

Synthesis, antioxidant and antibacterial activities of 3-nitrophenyl ferrocene

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Abstract. The current work aims in its first part to synthesize 3-nitrophenylferrocene after diazotizing nitroaniline in the meta position by the sodium nitrite and the formation of the corresponding diazonium salt: 3-nitrobenzodiazonium sulfate, then the salt in solution was added to the ferrocene for the purpose of introducing the nitrophenyl moiety thereon (arylation) and the formation of 3-nitrophenylferrocene. The second part is devoted to the study of the antioxidant activity of 3-NPF by applying the trapping test of superoxide radical using cyclic voltammetry, the free radical DPPH trapping test by spectrophotometry. The results showed that 3-nitrophenylferrocene has a scavenging effect of DPPH radical with IC₅₀= 1.44mg/ml, superoxide radical with IC₅₀=5.38mg/ml. The third part is devoted to the study of antibacterial activity of the synthesized compound tested on four strains of bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Klebsiella pneumoniae*. The obtained results clearly showed that 3-nitrophenylferrocene has low activities on the four bacterial strains with diameters of inhibition zones do not exceeding 17 mm at concentrations of 25mg/ml.

1. Introduction

Ferrocene synthesised by Kealy and Pauson in 1951 [1] is an organometallic sandwich compound with iron in the formal oxidation state of +2, it has been object of continuous interest both in fundamental and applied research areas for many years. Recent advanced applications of Ferrocene have been also proposed in electronics, material sciences and medicinal chemistry [2]. Research has shown that ferrocene derivatives exhibit cytotoxic [3-4], anti-tumor [5, 6], antifungal [7], antibacterial [8] and antioxidant activities [9-11].

Oxidative stress is the cause of many diseases, including cancer, cardiovascular disease [12]. The body's defence system against cell damage induced by the high level of oxidative stress includes endogenous non-enzymatic natural antioxidants: glutathione and exogenous: ascorbic acid (vitamin C), tocopherol (vitamin E), and enzymatic antioxidants: superoxide dismutase, catalase and glutathione



reductase [13]. Researchers do not cease to develop new synthetic molecules that exhibit antioxidant activity to be used in many industries such as the food industry, cosmetics industry ...etc.

In this work, we synthesized 3-nitrophenylferrocene (3-NPF) and studied his antioxidant and antibacterial effect. The antioxidant activity of this compound is measured by an electrochemical method (Superoxide test) and chemical method (DPPH test), the antibacterial activity is tested on four strains of bacteria: Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis and Klebsiella pneumoniae.

2. Materials and methods

2.1. Reagents

3-nitroaniline, Sodium nitrite, Magnesium sulfate, Ether, Petroleum ether, Sulphuric acid (98%), Methanol (99%), Dimethylformamide (99.9 %) were acquired from Biochem Chemopharma (Canada), Dimeyhulsulfoxide (99.9 %) , Tetrafluoroborate tetrabutylammonium (99%) from Sigma-Aldrich, α -Tocopherol (97%) , 1,1- Diphenyl -2- picrylhydrazyl (DPPH) (99%) from Merck.

2.2. Synthesis

The overall synthesis strategy of 3-NPF is represented by the scheme illustrated in figure 1.

A mixture of 10.7g (0.077mol) of 3- nitroaniline, 16.7 ml of concentrated sulphuric acid and 22.90 ml of water was stirred for 1 hour then it was cooled to 0-5°C, diazotized with a solution of 5.5g (0.08 mol) of sodium nitrite in 12.25 ml of water. The cold solution was filtered and the filtrate was added with stirring to a solution of 13.08g (0.070mol) of ferrocene in 316.45 ml of ether. The mixture was stirred for 3 h at room temperature. The two layers were separated. The aqueous layer was extracted with Et₂O, and then the organic layer washed with water, dried with MgSO₄. Concentration in vacuo and purification by column chromatography gave 3-NPF as orange plates with Y= 56%. (R_f= 0, 73).

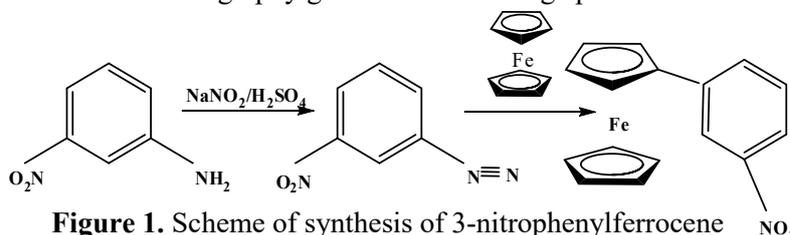


Figure 1. Scheme of synthesis of 3-nitrophenylferrocene

3. Antioxidant activity

3.1. Scavenging activity of free radical DPPH

The inhibitory activity of DPPH radical is evaluated using the stable radical DPPH and following the method described by Brand- Williams and al [14] with some modifications. 3 - NPF in 0.1ml of DMSO was added to 1.9 ml of DPPH solution in methanol. The samples are incubated for 30 minutes and the absorbance of DPPH solutions was measured at 517nm. Results are expressed and calculated using the equation (1):

$$I (\%) = \frac{A_0 - A_s}{A_0} \times 100 \quad (1)$$

I (%): inhibition capacity of DPPH, A₀: absorbance of the DPPH solution without sample

A_s: absorbance of the test sample mixed with DPPH solution.

3.2. Scavenging activity of superoxide anion radical (O₂^{•-})

The inhibitory activity of the superoxide radical anion is determined by cyclic voltammetry, this test is based on the method of Bourvellec and al with some modifications [15].

Voltammetric measurements are carried out using 40 VoltaLab PGZ301 model (Radiometer Analytical SAS), a three-electrode system in an electrochemical cell of volume V = 25 ml. A saturated

calomel electrode (SCE) is used as a reference electrode, a platinum wire as an auxiliary electrode and a glassy carbon electrode about 0.013cm² section as the working electrode. The data acquisition is performed by a computer Pentium IV (3.0GHz CPU and 1 GB RAM) using VoltaMaster 4 software, Version 7.08 (SAS analytical radiometer).

Before use, the working electrode was polished, washed with distilled water and dried with absorbent paper. This cleaning procedure is always applied before each electrochemical measurement. The superoxide anion radical is generated by the commercial molecular oxygen dissolved in DMF containing 0.02M of Bu₄NBF₄ at room temperature (28 ± 1 ° C). The scanning rate is maintained at 100 mV / s. The range of applied potential was -1.6V to 0.0V vs SCE.

3-NPF is added to the superoxide radical dissolved in DMF and voltammograms were recorded (figure 4).

The studied compound's ability to scavenge superoxide radicals (O₂ ·⁻) is calculated using the following equation (2):

$$I(\%) = \frac{Ip_0 - Ip_s}{Ip_0} \times 100 \quad (2)$$

wherein: Ips and Ip0 are the intensity of the anodic peak current of the oxygen respectively without and with test compound.

I (%): Inhibitory capacity of superoxide.

4. Antimicrobial activity

4.1. Microorganisms

Four strains of bacteria were used in this study including Escherichia coli ATCC25922, Pseudomonas aeruginosa, Klebsiella pneumoniae ATCC700603, Enterococcus faecalis ATCC29212.

4.2. Agar well diffusion assay

The qualitative antibacterial assay of 3-NPF was carried out by the disc diffusion method [16], all the bacterial strains mentioned above were incubated at 37°C for 24 h by inoculation into Mueller-Hinton (M-H) broth. The samples were prepared by dissolving 3-NPF in DMSO to obtain solutions with concentrations: 0.2 mg/ml, 2, 10, 20,25mg/ml. A bacterium was spread on the Muller-Hinton Agar (MHA) medium in a petri dish, which was incubated at 37°C for 24h to obtain uniform colonies of the bacterium. Then 4-5 morphologically identical colonies of the bacterium were put into a test tube containing autoclaved normal saline solution (5ml). Then the bacterial suspension was transferred into petri dishes that contained autoclaved MHA medium. We file on the surface of the Petri dishes filter paper disks of diameter 5 mm impregnated with the 3 - NPF solutions prepared at various concentrations. After incubating the petri dishes at 37°C for 24h, inhibition zones (ZI) were measured. Each test was repeated at least for three times and the mean was calculated.

5. Results and discussion

5.1. Synthesis

The synthesis of 3-nitrophenylferrocene takes place in two stages, the first stage being a diazotization of 3-nitroaniline by sodium nitrite and the second step is an arylation of ferrocene whose objective is the introduction of nitrophenyl group on the latter by diazonium salt intermediate; nitrobenzenediazonium sulphate formed in the first step.

This compound was synthesized according to the following procedure, 10.7 g of 3-nitroaniline dissolved in 22.90 ml of water + 16.7 ml of H₂SO₄ and diazotized with 5.5 g of sodium nitrite. 13.08 g of ferrocene in 316.45 ml of ether was added to the filtrate (3-nitrobenzenediazonium sulphate). After 3 hours of stirring under the room temperature, the organic phase is recovered, washed with water,

dried and evaporated to obtain the reaction product. Column chromatography and recrystallization in methanol allows recovering the products as red orange solid.

Its proton NMR spectrum shows different peaks, a peak at 4.05 ppm corresponding to 5H of the pentadienyl (C_5H_5) ferrocene ring, a peak at 4.40 ppm corresponds to 2H linked to the C2 or C3 atom, a peak at 4.71 ppm corresponding to 2H linked to the C3 atom or the C2 atom, a triplet at 7.43 ppm corresponds to a proton linked to the C9 atom with a coupling coefficient j equal to 7.98 Hz, a doublet at 7.74 ppm corresponds to a proton linked to the C10 atom with a coupling coefficient equal to 7.70 Hz, a doublet at 8.00 ppm corresponds to a proton bound to the C8 atom with a coupling coefficient equal to 8.34 Hz and a singlet peak corresponds to a proton linked to the C8 atom, atom C6.

By combining the information extracted from the ^{13}C and DEPT NMR spectra with those obtained from the proton spectrum of the compound, we can deduce that the compound does not contain CH_2 , and CH_3 , it's contains 7 (CH), 3 (C) and 13H.

5.2. Antioxidant study

5.2.1. Scavenging activity of free radical DPPH

Free radical scavenging activity of 3-NPF was determined through DPPH assay. DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical. It accepts an electron or hydrogen to form a diamagnetic species, and as a result, its purple colour (λ_{max} 517 nm) changes into pale yellow. When a sample containing a substance with radical scavenging potential is mixed with DPPH, reaction takes place and concentration of DPPH decreases causing a decrease in absorbance at 517 nm. Therefore, a stronger free radical scavenger causes a greater decrease in the absorbance [17]. The concentration of antioxidant required to reduce 50% of the initial concentration of oxidizing agent (DPPH) expressed by IC50 is a widely used parameter for measuring the antioxidant effect. The IC50 values were calculated graphically by plotting percentage inhibition against concentration of test compound. A low IC50 means high antioxidant activity. All measurements were performed in triplicate to ensure reproducibility of the results. The results obtained are shown in table 1.

5.2.2. Scavenging activity of superoxide anion radical ($O_2^{\cdot -}$)

The superoxide anion is a weak oxidant, but it is capable to produce powerful and damaging hydroxyl radicals and singlet oxygen, both of which contribute to oxidative stress. In this work, we chose the superoxide due to its presence in the human body as well as its long half-life. The superoxide anion radical was generated by one electron reduction of the commercial molecular oxygen (O_2) dissolved in DMF at room temperature ($28 \pm 1^\circ C$). The resultant CV response of the medium (DMF+0.02 M Bu4NBF4) and the superoxide radical in the same medium are shown respectively in figure 2 and figure 3.

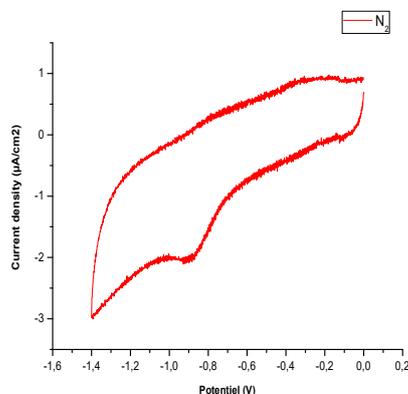


Figure 2. Cyclic voltammogram of the medium (DMF+0.02 M Bu4NBF4).

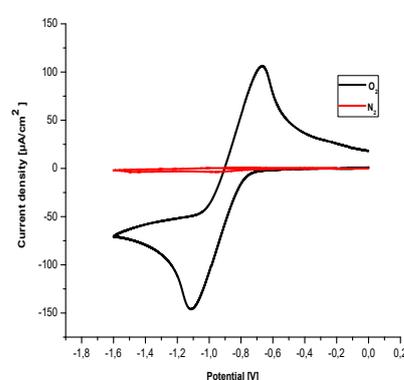


Figure 3. cyclic voltammogram of $O_2^{\cdot -}$ in DMF + 0.02 M Bu4NBF4, on CV as working electrode at $28^\circ C \pm 1 C$ with scan rate of 100 mV/s.

- *α -Tocopherol*

The figure 4 illustrates the cyclic voltammograms of $O_2^{\cdot -}$ in the presence of different concentrations of α -Tocopherol in DMF.

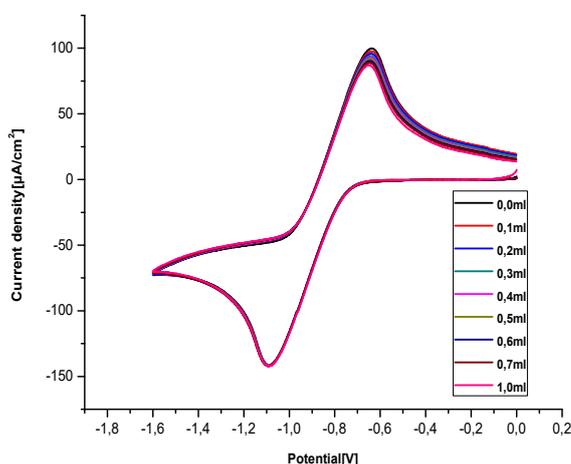


Figure 4. Voltammograms of $O_2^{\cdot -}$ in the presence of different concentrations of α -Tocopherol in DMF + 0.02 M Bu_4NBF_4 on CV as the working electrode at $28^\circ C \pm 1$ with a scan rate of 100mV / s.

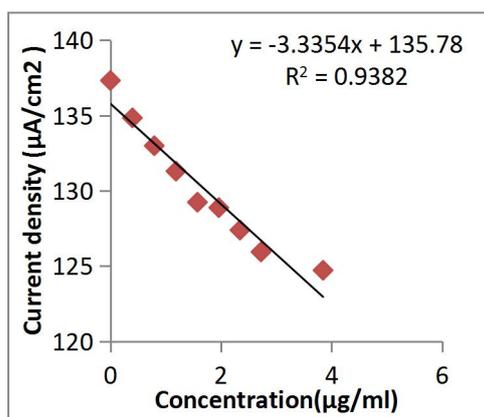


Figure 5. Change in current intensity as a function of the concentration of α -Tocopherol.

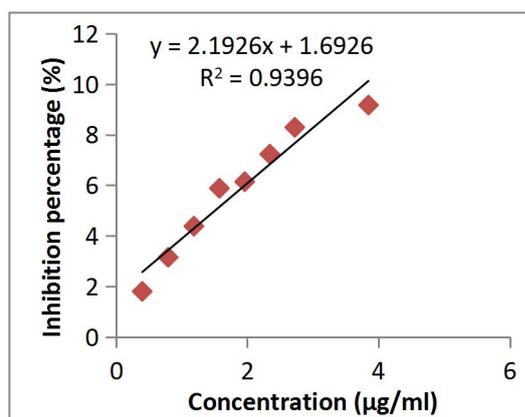


Figure 6. percent inhibition depending on the concentration of α -Tocopherol.

Figures 5 and 6 illustrate the change in current intensity as a function of the concentration of α -Tocopherol and the percent inhibition evolution depending on the concentration of α -Tocopherol.

- *The studied compound: 3-nitrophenylferrocène*

The figure 7, 8 and 9 present respectively the cyclic voltammograms of $O_2^{\cdot -}$ in the presence of different concentrations of 3-NPF in DMF, the change in current intensity as a function of the concentration of 3-NPF and the percent inhibition evolution depending on the concentration of 3-NPF.

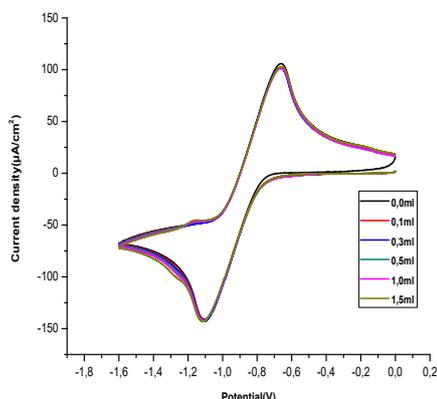


Figure 7. Voltammograms of $O_2^{\cdot -}$ in the presence of different concentrations of 3- NPF in DMF + 0.02 M Bu_4NBF_4 on CV as the working electrode at $28^\circ C \pm 1$ with a scan rate of $100mV / s$.

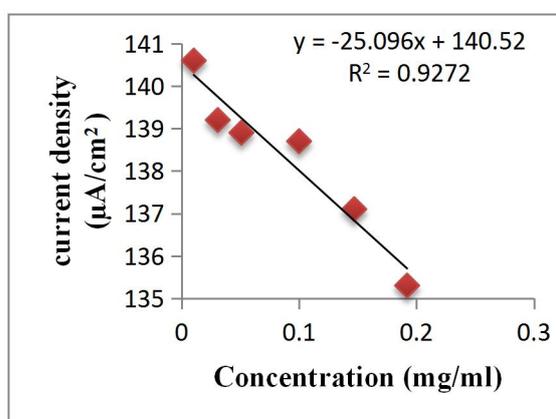


Figure 8. Change in current intensity as a function of the concentration of 3- NPF.

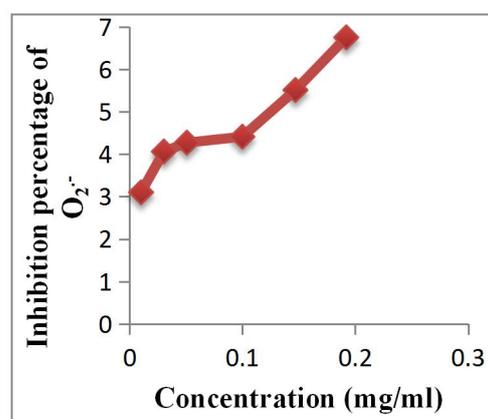


Figure 9. percent inhibition evolution depending on the concentration of 3-NPF.

The decrease of the anodic peak current of $O_2^{\cdot -}$ suggests that the ferrocene derivative reacts irreversibly with $O_2^{\cdot -}$.

For each antioxidant, a series of values is determined from the CVs recorded for increasing antioxidant concentrations (table1).

From the table 1, It can be seen that the IC50 values of 3-NPF showed an antioxidant capacity with an IC50 of 5.38mg/ml for the superoxide radical and an IC50 of 1.44 for the DPPH radical.

Table 1. IC50 values obtained by the DPPH test and superoxide test.

Compound	Methods	Equation	R ²	IC50(mg/ml)
3-NPF	DPPH	Y=36.30x-2.481	0.983	1.44
	$O_2^{\cdot -}$	Y=17.293x+3.152	0.994	5.38
α -tocopherol	DPPH	Y=1.56x+29.093	0.995	0.0134
	$O_2^{\cdot -}$	Y=2.955x+2.269	0.961	0.016

5.3. Antibacterial study

Antibacterial activity of 3-NPF was determined against four strains of micro-organisms. Mean zones of inhibition were calculated and the results are displayed in figure 10.

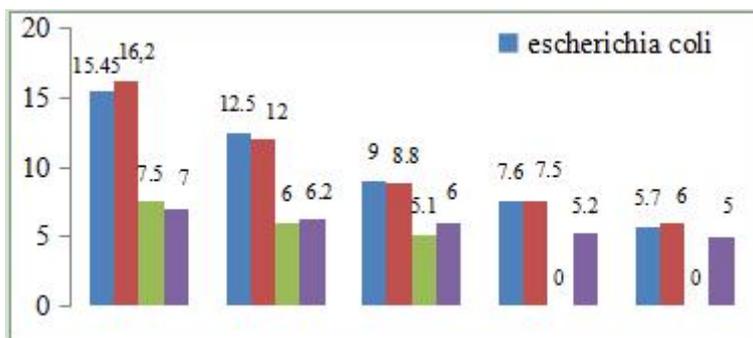


Figure 10. Mean inhibition zones of the bacteria at different concentrations of 3-NPF.

We notice from the results that the four bacterial strains are insensitive towards 3-nitrophenylferrocene, 3-NPF has an important activity on *Escherichia coli* with inhibition zone diameter of 15.45 mm for 25mg / ml and 5.7 mm to 0.2 mg / ml. For *Pseudomonas aeruginosa*, 3-NPF has antibacterial activity against it close to that obtained with *Escherichia coli*, and it results in the same inhibition zone diameters for all concentrations. 3-NPF has negligible activity on *Enterococcus faecalis* and *Klebsiella pneumoniae*.

6. Statistical analysis

The results were presented as the mean values \pm SD. Correlation analyses between the antioxidant activities were carried out using the correlation and regression program in the EXCEL program.

7. Conclusion

In conclusion, the antioxidant activity of 3-nitrophenylferrocene was assessed by using electrochemical and chemical methods. 3-nitrophenylferrocene has an IC₅₀ equal to 1.44 mg / ml evaluated by the DPPH test and IC₅₀ equal to 5.38 mg / ml measured by the superoxide scavenging test. Our results indicate that 3-NPF has a low activity to trap the radical 2,2-diphenyl-1-picrylhydrazyl and superoxide. The results of the antibacterial activity showed that 3-nitrophenylferrocene has low activities on the four bacterial strains with diameters of inhibition zones do not exceeding 17 mm at concentrations of 25mg / ml.

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