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Electrochemical Synthesis of L-Histidinol Using Solvated Electrons

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The feasibility of the preparation of L-histidinol (I) by reduction of L-histidine methyl ester (II) using an electrogenerated solvated electrons solution has been studied in a laboratory scale reactor. The solvated electrons solution was obtained by electrolysis of a solution of LiCl in $EtNH_2$ that permits an easier handling than NH_3 or $MeNH_3$. All components of the electrochemical setup have been optimized for the reaction conditions used. The influence of some reaction variables on the yield of (I) has been studied. Thus, increasing current intensity and temperature enhances the yield of L-histidinol. Nevertheless, the volatility of ethylamine limits the increase of the intensity current and the temperature. A fractionated feeding procedure of (II) was shown to be desirable. The work-up and isolation procedure of (I) has also been described. It includes an electrodialysis process that makes easier the isolation of (I), as well as the recovered LiCl could be recycled as supporting electrolyte.
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L-Histidinol (**I**), a structural analogue of the essential amino acid L-histidine is an effective drug in the fight against cancer and an inhibitor of protein biosynthesis. (**I**) increases the vulnerability of human cells affected by leukemia under the influence of several anti-carcinogenic drugs, and it acts as an intracellular histamine antagonist.[1]

Procedures described in the literature for the synthesis of (**I**) are based on the reduction of benzoyl and silylated derivatives of L-histidine or its methyl ester.[2-4] The procedure carried out in these reactions always involves the use of metal hydrides such as $LiAlH_4$[2,3] or Red-Al,[4] in ether or tetrahydrofuran (THF). This method suffers from disadvantages associated with the use of metal hydrides in the presence of ethers. Use and storage of these reagents are dangerous. Furthermore, a stepwise preparation of a derivative is needed. However, the literature does not describe any electrochemical procedure, usually characterized by its useful and simple methodology. This work describes a method of preparation of L-histidinol, based on the electrochemical reduction of L-histidine methyl ester (**II**). This method avoids the use of metal hydrides.

Preliminary voltammetric studies carried out in our laboratory, based on the direct reduction of L-histidine in strongly acid aqueous media by the use of lead or graphite electrodes, gave no satisfactory results.

The direct reduction of nonactivated esters, derived from carboxylic acids is not easy electrochemically[5] but several indirect methods have been developed to reduce these compounds at reasonably low potentials.[6-9] Some of these methods present the serious disadvantage of the low solubility of (**II**) in the reaction solvents.[8,9]

On the basis of the literature[6] and previous studies developed at this laboratory,[10] we thought it advisable to use an indirect reduction based on the electrochemical generation of solvated electrons. Although the use of ammonia,[7,11-14] methylamine,[6,15-19] ethylenediamine,[20,21] and HMPA[22-26] has been studied as stabilizer solvent to generate chemically and electrochemically solvated electrons (e_s^-) the use of $EtNH_2$ has not been adequately studied for this aim.[27,28] $EtNH_2$ (boiling point of 17°C at atmospheric pressure) allows an easier handling than ammonia or methylamine. Thus, the electrolysis of a LiCl in $EtNH_2$ solution allows us to obtain a solvated electrons solution capable to carry out the carboxylic acid ester reduction.

In this paper, we show a new method to obtain (**I**) based on the reduction of (**II**) by electrogenerated solvated electrons. The final reaction mixture contains the supporting electrolyte, LiCl, (**I**) and other reaction by-products. An electrodialysis process was used to remove LiCl from the reaction mixture. This removal makes easier the isolation of (**I**), and the LiCl, which could be recycled, is recovered.

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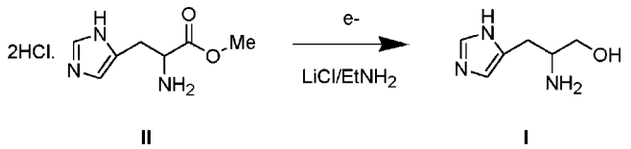
Experimental

Chemicals.—The catholyte and anolyte were prepared with LiCl PRS from Panreac and liquefied $EtNH_2$ from Praxair (97%). L-Histidine methyl ester dihydrochloride ⩾99% (AT) and L-histidinol dihydrochloride ⩾99% (AT) used as standard was supplied from Fluka. i-PrOH ⩾99% from Fluka.

Instrumentation.—The electrolysis process was carried out using a power supply Horizon Electronics model MSR 28 15. The electrodialysis was carried out using a power supply Promax model FAC303B. For HPLC analyses an isocratic Shimadzu pump model LC-10AT VP, and a HP Lichrosorb RP.18 column, dimensions: 5 μm, 200 × 4.6 mm were used. As eluant, phosphate buffer pH 3, flow: 1 mL/min and as detector an Ultraviolet HP series 1100 detector, wavelength of 210 nm were used. Lithium analyses were performed by an atomic absorption spectrometer from Varian model 220FS, by emission technique.

Reactors and experimental setup.—Electrolysis.—The experimental setup shown in Fig. 1 was used to carry out the electrosynthesis process. An electrochemical filter-press reactor described in an earlier paper[29,30] with a three-dimensional (3D) nickel (99.0% purity, Goodfellow) cathode and a 3D carbon anode (RVC 4002 carbon felt, Le Carbon Lorraine), both with a geometric area of 63 cm^2 was used. Polypropylene frames as flow distributors, EPDM gaskets and a nonselective polyethylene separator were used. The experimental setup comprises two jacketed glass solution reservoirs (350 mL) for anolyte and catholyte, the electrochemical filter-press reactor previously described and two circulation pumps (Nikkiso Eiko Co. Ltd model CP08-PPRV-24). The cooling bath temperature was lower than -5°C. All interconnecting tubing was made of glass and PTFE with an internal diameter of ~12 mm. A deaeration procedure of experimental setup was carried out with nitrogen.

Electrodialysis.—The experimental setup shown in Fig. 2 was used to carry out the electrodialysis step. We used a filter-press reactor described in an earlier paper,[31] with a stainless steel cathode and a platinized titanium anode (I.D. Electroquímica S.L.), both with a geometric area of 63 cm^2. The electrodialysis cell consists of three compartments: a central one and two lateral ones. The central compartment, diluate, contains the solid coming from the electrolysis process previously dissolved in ~400 mL of distilled water. The two lateral compartments contain the concentrate. As initial concentrate we used a NaOH 0.1 M solution. A cation exchange membrane MC-3470 (Sybron Chemicals Inc.) and an anion exchange membrane MA-3475 (Sybron Chemicals Inc.) were employed, both of



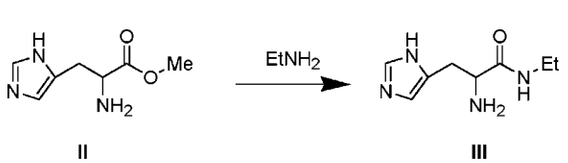
Scheme 1. Electrochemical preparation of (I) from histidine methyl ester.

63 cm^2 of projected area. All interconnecting tubing was made of flexible PVC having an internal diameter of ~12 mm.

*Electroreduction of (***II***).*—The catholyte and anolyte tanks were filled up with 250 mL of a $LiCl/EtNH_2$ solution (*ca.* 1 M) prepared separately in an ice bath. Then, the pumps of experimental setup were turned on, and a controlled current intensity was passed until the appearance of a blue coloration, indicative of solvated electrons generation. Then, L-histidine methyl ester dihydrochloride was immediately added, beginning the electrosynthesis process, developed as well at controlled current intensity. The ester addition causes an immediate discoloration of the catholyte because of the consumption of solvated electrons. The electrolysis was performed until the blue color again appeared in the catholyte. Next, the catholyte tank was unloaded and then, an amount of NH_4Cl was added (equivalent to the maximum theorical expected amount of lithium electrogenerated in catholyte). Afterward, $EtNH_2$ was removed at reduced pressure, and a solid residue was obtained. Then, 50 mL of *i*-PrOH were added and the solvent removed again at reduced pressure until dryness. This last process was repeated five times to remove the remaining $EtNH_2$ from the solid reaction mixture. Before the last evaporation, the pH of dissolved solid in *i*-PrOH was ~7 (by wet pH paper). Finally an aqueous sample was analyzed by HPLC.

L-histidinol isolation procedure.—Extractive work-up did not give satisfactory results because of the high solubility of (**I**) in water. The reaction mixture obtained in the electroreduction step contains a great amount of supporting electrolyte, LiCl, which makes difficult the isolation process of (**I**) by column chromatography. The electrodialysis technique allows the removal of LiCl as well as the recovery of a great amount of it. The electrodialysis was carried out at controlled potential of 12 V at room temperature (initial current 1.5 A). The experiment finished after passing an equivalent charge of 1 F/mol of lithium in initial diluate. An 86% of LiCl has been removed. A loss of 26% of (**I**) in the diluate has been observed. After the electrodialysis step, the water from the final diluate was removed at reduced pressure. The mixture obtained was dissolved in methanol, basified with NaOH, and then purified by flash chromatography using mixtures of ethyl acetate:methanol of increasing polarity. Samples taken were analyzed by HPLC gathering those which contain (**I**). Subsequently, HCl was added to obtain L-histidinol dihydrochloride and the solvents were then removed at reduced pressure. HPLC, spectral and optical data of the obtained product coincide with that of standard supplied from Fluka.

In a typical reaction 6.2 mmol of (II) was electroreduced and 18.5 g of solid reaction mixture, after removing the $EtNH_2$, were obtained (1.7 mmol of (I), HPLC). This mixture was used in the electrodialysis procedure obtaining 1.8 g of the crude product (1.3 mmol of (I), HPLC). Further purification by column chromatography yield 1.1 mmol of (I).



Scheme 2. Formation of the amide (III).

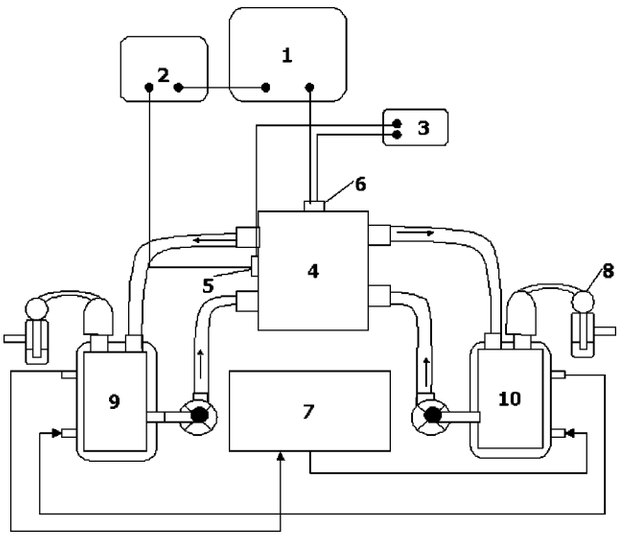


Figure 1. Experimental setup for electrochemical reduction of (II) by e_s^-. (1) Power supply, (2) charge integrator, (3) voltmeter, (4) filter press cell, (5) anode, (6) cathode, (7) cooling bath, (8) gas wash bottle, (9) anolyte tank, and (10) catholyte tank.

Results and Discussion

A series of galvanostatic runs were carried out to asses the effect of operating parameters such as temperature, current intensity, charge passed and the ester feeding procedure to the catholyte in the electroreduction process of (**II**).

The yield of L-histidinol, $\Theta_{His\text{-}OH}$, is defined as follows

$$\Theta HisOH = \frac{n1}{n2} x100 \qquad [1]$$

where n_1 is the obtained mol of (**I**) and n_2 is the added mol of (**II**).

Influence of charge passed.—The total charge passed is a parameter of great importance in the electrochemical synthesis reactions, as it can report on the number of electrons involved per mol of (**II**) in the reaction mechanism and the efficiency of the process by the current yield calculation. Obviously, the passed charge is proportional to the global solvated electrons generated during the reduction reaction. When (**II**) was used, 2 F/mol were necessary to reduce the acid protons of the molecule and 4 F/mol to reduce carboxylic group to alcohol. In most of the reactions carried out at this work, the final point of the reaction, coincident with the reappearance of solvated electrons, shows a global consumption of 6 F/mol. However, a study of the reduction of (**II**) at different charge passed values (Q_T) was first performed so that we may understand the influence of these parameter on the reaction system. The need of passing a higher charge values for appearance of the solvated electrons can be due principally to moisture or to the existence of another undesirable reduction process. Table I shows the yield obtained for a set of reactions.

Table I reveals that an increase in charge passed does not have a great effect on the yield of (**I**). As no initial product was detected, we conclude that a great amount of this charge is employed in other side reactions. Moreover, (**I**) seems to be stable in this reaction media because no significant drop in the yield was observed even when a charge more than a 30% of the theoretical necessary charge was passed. The stability of (**I**) in the reaction media was previously assessed by adding (**I**) to a e_s^- solution generated dissolving lithium in ethylamine.

If a certain amount of charge is passed, Q_p, before the addition of (**II**), then a greater concentration of solvated electrons, presumably, will be accumulated whenever the solvated electrons do not react

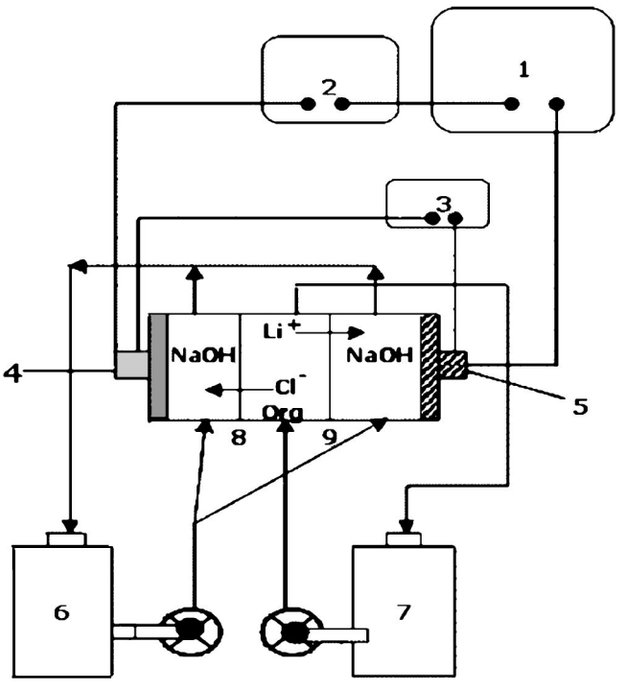


Figure 2. Experimental setup for electrodialysis process. (1) Power supply, (2) charge integrator, (3) voltmeter, (4) anode, (5) cathode, (6) concentrate tank, (7) diluate tank, (8) anion exchange membrane, and (9) cation exchange membrane.

with the solvent. Table II shows the influence of Q_p, on the yield of (**I**).

An additional chromatographic peak belonging to a new reaction product (**III**), was observed in the HPLC analysis of the reaction mixture while the previous charge variable was studied. Table II shows that the chromatographic area corresponding to this new product increases when a higher previous charge is passed. Moreover, a decreases of yield of (**II**) was observed. It is obvious that the appearance of this compound is clearly enhanced with increasing the Q_p. These results are reported in Table II.

To identify the new product appeared and its formation mechanism, the following reaction was carried out. A solution of 1.5 g of (**II**) in 150 mL of $EtNH_2$ was stirred at 0°C overnight and the solvent was then removed at reduced pressure. The HPLC analysis showed the appearance of only one peak on the chromatogram, whose retention time coincided with that of (**III**). This experience proved that (**III**) was generated by chemical reaction, not electrochemically.

The NMR spectra confirmed that the new product was *N*-ethyl-2-amino-3-(1*H*-5-imidazolyl)propanamide.

^{1}H RMN (CD_3OD) δ 8.6 (s, 1H, ArH), 7.9 (s, 1H, ArH), 4.8 (dd, J = 6.3, 7.8 Hz, 1H, C*H*NH_2), 4.0 (dd, J = 3.1, 7.8Hz, 1H, CH_2CH), 3.8 (m, 3H, CH_2CH and CH_2CH_3) and 1.9 (t, J = 7.2Hz, 3H, CH_3)

^{13}C-RMN (CD_3OD) δ 172.4 (C = O), 136.4, 133.9, 117.9 (ArC), 55.3 CHNH) 35.0, 31.7 (*C*H_2CH and *C*H_2CH_3) and 12.7 (CH_3).

The charge passed before the addition of (**II**) increases the formation of the $EtNH^-$, which is a better nucleophyle than $EtNH_2$. and it makes more extensive the formation of (**III**). This effect can be observed when the previous equivalent charge passed is more than 2 F/mol. When a lower previous charge is passed, the acid protons of (**II**) neutralize the amidure generated.

Influence of the concentration of L-histidine methyl ester.—It is well known that there are many synthetic procedures where a high concentration of some reactive is necessary in order to optimize the reaction yield. For this purpose, we have tried to study the influence of $[(\mathbf{II})]/[e_s^-]$ ratio on the reaction. To reach this aim, some reactions were carried out using the same total amount of (**II**) (6.2 mmol) but in several additions. The additions were performed when the blue color of the catholyte reappeared after the previous addition. Performing the reactions by a multiple addition avoids long and abrupt discolorations of the media so a high e_s^- concentration always remains in the catholyte solution. Table III shows the influence of the fractionated addition of the initial product.

The results show an improvement in the yield of (**I**) when the total amount of (**II**) is added in three times during the process.

Influence of the current intensity.—Another important parameter is the current intensity, as it provides a way of varying the rate of solvated electrons production. So, modifying the current intensity allowed us to change the $[(\mathbf{II})]/[e_s^-]$ ratio. Three different current intensities were studied (I = 3, 6, and 9 A). The results can be seen in Table IV.

Table IV shows that the reactions developed at the same intensity do not differ substantially in their (**I**) yields, but an increase in the current intensity causes an increase in the yield of (**I**). Nevertheless, a higher current intensity could not be used because of the volatility of the $EtNH_2$. Note that high current intensity increases the temperature in the reactor by the Joule's effect.

Table I. Influence of the total charge on the (I) yield

Entry	Q_t (F/mol)	Q_p[a] (F/mol)	Yield[b] (%)
1	6.0	0	15
2	6.9	0	18
3	8.1	0	15

[a] Charge passed before the ester addition.
[b] L-Histidinol yield on the basis of analysis by HPLC. System parameters: cooling bath temperature −10°C, current intensity 3 A, L-histidine methyl éster 6.2 mmol. 1 addition of ester.

Table II. Influence of the previous charge flown before the addition of (II) on the yield of (I).

Entry	Q_t (F/mol)	Q_p[a] (F/mol)	New product[b]	Yield[c] (%)
1	6.9	0	5.0	18
2	5.7	1.5	13.5	14
3	6.0	2.3	16.0	14
4	6.6	3.1	28.4	12
5	7.1	4.6	63.8	6

[a] Charge passed before the ester addition.
[b] Percentage of chromatographyc area, HPLC.
[c] L-Histidinol yield on the basis of analysis by HPLC System parameters: cooling bath temperature −10°C, current intensity 3A, (**II**) 6.2 mmol and 1 addition of ester.

Table III. Influence of the way of ester addition in 1 or 3 times.

Entry	Q_t (F/mol)	Q_p [a](F/mol)	Additions	Yield[b] (%)
1	6.0	0	1	15
2	5.7	1.5	1	14
3	5.2	0.1	3	21
4	6.3	1.5	3	26

[a] Charge passed before the ester addition.
[b] L-Histidinol yield on the basis of analysis by HPLC of crude. System parameters: cooling bath temperature −10°C, current intensity 3 A, (**II**) 6.2 mmol.

Table IV. Influence of the current intensity on the (I) yield.

Entry	1 (A)	Q_t (F/mol)	Q_p[a] (F/mol)	Yield[b] (%)
1	3	5.5	1.2	17
2	3	5.2	0.1	21
3	6	6.5	0.2	34
4	6	6.1	0.3	33
5	9	7.9	0.3	44
6	9	8.7	0.3	42

[a] Charge passed before the ester addition.
[b] L-Histidinol yield on the basis of analysis by HPLC of crude. System parameters: cooling bath temperature −10°C, (**II**) 6.2 mmol and 1 addition of ester.

Influence of the temperature.—Finally, the influence of reaction temperature was studied. To select different reaction temperature, an appropriate cooling bath must be employed. Although the temperature near the electrodes, in the electrochemical reactor, is most likely higher than in the bulk solution, when some reactions were carried out at the same current intensity the temperature of the cooling bath should be proportional to the reaction temperature.

Table V shows that the yield of (**I**) was increased when higher reaction temperatures were used. Nevertheless, it is not possible to carry on increasing the temperature because of the high volatility of the solvent.

Conclusions

The simplest method for obtaining (**I**) from histidine, a direct electrochemical reduction, unfortunately has not been accomplished by the studied lead or graphite electrodes in acidic aqueous medium. Nevertheless, the present investigation demonstrates the feasibility of the synthesis of (**I**) using an electrogenerated solution of e_s^-. The principal advantage of this method compared with those reported in the literature is the avoidance of the use of metal hydrides. Moreover, the procedure for electrogenerating e_s^- solutions by electrolysis of a LiCl in $EtNH_2$ solution is general and can be used for other processes. Optimized conditions for the synthesis were established and construction of a simple experimental setup for electrogenerating e_s^- solutions was achieved. Reduction of (**II**) was carried out using 6 F/mol and no great effect was observed when more charge was passed. (**I**) showed a good stability in e_s^- solutions. A high formation rate of the amide (**III**) was observed when an accumulation of e_s^- in the catholyte solution by passing a previous charge was done, this might be due to the generation of $EtNH^-$. The addition of (**II**) within three times enhances the yield of (**I**). Thus, a slowly and continuous addition of (**II**) should be desirable. Increasing current intensity and temperature enhances the yield of (**I**). Nevertheless, the volatility of ethylamine limits the increase of the intensity current and the temperature. The reaction is clearly affected by moisture and higher charge is needed to be passed for achieving the final point of the reaction.

Using the obtained optimized conditions a 44% yield can be reached. The electrodialysis stage used during the isolation procedure allows the LiCl to be recycled.

Table V. Influence of the temperature on the yield of (I).

Entry	Q_t (F/mol)	Q_p[a] (F/mol)	T[b] (°C)	Yield[c] (%)
1	8.7	0.9	−20	27
2	6.6	0.3	−19	24
3	6.1	0.4	−14	27
4	7.9	0.4	−11	28
5	8.7	0.3	−10	42
6	11.6	0.3	−8	44

[a] Charge passed before the ester addition.
[b] Cooling bath temperature.
[c] L-Histidinol yield on the basis of analysis by HPLC of crude. System parameters L-histidine methyl ester: 6.2 mmol added in 3 times. Current intensity: 9 A.

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