first step a mild nitration agent, such as nitric acid in glacial acetic acid, was used and in the second step a more powerful nitration agent, concentrated nitric acid in sulphuric acid, was used. Attempts to obtain the dinitro compound **2** in a single nitration step failed, with either the first or the second nitration agents. Thus, nitric acid in glacial acetic acid, even after a long time or after increasing the temperature, afforded only traces of **2**; by using nitric acid in sulphuric acid directly, a violent reaction occurred, which could be handled safely. Compounds **3** and **4** were obtained in the classical way, while compound **5** was obtained as a by-product. Regarding compounds **6–8**, they were obtained by the well-known procedures shown in Scheme 1 (with the exception of compound **9**, for which the synthesis failed).[11,13]

3.2. Spectral and Acidic Properties of Compounds 6 and 8

Compounds **6** and **8** were chosen to be tested as pH indicators. Both compounds contain a methoxyamino group, in which the N-H proton can be easily removed by a base; this process is accompanied by a colour change. Similar literature compounds have pK_a values between 4 and 11, and they change colour from yellow (in acidic media) to red, green or blue (in basic media), depending on their structure.[14–16] For compounds **6** and **8**, the pK_a values were determined by means of a potentiometric titration method in a mixture of methanol/water (1/1, v/v), as previously described.[15,16] The solvent mixture (methanol/water) was used due to the low solubility of these compounds in pure water. Thus, for **6** the value of the pK_a determined was 7.8, while for **8** the value found was 6.5. The nitronyl-nitroxide moiety has a bigger influence on the acidity of the –O-NH- group, compared with the nitrone moiety, thus increasing the acidity.

The colour changes due to the pH changes, in the same water/methanol mixture, were as follows: for compound **6**, at acidic pH, the colour is yellow with λ_{max} = 390 nm, while at a basic pH the colour is greenish, with λ_{max} = 575 nm; for compound **8**, at acidic pH, the colour is yellow, with λ_{max} = 380 nm, while at basic pH the colour is blue, with λ_{max} = 595 nm. These colour changes may allow compounds of this type to be used as pH indicators.

3.3. EPR Spectroscopy and Spin Trapping

Compound **6** acts as a classical spin trap for short-lived radicals; thus, when a peroxide (*t*-butylperoxide) was irradiated with UV light in the presence of the spin trap **6**, the formation of the *t*-butoxyl radical spin adducts was shown by EPR spectroscopy (Figs 1 and 2).

The spectrum (recorded in benzene) shows a mixture of two spin adducts, the first one being the spin adduct with the *t*-butoxyl radical, and the second one with the methyl radical (formed *via* disproportionation of the *t*-butoxyl radical). Simulation with the WinSim software package[17] gave a very good fit, with EPR hyperfine coupling constants of a_N = 14.09 G and a_H = 1.80 G for the first spin adduct and a_N = 14.07 G and a_H = 3.38 G for the second spin adduct.

Compound **8** is a stable free radical of nitronyl-nitroxide type, in which the EPR hyperfine coupling constants are equal (with a value of 8.29 G, spectrum recorded in DCM), due to the interaction of the unpaired electron with two equivalent nitrogen atoms, leading to an EPR spectrum consisting of 5 lines, with an intensity ratio of 1:2:3:2:1 (Fig. 3, left). In addition, oxidation of **6** with solid lead dioxide in DCM led to the formation of the corresponding unstable aminoxyl radical, as shown by the EPR spectrum (Fig. 3, right); in about 1 h, the EPR signal was completely lost.

In conclusion, a new compound which contains in the same molecule an acid–base sensitive moiety and a nitrone spin-

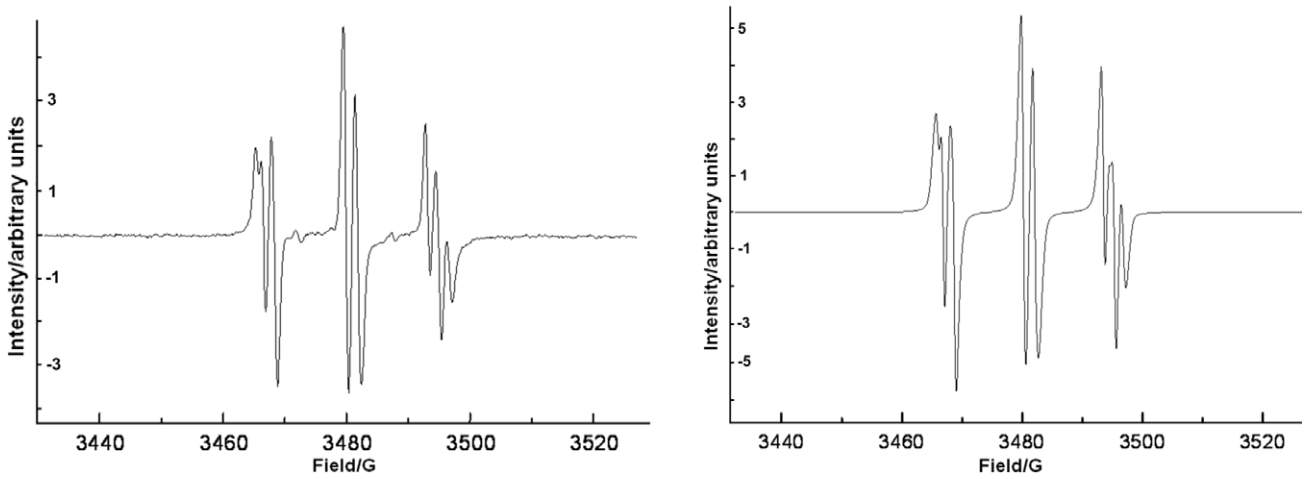


Figure 2. Experimental EPR spectrum (in benzene) obtained by irradiation of *t*-butylperoxide in the presence of **6** (left) and simulation as a mixture of two adducts (right).

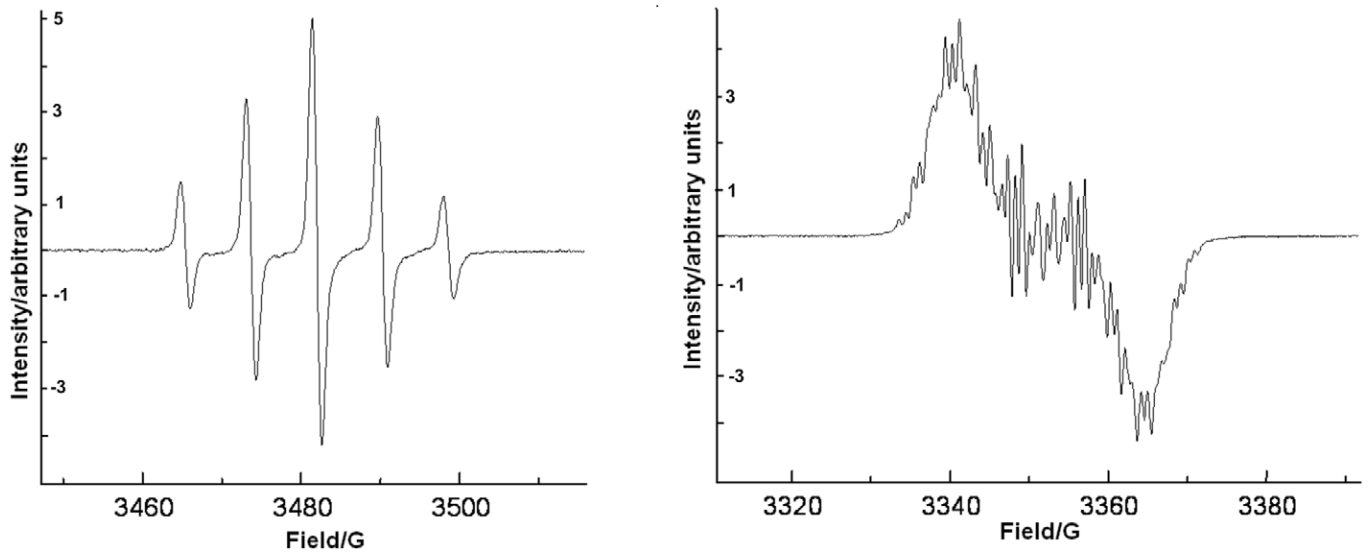


Figure 3 EPR spectrum of **8** in DCM (left) and EPR spectrum obtained by oxidation of **6** in DCM (right).

trapping moiety has been synthesized and characterized. Removal of the acidic proton by a base is accompanied by a colour change; thus, the compound acts as a pH indicator. The nitrone moiety can trap short-lived radicals, with the formation of a spin adduct which is easily characterized by EPR spectroscopy; in this way, the compound acts as a spin-trapping agent. This compound may be used as a dual sensor, to monitor both pH changes and free radicals.

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