Stereoselective Electrochemical Reduction of Imazapyr in Aqueous Media Without Chiral Auxiliaries

J. D. Mozo, a,*, M. L. López-López, a J. L. Oloqui-Sariego, b V. M. Molina, b J. Maraver, a and J. Carbajo a

aDepartamento de Ingeniería Química, Química Física y Química Orgánica, Facultad de Ciencias Experimentales, Universidad de Huelva, E-21071 Huelva, Spain
bDepartamento de Química Física, Facultad de Química, Universidad de Sevilla, E-41012 Sevilla, Spain

The electrochemical reduction of imazapyr at the static mercury drop electrode was studied by cyclic voltammetry as a function of pH in aqueous buffered media. The process leads to the 2,3-C≡N double bond reduction in the imidazoline moiety in all media. The products have been isolated by controlled-potential electrolyses and identified by high performance liquid chromatography–tandem mass spectrometry measurements and 1H-NMR, 13C-NMR, and IR spectra. Although no chiral auxiliary was used, a moderate diasteroisomeric excess was observed. The diasteromeric ratio depends on pH of the electrolyses.

© 2010 The Electrochemical Society. [DOI: 10.1149/1.3466872] All rights reserved.

Manuscript submitted January 14, 2010; revised manuscript received June 16, 2010. Published August 2, 2010.

Imidazolinones are a family of compounds inaugurated in 1981 by the American Cyanamid with the synthesis of the herbicide imazapyr (see Scheme 1) and a set of others whose herbicide activity was modulated by adding a substituent on position 5 of the pyridine moiety or, alternately, interchanging the aromatic system with benzene or naphthalene. Further studies demonstrated that this type of chemical exhibits anticonvulsives activity, antimicrobial efficiency over infectious diseases, and the ability to act as chelating ligands with various metal ions. Their organometalllic complexes may show specific chiral catalytic activity in the homogeneous asymmetric syntheses or spin-crossover characteristics that make them suitable for developing molecular switches, sensors, organic displays, or data storage devices.

Synthesis and characterization of related compounds with similar structures and their respective chelate complexes with some metal ions using structural analysis by 1H-NMR, 13C-NMR, optical rotation, absorption, electron paramagnetic resonance spectroscopy, X-ray diffraction, elemental analysis, and others have been reported.

The degradation of imidazoline herbicides has been studied extensively in natural media. This degradation is induced by sunlight and other soil components. This degradation is induced by sunlight and other soil components. In most cases, the stereochemistry of the reduction process.

Control of the synthesis process of organic compounds is one of the most important fields in organic chemistry and is well-developed today. However, the stereochemical aspects of organic electrochemistry have advanced slowly because the manner of stereocontrol in electrochemical reactions is highly complex. Stereocchemical results are influenced by a wide variety of experimental conditions such as temperature, electrode material, the nature of the electrolyte solution, and electroactive species. In all cases reported, the desired selectivity in an electrochemical process is achieved by using a chemical inducer of chirality and enhanced by modulating the experimental conditions of reaction. Although Mikysek et al. did not report anything about the diastereoselectivity, (i) the influence of pH on the electrochemical behavior, (ii) the presence of multiple ionizable substituents in the molecule structure (all the nitrogen atoms in the imidazolyl–pyridine derivative), and (iii) the existence of an asymmetric carbon may induce selectivity in the reduction of a prochiral carbon (C2 in the imidazolinic moiety). To show this hypothesis, we studied the electroreduction of a commercial imidazolinone molecule (imazapyr) in a wide range of pH.

The aim of this paper is to contribute to a better understanding of the reduction mechanism of imidazolinones and to show the possibility of achieving selectivity in an electrochemical process in the absence of auxiliaries.

Experimental

Reagents and solutions.—Imazapyr (Pestanal) was purchased from Riedel as analytical standard (purity > 99.9%) and was used without further purification. All the other reagents employed were of analytical grade.

All solutions were prepared using low conductivity water (18 MΩ cm) purified by a Millipore Milli-Q water system. Stock solution of 0.1 M Britton–Robinson buffer was prepared, and ionic strength was adjusted with 0.1 M tetraethylammonium hydrogen sulfate (TEAHSO4). pH of the working solutions was adjusted with solid NaOH or small additions of concentrated commercial HCl.

Imazapyr solutions were stored in closed recipients and protected from sunlight to avoid the decomposition of the herbicide. The solutions were rejected after 3 days.

All the experiments were carried out at room temperature except for the cyclic voltammetry (CV) experiments, which were at 25.0 ± 0.1°C in a cell with a water jacket. For the electrochemical experiments, oxygen was removed by bubbling nitrogen through the solutions.

Organic Chromasolv grade solvents were used for the purification and separation of the products.

Scheme 1.

* E-mail: j.daniel.mozo@diq.uhu.es

Reagents and solutions.—Imazapyr (Pestanal) was purchased from Riedel as analytical standard (purity > 99.9%) and was used without further purification. All the other reagents employed were of analytical grade.

All solutions were prepared using low conductivity water (18 MΩ cm) purified by a Millipore Milli-Q water system. Stock solution of 0.1 M Britton–Robinson buffer was prepared, and ionic strength was adjusted with 0.1 M tetraethylammonium hydrogen sulfate (TEAHSO4). pH of the working solutions was adjusted with solid NaOH or small additions of concentrated commercial HCl.

Imazapyr solutions were stored in closed recipients and protected from sunlight to avoid the decomposition of the herbicide. The solutions were rejected after 3 days.

All the experiments were carried out at room temperature except for the cyclic voltammetry (CV) experiments, which were at 25.0 ± 0.1°C in a cell with a water jacket. For the electrochemical experiments, oxygen was removed by bubbling nitrogen through the solutions.

Organic Chromasolv grade solvents were used for the purification and separation of the products.
Table I. Relative yields of products I and II in acidic and alkaline media detected by several techniques.

<table>
<thead>
<tr>
<th>pH</th>
<th>Electrolysis potential (mV)</th>
<th>Number of electrons</th>
<th>HPLC/MS</th>
<th>^1H-NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>% I ′</td>
<td>% I ′′</td>
</tr>
<tr>
<td>1.2</td>
<td>~650</td>
<td>1.82</td>
<td>56.43</td>
<td>43.57</td>
</tr>
<tr>
<td>10.9</td>
<td>~1450</td>
<td>1.90</td>
<td>34.96</td>
<td>65.04</td>
</tr>
</tbody>
</table>

a Relative intensity of chromatographic signal at 1.8 min.
b Relative intensity of chromatographic signal at 6.1 min.
c Diastereomeric ratio: %I′′/%.d Relative intensity of ^1H-NMR signal at δ = 1.08 ppm (CH₃).
e Relative intensity of ^1H-NMR signal at δ = 1.20 ppm (CH₃).

Isolation of the products.— After electrosytes, pH of the solution was adjusted to 4.1 to neutralize the charges of the products and the reagent remaining. These solutions were evaporated to dryness; the residue was extracted with chloroform. The extract was then reduced to dryness again and was methylated with an excess of 0.1 M trimethylsilyldimethyl ammonium hydroxide (TMAH) in methanol and re-evaporated to dryness. Excess TMAH was removed by percolating the extract in a silica gel (Merck 1.0935.1000) column (inner diameter = 2 cm; h = 10 cm) and a chloroform/methanol (12:1, v/v) mixture. Methylated derivatives of the compounds I and II (see Scheme 1 and Preparative electrosytes experiments section concerning the nature of products), called Met-I and Met-II, were eluted together by washing the column with pure methanol.

Chromatographic method and the resultant data.— The flow rate for the high performance liquid chromatography (HPLC) was 300 μL/min. Analysis was carried out using a gradient solvent program. The initial composition of the mobile phase was 10% acetonitrile and 90% water, both acidified with 0.1% of formic acid. An isocratic regime was maintained for 2 min. The gradient was programmed to linearly increase the amount of acidified acetonitrile up to 60% in 28 min, and then an isocratic regime was maintained for 5 min. To clean the column, the amount of acidified acetonitrile was kept constant at 100% for 5 min, and after, 10% of acidified acetonitrile for 2 min.

Chromatograms of electrolyzed crude solutions were recorded with the MS detector tuned to the m/z ratio corresponding to molecular ions (M + 1)⁺ of imazapyr (M = 261) or reduced imazapyr (M = 263), respectively. In the first case, only one peak was observed at ~9.8 min, which corresponds to the nonelectrolyzed imazapyr. The similarity between the mass spectrum for this peak and that for the imazapyr standard in the same conditions confirms this assignment. In the second case, the chromatogram of the reduction products was obtained. There are two peaks at 1.8 and 6.1 min, which correspond to the diastereoisomers of the reduced imazapyr (this molecule has two chiral centers). The assignment of these peaks to diastereoisomers is confirmed by their equal mass spectra.

Similar results were obtained when the imazapyr was electrolyzed in acid and alkaline media, except for the relative intensities of chromatographic peaks, which depend on pH. The results are shown in Table I.

NMR analysis and the resultant data.— The ^1H- and ^13C-NMR spectra (δ in ppm, J in Hz) of hexadeuterated dimethyl sulfoxide solutions of Met-I and Met-II were measured (400.2216 MHz for ^1H and 100.6433 MHz for ^13C; reference for δ = ^1H traces of deuterated solvent). The analyses of the mixture of the products gave the following results (the assignment of signals to products Met-I or Met-II is based on their relative intensities; all signals appear in pairs of equal multiplicity and similar displacement): (i) Product Met-I: (2-(4-isopropyl-1,3,4-trimethyl-5-oxo-imidazolidine-2-yl)-nicotinic acid (δ = 9.2-NMR (δ): 0.88 (d, 3H, J = 6.58, CH₂ isopropyl), 0.91 (d, 3H, J = 6.58, CH₃ isopropyl), 1.08 (s, 3H, CH₃), 1.75 (hpt, 1H, J = 6.71, CH isopropyl), 2.48 (s, 3H, NCH₃), 6.34 (bs, 1H,
Results and Discussion

CV analysis.— The reduction of imazapyr was studied by using the CV technique in a wide range of pH values and sweep rates. The analysis of voltammograms over the whole pH range shows that the reduction process is highly dependent on pH. This can be shown by plotting the peak potential ($E_p$) of the first voltammetric wave against this parameter (Fig. 1). As can be seen, there are three ranges of pH in which the $E_p$ behavior is different, which will be described below.

In all media, the number of electrons was two per molecule electrolyzed, and the products were those derived from the double bond 2,3-C=\(-\)N reduction. As seen in the Experimental section, the preparative electrolyses were performed at pH 1.2 and 10.9. The number of electrons was determined from the total charge and the amounts of reagent electrolyzed. To obtain the number of electrons in those media whose pH values ranged from 1 to 6 and from 10 to 13, the area under the first voltammetric wave was calculated (Table II), with a similar value achieved in all cases. This fact confirms that the total number of electrons interchanged remains constant across the range of pH studied.

Acid media (pH < 6).— In this range of pH, imazapyr gave two relevant reduction waves without oxidation, as shown in Fig. 2. The peak at the least negative potential, the so-called A, was analyzed. This wave shifts to a more negative potential with increasing pH, as can be seen in Fig. 1. A slope of $\approx 70$ mV/pH unit is observed at pH values from 1.0 to 6.0. This behavior indicates that two protons were involved in the whole reduction process.

2-H), 7.32 (d, 1H, $J = 5.02$, 5'-H), 8.05 (dd, 1H, $J_1 = 7.87$, $J_2 = 1.68$, 4'-H), 8.51 (m, 1H, 6'-H) and $^{13}$C-NMR $= 17.1$ (CH$_2$-i), 18.4(CH$_3$-i), 21.5(CH$_3$), 27.7(NCH$_3$), 33.9 (CH-i), 64.7 (C-4), 71.0 (C-2), 123.8(C-5'), 138.0(C-3'), 138.2(C-4'), 149.3(C-6'), 154.4(C-2'), 170.8(CO$_2$H), 177.4 (C-5) and (ii) product Met-II: (2-(4-isopropyl-1,3,4-trimethyl-5-oxo-imidazolidine-2-yl)-nicotinic acid) $[^1]$c-NMR (δ): 0.83 (d, 3H, $J = 6.58$, CH$_3$ isopropyl), 0.88 (d, 3H, $J = 6.58$, CH$_3$ isopropyl), 1.20 (s, 3H, CH$_3$), 1.87 (qpt, 1H, 1H, $J = 6.71$, CH isopropyl), 2.46 (s, 3H, NCH$_3$), 6.36 (bs, 1H, 2-H), 7.30 (d, 1H, $J = 5.02$, 5'-H), 8.01 (dd, 1H, $J_1 = 7.87$, $J_2 = 1.60$, 4'-H), 8.51 (m, 1H, 6'-H) and $^{13}$C-NMR $= 17.1$ (CH$_2$-i), 18.5(CH$_3$-i), 22.9(CH$_3$), 27.3(NCH$_3$), 35.2 (CH-i), 64.8 (C-4), 72.8 (C-2), 124.0(C-5'), 137.9(C-3'), 138.3(C-4'), 149.3(C-6'), 154.5(C-2'), 170.8(CO$_2$H), 177.2 (C-5).

The results obtained at different pH values indicate dependence between the intensities of the peaks of products I and II and pH of the electrolyses. This dependence is the same as that observed in the chromatographic peaks and is reported in Table I for comparison.

IR spectroscopy.— The IR spectrum of the product mixture (nujol mull) was measured giving the following results (wavenumbers in cm$^{-1}$): 3288, 2924, 1678, 1598, 1458, 1379, 1258, 1152, 1077, and 799.

UV-vis spectroscopy.— The absorption spectra in the 200–350 nm range were recorded as a function of pH. The working solution was imazapyr $8 \times 10^{-5}$ M in 0.1 M Britton–Robinson buffer and 0.1 M TEAHSO$_4$. In strongly acidic medium, the spectra present a single band whose maximum absorption is at 236 nm. From slightly acidic medium, two absorption bands arise with maxima centered at 224 and 267 nm.

Polarimetry and CD.— The polarimetric experiment was made at 253.7 nm using an aqueous solution of imazapyr $10^{-3}$ M at room temperature. No change in the angle of rotation was observed, which shows that commercial imazapyr is a racemic mixture.

To check this last result, CD spectra were performed in the 200–320 nm range at 25°C using different aqueous solutions of imazapyr whose concentration varied from $10^{-5}$ to $10^{-3}$ M. For each spectrum, 5–10 runs were averaged with a 5 min equilibration interval before the scans. Again, the results confirm the racemic composition of the reactant because no CD bands were observed.

Table II. Peak magnitudes of first cathodic wave and computed area for media at several pH values.

<table>
<thead>
<tr>
<th>pH</th>
<th>Peak potential (mV)$^a$</th>
<th>Current density peak (mA/cm$^2$)$^a$</th>
<th>Area (µA/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>−519</td>
<td>−4.99</td>
<td>1611.8</td>
</tr>
<tr>
<td>2.98</td>
<td>−655</td>
<td>−4.33</td>
<td>1617.9</td>
</tr>
<tr>
<td>5.00</td>
<td>−810</td>
<td>−3.18</td>
<td>1621.3</td>
</tr>
<tr>
<td>10.02</td>
<td>−1274</td>
<td>−2.75</td>
<td>1769.4</td>
</tr>
<tr>
<td>11.00</td>
<td>−1301</td>
<td>−2.78</td>
<td>1769.6</td>
</tr>
<tr>
<td>12.11</td>
<td>−1360</td>
<td>−2.78</td>
<td>1747.1</td>
</tr>
</tbody>
</table>

$^a$ Data from first voltammetric reduction wave.

Figure 1. Peak potential of the first voltammetric wave. Imazapyr $1 \times 10^{-5}$ M, $v = 2.0$ V/s

Figure 2. Cyclic voltammograms of imazapyr $1 \times 10^{-3}$ M at several pH values in acidic media, $v = 2.0$ V/s in all cases.
These results and analysis of the final products are consistent with the mechanism proposed by Mikysek et al. for similar molecules (IMZH\(^+\) + 2e\(^-\) + 2H\(^+\) \\\rightleftharpoons IMZH\(^2+\)) in which IMZH\(^+\) is the imazapyr protonated in 3N and IMZH\(^2+\) corresponds to products I and II protonated in same position.

The pattern of the voltammetric wave also becomes broader and lower with increasing pH. This change in shape can be explained by the adsorption of imazapyr over the mercury electrode and by the pH dependence on this process. However, the number of electrons remains constant.

Neutral and weakly alkaline media (6 < pH < 10). In this region, wave A goes down and, at the same time, splits into a new wave (B) at more negative potentials, whose intensity increases with pH (Fig. 3). Slight changes in the peak potential of wave A are observed across this range of pH, while the peak potential of wave B remains constant. According to the analysis of \(I_L\) convoluted, these waves can be associated to the reduction processes of two chemicals involved in an acid/base equilibrium. As can be seen in the insert of Fig. 3, the addition of intensities \((I^2 + I^3)\) remains constant throughout this pH range. This behavior is related to a protonation equilibrium before the reduction, as will be explained later.

Meanwhile, an oxidation wave appears whose peak potential shifts to more negative values. The behavior of the oxidation wave with the reverse potential permits us to confirm that it is the anodic process associated to wave B.

Strongly alkaline media (pH > 10). In this media, the reduction peak A disappears along with the others at the most negative potential (see Fig. 4). The intensity of the oxidation wave decreases until it disappears in highly alkaline media and its peak potential shifts to more negative values. The formal standard potential of wave B shifts to more negative values at 50–60 mV/pH unit for pH ranged from 11.0 to 13.0. This behavior indicated that two protons are involved in the whole reduction process. The intensity of wave B is determined by the effective concentration of the chemical species, which is reduced in alkaline media, as in an amperometric titration (insert of Fig. 4). Unlike the behavior observed in the pH range above, the peak intensity reached its maximum value and remained constant throughout the interval. The evolution of the intensities of waves A and B enables the calculation of the \(pK_a\) of protonation equilibrium IMZ + H\(^+\) \\\rightleftharpoons IMZH\(^+\). Wave B corresponds to the reduction of IMZ in alkaline media, and the reduction of IMZH\(^+\) in acid media results in wave A. A value of \(pK_a = 8.33\) has been obtained from the pH value where intensities of both waves A and B are the same. An analysis of the behavior of wave B and its associated oxidation was made to obtain information about the reduction mechanism in this pH range. According to the criteria published by Nicholson and Shain, the evolution of \(E^o/\Delta E\) with log(\(v\)) corresponds to an EC\(_i\)-type mechanism. According to the experimental results of the electrolysis and the slope of \(E_p/\Delta E\), the electron transfer step IMZ + 2e + 2H\(^+\) \\\rightleftharpoons IMZH\(^2+\) can be proposed for alkaline media, which is in line with Mikysek et al. The IMZ reactant is the imazapyr present in alkaline media whose 3N remains unprotonated. The compound IMZH\(^2+\) corresponds to the products I and II and can be reoxidated by taking into account the oxidation wave observed. This anodic wave corresponds to the oxidation of the reduced IMZH\(^2+\).
are summarized in Table III. The $pK_a$ of imazapyr and its assignment to functional groups. Values published and measured in different aqueous solutions.

<table>
<thead>
<tr>
<th>Group</th>
<th>Theoretical</th>
<th>$I = 0$ M $^a$</th>
<th>$I = 0.1$ M $^b$</th>
<th>CV</th>
<th>UV-vis</th>
</tr>
</thead>
<tbody>
<tr>
<td>3N-imidazole</td>
<td>1.33</td>
<td>1.81</td>
<td>1.88</td>
<td>1.78</td>
<td>—</td>
</tr>
<tr>
<td>Carboxyl</td>
<td>3.44</td>
<td>3.64</td>
<td>3.60</td>
<td>3.40</td>
<td>—</td>
</tr>
<tr>
<td>2,3-C=N</td>
<td>—</td>
<td>—</td>
<td>8.33</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>N-lactame</td>
<td>12.35</td>
<td>11.34</td>
<td>10.81</td>
<td>11.07</td>
<td>—</td>
</tr>
</tbody>
</table>

$^a$ Reference 22.
$^b$ Reference 21.

2,3-C=N group. Because the final products are the same in the entire pH range, the chemical step may be a type of reaction that involves a homogeneous interchange of protons.

The proposed mechanism is consistent with the results obtained by digital simulation. The fit was carried out by using the Digisym program. The best fit in all pH (see Fig. 5) was obtained by an EC1 mechanism. The standard charge-transfer rate constant ($k_1$) has a value of $1.2 \times 10^{-4} \pm 0.1 \times 10^{-4}$ cm/s in pH ranging from 11 to 13, the rate constant for the homogeneous chemical step ($k_2$) increases with pH from 3 s$^{-1}$ for the same pH range, and the formal standard potential shifts similarly to the experimental peak potential.

Absorption spectroscopy experiments.— The IMZ molecule has several groups susceptible to proton dissociation (see Scheme I) whose $pK_a$ values have been measured and assigned by other authors$^{21,24}$ in aqueous solutions with an ionic strength ($I$) lower than 0.1 M. In this study, a higher value of $I$ is set ($\sim 0.4$ M); therefore, a series of spectroscopic experiments were made to obtain the $pK_a$ values for IMZ, as described elsewhere.$^{25}$ All these results are summarized in Table III.

As can be seen, the $pK_a$ value reported in the Strongly alkaline media section does not agree with the spectroscopic ones. This difference suggests that the protonation equilibrium determined by the voltammetry experiments does not correspond to either functional group that interchanges protons. The assignment of $pK_a$ to the group that is reduced (the 2,3-C=N double bond) is consistent with this line of argument.

Preparative electrolyses experiments.— To identify the reaction products unambiguously, bulk electrolyses experiments were carried out at controlled potential. The products obtained in acid and alkaline-buffered media were separated and identified by high performance liquid chromatography–tandem mass spectrometry, NMR, and IR analysis.

In both media, only a mixture of products I and II was detected. According to the structural analysis, these compounds are two diastereomers obtained as a result of the reduction of the 2,3-C=N double bond of the imidazole moiety of imazapyr by consuming two electrons and two protons per molecule reduced (Scheme I).

The relative quantities of both products obtained are pH-dependent, and the yields are summarized in Table I. In addition, there is a change in color of the products dissolved, yielding a pale yellow solution in acid and orange in alkaline.

As seen in Table I, while both products are obtained in almost the same proportion in acid media, there is a diastereomeric excess of product II in alkaline media. That is, the electrochemical reduction of imazapyr in alkaline media shows some selectivity that favors the formation of one of the stereoisomers. Moreover, selectivity has been achieved in the electroreduction although no chiral inducer was added to the medium and without the use of an enantiopure reagent.

Results seem to indicate that the reaction medium, the presence of different ionizable groups, and/or the existence of an asymmetric carbon in the reagent are sufficient to induce selectivity in the reduction studied. This result opens the way for extensive research of this behavior to discern the causes of the asymmetry. Our current efforts are directed toward the synthesis of similar compounds, in which different substituents are introduced, to study their electrochemical reduction in different conditions and analyze the influence of structural and electronic changes on the stereoselectivity of the products obtained.

This behavior also suggests the possibility of modulating the experimental conditions to contribute to the formation of one or other product, that is, the possibility of chiral synthesis.

Conclusion

Imazapyr can be reduced in aqueous-buffered media over a mercury drop electrode. The products of the reaction from the first voltammetric wave correspond to the reduction of the 2,3-C=N double bond in the imidazole ring, obtaining two diastereomers. This process is pH dependent, showing three different regions of behavior. The EC1 mechanism is proposed in all media.

It is possible to achieve some stereoselectivity and, perhaps, to improve it by changing the experimental conditions of reduction without using any chiral auxiliary; this should be due to the effect of pH on the reduction and because imazapyr is adsorbed only at acidic pH values.

Attending to the pharmaceutical and herbicidal properties of this kind of molecules, this optimization can be of interest because the biological activity often resides only in one of the enantiomers.

Acknowledgments

This research was supported by grants from the DGICYT (CTQ2004-00362) and the PAI + D + I from the Junta de Andalucía. The authors are grateful to Professor J. Fernández Arteaga for the helpful debate on spectra and structural analysis. Also, the authors thank Professor T. R. Belderrain for the use of NMR and IR spectrometers.

*Universidad de Huelva assisted in meeting the publication costs of this article.*

References