Hydroxylation of aromatic drugs by the electro-Fenton method. Formation and identification of the metabolites of Riluzole

Mehmet A. Oturan,[a]* Jean Pinson,[a] Nihal Oturan[b] and Dominique Deprez[c]

[a] *Laboratoire d'Electrochimie Moléculaire (CNRS UMR 7591), Université Paris7-Denis Diderot, 75251 Paris Cedex 05, France. E-mail: oturan@paris7.jussieu.fr*
[b] *Université de Marne la Vallée, Cité Descartes, Bat. Lavoisier, 77420 Champs sur Marne, France*
[c] *Rhône-Poulenc Rorer, Centre de Recherche de Vitry-Alfortville, 13 quai Jules Guesdes, BP14, 94403, Vitry sur Seine, France*

Received (in Strasbourg, France) 22nd April 1999, Accepted 14th June 1999

The electro-Fenton method permits hydroxyl radicals to be produced by simultaneous electrochemical reduction of dioxygen and ferric ions. These hydroxyl radicals react with the neuroprotective drug Riluzole to give four identified hydroxylated compounds. These hydroxyl compounds are identical to the natural metabolites, the electrochemical behaviour of which is investigated. The electrochemically assisted Fenton reaction could therefore provide a convenient method for obtaining metabolites of aromatic drugs.

The electro-Fenton method permits hydroxyl radicals ($OH^{\cdot}$) to be generated by the simultaneous electrochemical reduction of dioxygen and catalytic amounts of ferric ion in an acidic aqueous medium, on a carbon electrode.[1–3] An acidic aqueous medium prevents the precipitation of iron hydroxide and allows the electrolysis to be performed without additional supporting electrolyte.

$$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2$$

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$$

$$Fe^{3+} + e^- \rightarrow Fe^{2+}$$

Hydroxyl radicals are powerful hydroxylating agents and their reaction with aromatic compounds provides hydroxylated derivatives,[2,4–6] as shown, for example, on benzoic and salicylic acids.

Metabolites of aromatic drugs are often hydroxylated compounds[7–9] and we want to show in this report that the electrochemically assisted Fenton reaction offers an easy and fast way of preparing such metabolites in small amounts, sufficient, however, for identification. We will consider the example of Riluzole,[10,11] a neuroprotective drug that has proved to be efficient against amyotrophic lateral sclerosis (ALS).

In an acidic aqueous medium (pH 2, room temperature), saturated with dioxygen and containing Riluzole (1–5 mM) and ferric chloride (1 mM), the potential of a carbon working electrode was set at −0.5 V *vs.* SCE, a potential at which both dioxygen and ferric ions are reduced.[12] Aliquots were withdrawn at different charge amounts and analysed by high performance liquid chromatography (HPLC) using a Shadon ODS Hypersil C-18 reversed phase column (250 mm × 4.6 mm i.d.; 5 μm mean particle diameter). The column was eluted with methanol–phosphate buffer (Na_2HPO_4, 0.01 M)–acetic acid (60 : 40 : 0.2 v/v) with a flow rate of 0.7 mL min^{-1}. The detection was performed by UV absorption at 263 nm. The HPLC retention times (Fig. 1) were 24.80 min for Riluzole, 22.62 min for **II**, 16.70 min for **III** and 9.95 min for **IV** and **V**. To analytically separate **IV** and **V**, the column was eluted with a linear gradient composed of solvent A (acetonitrile–water–phosphoric acid, 20 : 80 : 0.2 v/v) and solvent B (acetonitrile–water–phosphoric acid, 90 : 10 : 0.2 v/v) at mL min^{-1}. The percentage of B was 10 at initial time, 10 at 5 min, 90 at 45 min and 10 at 60 min. The retention times under these conditions were 25.60 min for **V** and 28.50 for **IV**.

The reaction products were identified by comparison with authentic samples using HPLC and HPLC-mass spectrometry (LCQ2 system, electrospray HPLC/MS/MS, chemical ionization; $[M + 1]^+$ at 235 for Riluzole and at 251 for the four hydroxylated metabolites). In this way, we could identify the four known metabolites of Riluzole (Scheme 1). The maximum yield of the metabolites was obtained after 300 C had been passed through the solution (125 mL of a 1 mM Riluzole solution, reaction time about one hour): **II** (3%), **III** (7%), **IV** (4%), **V** (3%). These monohydroxylated derivatives underwent further hydroxylation and ring opening reactions. It has been shown previously that CO_2 and H_2O are the final oxidation products of organic compounds by hydroxyl radicals.[13,14] The polyhydroxylated and ring opened compounds are observed as a series of peaks at the begining of the chromatogram.

The electrochemical behaviour of these four hydroxylated derivatives was examined by cyclic voltammetry on a glassy carbon electrode with a scan rate equal to 0.2 V s^{-1}. Riluzole itself at pH 6.5 (MeOH–H_2O, 50 : 50 v/v) shows a two-

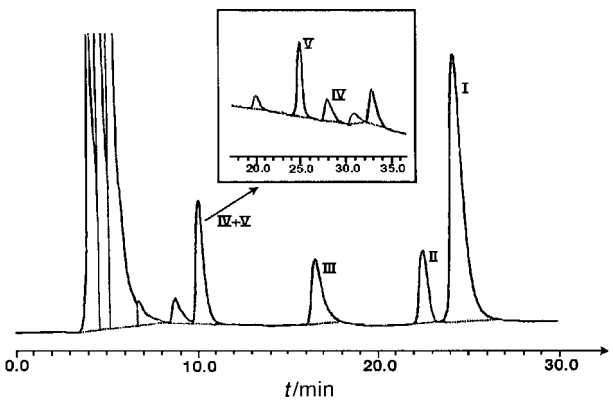
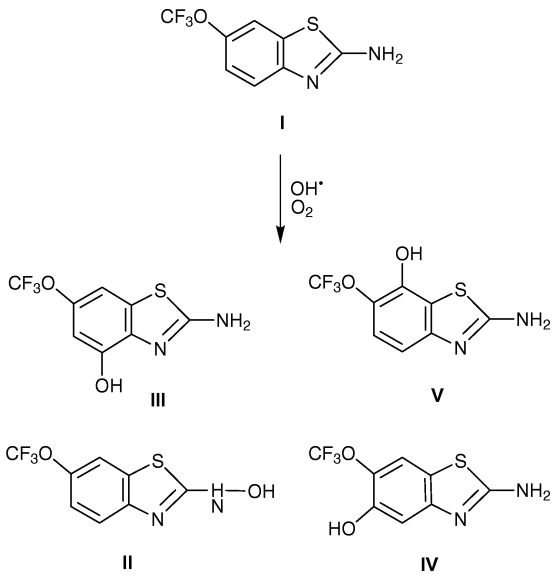
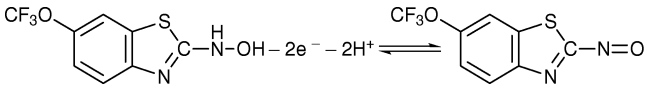


Fig. 1 Chromatogram of the electrolysis solution of Riluzole. Separation of **IV** and **V** was achieved under gradient conditions (see text for conditions).



Scheme 1

electron irreversible oxidation wave at +1.07 V *vs.* SCE, leading to an azo dimer[5]. The ring hydroxylated compounds are irreversibly oxidized: **III** (E_p = +0.55 V *vs.* SCE), **V** (E_p = +0.65 V *vs.* SCE), and **IV** (E_p = + 0.67 V *vs.* SCE); they also present a broad wave at approximately +1.10 V *vs.* SCE. The first wave likely corresponds to the oxidation of the phenolic function while the second one located at the same potential as Riluzole itself should correspond to the oxidation of the amino function. The behaviour of **II** is different: it presents in the same medium a reversible wave located at E° = +0.44 V *vs.* SCE. The reversibility of this voltammetric wave can be confirmed by spectroelectrochemistry: **II** presents an absorption maximum at λ_{max} = 292 nm, which disappears upon oxidation at +0.5 V while a new one appears at 387 nm; if the potential of the grid is returned to −0.5 V the initial spectrum reappears, thus showing the reversibility of the system on a time scale of a few minutes. This reversible system can be confidently assigned to the oxidation of the hydroxylamine function into a nitroso group on the basis of the well established behaviour of hydroxylamines in aqueous medium:[15]



The hydroxylated derivatives of Riluzole are therefore more easily oxidized than the starting compound, in particular **II** whose behaviour is similar to that of an antioxidant.

In conclusion, the electrochemically assisted Fenton reaction, despite the low yields, appears as an easy and fast method to obtain hydroxylated derivatives of aromatic drugs, which may be identical to the biological metabolites. There is indeed a present lack of good methods to selectively oxidize aromatic molecules into phenols.

References

1 R. Tomat and A. Rigo, *J. Appl. Electrochem.*, 1985, **15**, 167 and references therein.
2 M. J. Clifton and A. Savall, *J. Appl. Electrochem.*, 1986, **16**, 812.
3 M. A. Oturan and J. Pinson, *J. Phys. Chem.*, 1995, **99**, 13948.
4 G. B. Buxton, C. L. Greenstock, V. P. Helman and A .B. Ross, *J. Phys. Chem. Ref. Data*, 1988, **17**, 513.
5 C. Walling, *Acc. Chem. Res.*, 1975, **8**, 125.
6 M. A. Oturan, J. Pinson, D. Deprez and B. Terlain, *New J. Chem.*, 1992, **16**, 705.
7 *Biotransformations: a Survey of the Biotransformations of Drugs and Chemicals in Animals*, ed. D. R. Hawkins, Royal Society of Chemistry, London, 1988–1993, vol. I–V.
8 *Oxygen Radicals in Biology and Medicine*, eds. M. G. Smic, K. A Taylor, J. F. Ward and C. von Sonntag, Plenum Press, New York, 1988.
9 B. Halliwell and H. Kaur, *Free Rad. Res.*, 1997, **27**, 239.
10 G. Bensimon, L. Lacomblez, V. Meininger, and the ALS/Riluzole study group, *New Eng. J. Med.*, 1994, **330**, 585.
11 P. Couratier, P. Sindou, F. Esclaire, E. Louvel and J. Hugon, *NeuroReport*, 1994, **5,** 1012.
12 M. A. Oturan, J. Pinson, M. Traikia and D. Deprez, *J. Chem. Soc., Perkin Trans. 2*, 1999, 619.
13 G. Merga, H.-P. Schuchmann, B. S. Madhava Rao and C. von Sonntag, *J. Chem. Soc., Perkin Trans. 2*, 1996, 1097.
14 Y. Sun and J. J. Pignatello, *Environ. Sci. Technol.*, 1993, **27**, 304.
15 W. Kemula and T. M. Krygowski, in *Encyclopedia of the Electrochemistry of the Elements, Organic Section*, eds. A. J. Bard and H. Lund, M. Dekker, New York, 1979, vol. 13, p. 78.

Letter 9/03791H