

The electrochemical oxidation of Riluzole, a neuroprotective drug: comparison with the reaction with oxygen derived radicals



Mehmet A. Oturan,^a Jean Pinson,^{*a} Mounir Traikia^a and Dominique Deprez^b

^a Laboratoire d'Electrochimie Moléculaire, Université Paris 7-Denis Diderot, Unité Mixte de Recherche Paris 7-CNRS, No. 7591, 2 place Jusieu, 75251 Paris Cedex 05, France

^b Rhone-Poulenc Rorer, Centre de Recherche de Vitry-Alfortville, 13 Quai Jules Guesdes, BP 14, 94403, Vitry sur Seine, France

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The electrochemical oxidation of Riluzole, a neuroprotective drug, is investigated. It leads to the formation of an azo dimer through a short lived radical cation. The same dimer is obtained by reaction with dioxygen in the presence of copper or in the presence of superoxide ion. The reaction with electrochemically generated hydroxyl radicals provides two hydroxylated derivatives which have been previously identified as metabolites of Riluzole.

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurological disorder neuropathologically characterised by a progressive degeneration of upper (cervical or bulbar) and lower (located in the lower part of the spinal cord) motoneurons. Riluzole [2-amino-5-(trifluoromethoxy)benzothiazole] **1** appears to slow the progression of this disease and may improve survival in patients with disease of bulbar onset¹⁻³ (ALS is initially located in the bulbar neurones). The origin of neuronal death is presently unknown but recent findings suggest that neurodegeneration could be related to an excitotoxic disorder⁴ (an excessive production of excitative amino acids which become toxic at high concentration). Physiological activation of motoneurons by glutamate (one of the main mediators of the nervous system) coupled to a lower activity of the enzyme cytoplasmic superoxide dismutase SOD1 (which is responsible for the destruction of superoxide ion $O_2^{\cdot-}$ in the cells) would lead to an excess of radicals in the cells leading to their death.⁵ Riluzole has been shown to interfere with the transmission of glutamate.⁶⁻⁸ In view of the possible implication of Riluzole in processes involving radicals, we decided to investigate the reaction of Riluzole with oxygenated radicals: $O_2^{\cdot-}$ and OH^{\cdot} as well as its electrochemical oxidation.

Experimental

The cyclic voltammetry experiments were performed at 20 °C with 3 mm diameter glassy carbon (GC) electrodes (Tokai Corp.) or on 1 mm diameter gold or platinum electrodes. These electrodes are carefully polished with 1 μ m diamond paste and ultrasonically rinsed before each experiment. The reference electrode was a saturated calomel electrode (SCE) separated from the solution by a salt bridge. The electronic set-up consisted of a Tacussel signal generator, a laboratory made potentiostat⁹ and a digital oscilloscope, Nicolet 310. High scan rate cyclic voltammetry experiments were performed with a 10 μ m platinum electrode sealed in soft glass.¹⁰ The signal generator was a Hewlett-Packard 3314A and the curves were recorded with a Nicolet 450 with an acquisition time of 5 ns per point.

Electrolyses were performed in a glassy carbon crucible used as an anode or with a carbon felt anode; the platinum cathode was separated from the anodic compartment by a no. 4 glass frit. In aprotic solvents acetonitrile (ACN), dimethyl sulfoxide (DMSO) and dichloromethane (CH_2Cl_2), NEt_4BF_4 and

NBu_4BF_4 (0.1 M) were used as supporting electrolytes. At the end of the electrolysis, the solution was poured in 200 cm^3 of water and extracted three times with 50 cm^3 of ether. The organic extract was dried and evaporated. The orange solid which was obtained presented a single spot by TLC (SiO_2 GF 254, CH_2Cl_2 -MeOH 95:5) with $R_F = 0.7$; it was recrystallised in a water-methanol solvent. Orange crystals; mp = 270 °C, visible UV spectrum: $\lambda_{max} = 420$ nm, $\epsilon = 3800$ $M^{-1} cm^{-1}$; mass spectrum (EI): $m/e = 464$.

The reaction with hydroxyl radicals was performed in the following way: to an aqueous solution of **1** (100 cm^3 , 8 mM) 5 mM of $FeCl_3$ were added. To prevent the precipitation of iron hydroxides the pH was adjusted either to a value lower than 2 or to a value close to 3 but with addition of EDTA. The solution was saturated with oxygen and the potential of the electrode was set at -0.5 V/SCE (V versus SCE), a potential at which both oxygen and the Fe^{3+} ions are reduced simultaneously. Separation of the products was achieved by flash chromatography on a 5 cm long column filled with silica 60 (Merck) and eluted with CH_2Cl_2 .

Analysis of the different solutions was also achieved by HPLC (Gilson 803C) on a 5 μ m Kromasyl RP C18 reverse phase 25 cm long column. For the dimer **2** the UV detector was set at $\lambda = 420$ nm; the elution solution was ACN-water 90:10 v/v (1 cm^3 min^{-1}). Under such conditions **2** was eluted after 12.24 min. For the separation of the products of reaction with hydroxyl radicals or superoxide ion, the detector was set at $\lambda = 263$ nm and the column was eluted (0.7 cm^3 min^{-1}) with: methanol 60%; Na_2HPO_4 (0.01 M) solution 40%; CH_3COOH 0.02%; with these conditions, the elution times were: 23.0, 16.0 and 11.0 min respectively for **1**, **4** and **5**.

Results and discussion

Electrochemical oxidation of **1**

In ACN + 0.1 M NEt_4BF_4 the cyclic voltammetry of **1** on a GC electrode presents (Fig. 1) a well defined irreversible peak at $E_p = +1.35$ V/SCE (scan rate $v = 0.2$ $V s^{-1}$). Similar voltammograms are observed on gold or platinum electrodes. By comparison with the one-electron reversible system of ferrocene we have measured the number of electrons transferred at the potential of the peak: $n = 0.58$ $F mol^{-1}$. Upon increasing the scan rate up to 1000 $V s^{-1}$, the wave remains irreversible. However the reversibility can be recovered at 18 000 $V s^{-1}$ on a

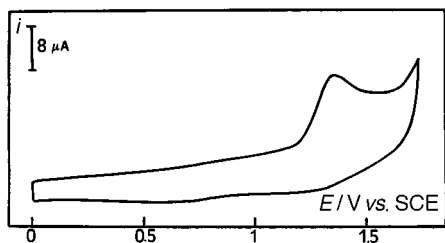


Fig. 1 Cyclic voltammetry of **1** ($C_0 = 1$ mM) in ACN + 0.1 M NBu_4BF_4 , 3 mm glassy carbon electrode; reference SCE, 0.2 V s^{-1} .

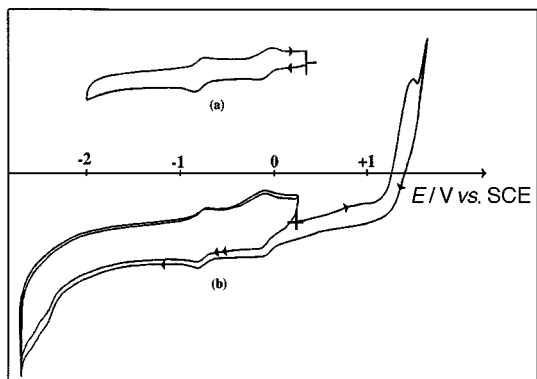


Fig. 2 Cyclic voltammetry on a glassy carbon electrode ($d = 3$ mm), reference electrode: SCE, $\nu = 0.2 \text{ V s}^{-1}$. (a) **2** alone ($C_0 = 1$ mM) in DMSO + NEt_4BF_4 (0.1 M), (b) solution obtained by electrolysis of **1** ($C_0 = 3$ mM) after 18.9 coulombs in DMSO + NEt_4BF_4 (0.1 M) in the presence of solid Na_2CO_3 .

Table 1 Cyclic voltammetry of **1**

	ACN	DMSO	CH_2Cl_2	H_2O
$E_p/\text{V vs. SCE}$	1.35	1.19	1.45	0.85
$n/\text{F mol}^{-1}$	0.58	0.84	0.64	2

micrometric electrode. It is therefore possible to measure the standard potential of the $1/1^{++}$ couple: $E^\circ = +1.43 \text{ V/SCE}$ as the half sum of the anodic and cathodic peak potentials as well as to estimate the life time of the radical cation: $\tau = 20 \mu\text{s}$. In DMSO the oxidation peak is located at $+1.19 \text{ V/SCE}$. On the cathodic and successive scans, as shown in Fig. 2, two reversible systems are observed, which can be assigned by comparison with the azocompound **2** isolated and identified after electrolysis (see below). A similar voltammogram was observed in dichloromethane. The potentials and number of electrons are gathered in Table 1.

In aqueous medium (pH = 7 buffer) an irreversible oxidation peak ($n = 2.0 \text{ F mol}^{-1}$ by comparison with methyl viologen) is observed at $E_p = +0.85 \text{ V/SCE}$ with gold, platinum or GC electrodes at $\nu = 0.2 \text{ V s}^{-1}$.

Electrolyses were performed in ACN, DMSO and CH_2Cl_2 but also in aqueous medium (dimethylformamide–pH 7 buffer: 75:25 v/v). In ACN and in aqueous medium the current decreases rapidly due to the fouling of the electrode. However analysis of the final solution by thin layer chromatography indicates that the same product is formed in the four solvents. Electrolysis of **1** in DMSO in the presence of solid sodium carbonate (which improves the yield of the electrolysis by trapping the protons liberated in the course of the oxidation) shows unambiguously the formation of dimer **2** by comparison with an identified sample (Fig. 2). Electrolysis of **1** in CH_2Cl_2 under the same conditions provides the dimer **2** which can be isolated in 30% yield and characterised by spectroscopy. The proton NMR shows the three aromatic protons (200 MHz, CDCl_3 , δ (ppm): 6.95 (1H); 7.48 (2H)). The assignment of the N=N bond by infrared spectroscopy is difficult for a symmetrical

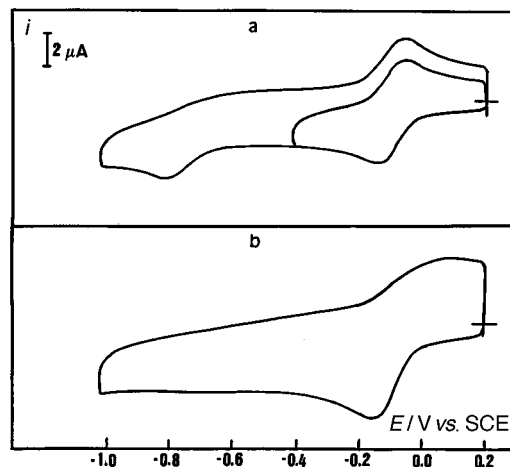


Fig. 3 Voltammogram of **2** in (a) DMSO + 0.1 M NBu_4BF_4 and (b) in the presence of an excess of acetic acid (0.17 M). GC electrode, reference SCE, $\nu = 0.2 \text{ V s}^{-1}$.

molecule as the absorption bands are weak and may be confused with the aromatic C=C vibrations. On the contrary, in the case of a conjugated N=N bond one can expect a rather strong band between 1465 and 1380 cm^{-1} in Raman spectroscopy. The strong band observed at 1385 cm^{-1} on the FT Raman spectrum (with excitation in the infrared at 1060 cm^{-1}) is therefore assigned to this vibration while the other strong band observed at 1175 cm^{-1} is related to the symmetrical vibration of the CF_3 group.

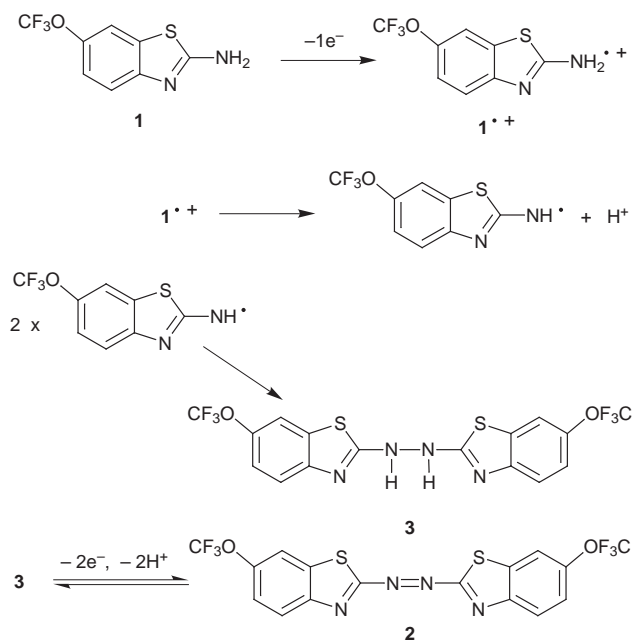
The same (as shown by TLC and HPLC) dimer **2** can be isolated through the oxidation of **1** by dioxygen in the presence of Cu(I) in pyridine at room temperature,¹¹ albeit in low yield (about 5%).

The cyclic voltammetry of **2** (Fig. 3) presents first reversible systems at $E^\circ = -0.14 \text{ V/SCE}$ (in ACN) and $E^\circ = -0.085 \text{ V/SCE}$ (in DMSO) and a second irreversible peak at $E_{pc} = -0.79 \text{ V/SCE}$ (in ACN) and $E_{pc} = -0.80 \text{ V/SCE}$ (in DMSO) corresponding to the reduction of **2** into a radical anion and a dianion. A similar voltammogram is observed at the end of the electrolysis of **1** in CH_2Cl_2 and in DMSO (Fig. 2) in the presence of carbonate, but in the last case both peaks are reversible, the acidic impurities of the medium being trapped by the carbonate. In the presence of acetic acid a single bielectronic wave is observed at -0.15 V/SCE in DMSO.

The preceding results suggest the following mechanism (Scheme 1) for the oxidation of **1**. The radical cation $1^{+\cdot}$ is formed through the transfer of one electron, it is then deprotonated either by the base of the buffer in aqueous medium or by the starting molecule in unbuffered aprotic media (ACN, DMSO, CH_2Cl_2). The resulting neutral radical dimerises to give an hydrazo compound which is more easily oxidised than the starting compound and leads to the final azo compound **2**. In buffered medium this mechanism corresponds to a consumption of 2 F mol^{-1} . In non buffered medium the starting molecule is protonated by the protons liberated during the oxidation, therefore 4 electrons are consumed for 6 molecules of **1** (2 molecules are oxidised and 4 are protonated) and a coulometry of 0.66 F mol^{-1} is expected. This is in fair agreement with the results observed in DMSO (0.84 F mol^{-1}), in ACN (0.58 F mol^{-1}) and in CH_2Cl_2 (0.64 F mol^{-1}). This mechanism is in agreement with the literature data concerning the electrochemical oxidation of aminobenzothiazole in aqueous^{12,13} or aprotic medium.^{14,15}

The final azo derivative **2** can undergo either a reversible $2e^-$, 2H^+ reduction in aqueous medium leading to the hydrazo compound **3** ($E^\circ = -0.050 \text{ V/SCE}$) or a reversible reduction to its radical anion $2^{\cdot-}$ ($E^\circ = -0.08 \text{ V/SCE}$) in aprotic medium.

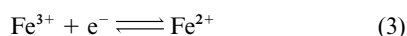
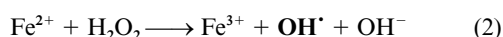
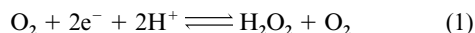
Both the oxidation by dioxygen and the electrochemical oxidation furnish the azo dimer as the resulting product. How-



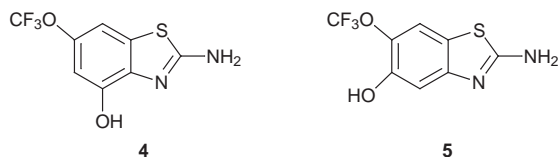
ever, it should be noted that Riluzole is rather difficult to oxidise ($E_p = +1.35$ V/SCE) and that only strong oxidants would be able to achieve this oxidation. Oxidation by dioxygen ($E^\circ = -0.75$ V/SCE in DMSO) is possible only in the presence of Cu(I) complexed by pyridine and Riluzole is most likely also involved in the complexation of copper.^{11,16–18} This complexation would lower the oxidation potential thus permitting the oxidation of Riluzole by dioxygen, a reaction which would also be possible in biological media in the presence of complexing metals.

Reaction with hydroxyl radicals

Hydroxyl radicals can be conveniently produced in controlled amounts by simultaneous electrochemical reduction of Fe^{3+} and dioxygen through the reactions (1)–(3).



This method has proved very efficient for the hydroxylation of inflammation inhibitors,¹⁹ of salicylic acid²⁰ and of benzoic acid.²¹ When subjected to such a hydroxylation, Riluzole furnishes two hydroxylated products **4** and **5** which could be identified by comparison with authentic metabolites. At the end of the electrolysis, the anode is covered with **2** but it should be noted that **2** is not produced if the hydroxyl radicals are formed by reaction of hydrogen peroxide with Fe^{2+} , indicating that **2** is produced by electrochemical oxidation at the unseparated anode.



Reaction with superoxide ion

Superoxide ion²² can be easily prepared by electrochemical reduction of dioxygen in aprotic media^{23,24} and its solutions in DMSO are relatively stable. Cyclic voltammetry of dioxygen in DMSO presents a reversible system ($\text{O}_2/\text{O}_2^{\cdot-}$, $E^\circ = -0.77$

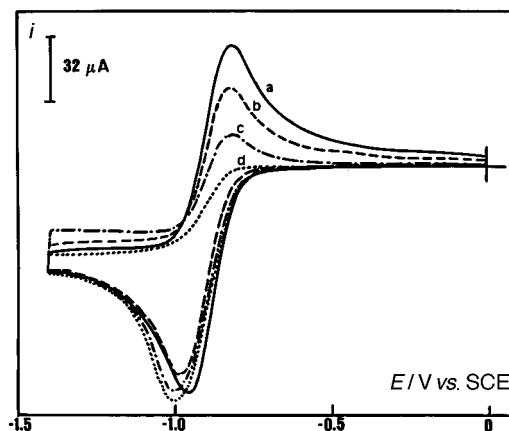


Fig. 4 Cyclic voltammogram of dioxygen (saturated solution in DMSO + 0.1 M NBu_4BF_4) in the presence of **1**; $C = 0$ mM (a); 2 mM (b); 4 mM (c); 6 mM (d). GC electrode, reference SCE, $\nu = 0.2$ V s^{-1} .

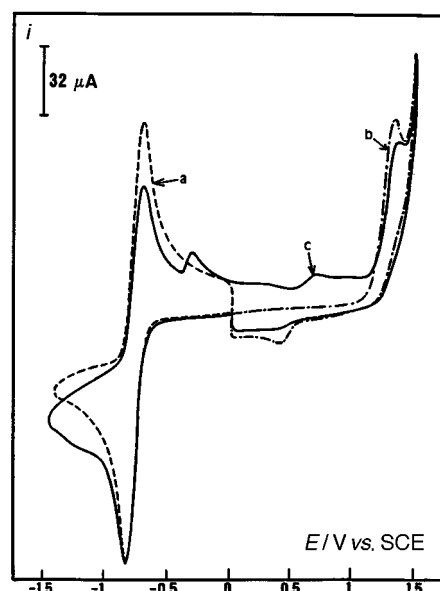


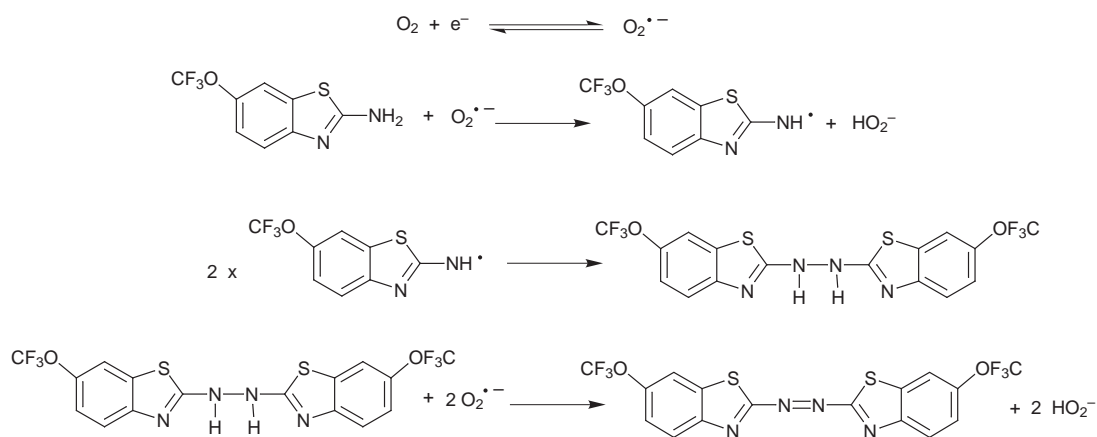
Fig. 5 Cyclic voltammogram of dioxygen alone (a); of **1** alone (b); of **1** ($C = 5$ mM) in the presence of saturated dioxygen (c). Solvent DMSO + 0.1 M NBu_4BF_4 ; GC electrode, reference SCE, $\nu = 0.2$ V s^{-1} .

V/SCE); upon addition of **1**, the reversibility of the system decreases as shown in Fig. 4. It can be also observed that the height of the irreversible voltametric peak of **1** located at $E_p = +1.3$ V/SCE decreases in the presence of superoxide ion (Fig. 5).

A preparative electrolysis was performed in DMSO at -1.0 V/SCE (at the reduction potential of dioxygen). Analysis of the final solution by TLC and HPLC indicated the presence of the dimer **2** along with two other unidentified products. The formation of **2** can be ascribed to a hydrogen atom abstraction as sketched in the following mechanism (Scheme 2) and as already observed with aromatic amines.^{25–27}

Conclusion

Riluzole is relatively difficult to oxidise as shown by its standard potential of $+1.43$ V/SCE in ACN and the radical cation obtained upon electron transfer has a lifetime of approximately 20 μs . However it can be oxidised by dioxygen when catalysed by copper. Both the electrochemical and the chemical oxidation provide an azo dimer **2**. This same dimer **2** is also obtained by reaction with superoxide ion through abstraction of a hydrogen atom. Hydroxyl radicals can be trapped by Riluzole to give two known metabolites.



Scheme 2

Acknowledgements

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Paper 8/07354F