The intersecting parabolas model predicts a barrier consistent with that of the statistical treatment. Quantitative deviations suggest that electronic coupling may prove to be important in understanding the barrier heights.

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Registry No. $C_6H_4XNO_2$ (X = H), 98-95-3; $C_6H_4XNO_2^{\bullet-}$ (X = H), 12169-65-2; $C_6H_4XNO_2$ (X = 3-Cl), 121-73-3; $C_6H_4XNO_2^{\bullet-}$ (X = 3-Cl), 34467-54-4; $C_6H_4XNO_2$ (X = 3-F), 402-67-5; $C_6H_4XNO_2^{\bullet-}$ (X = 3-F), 34470-17-2; $C_6H_4XNO_2$ (X = 4-F), 350-46-9; $C_6H_4XNO_2^{\bullet-}$ (X = 4-F), 34467-53-3; $C_6D_5NO_2$, 4165-60-0; $C_6D_5NO_2^{\bullet-}$, 65119-76-8.

Complete Conversion of L-Lactate into D-Lactate. A Generic Approach Involving Enzymatic Catalysis, Electrochemical Oxidation of NADH, and Electrochemical Reduction of Pyruvate

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Abstract: L-Lactate was converted into D-lactate with a yield better than 97%, the system involving stereospecific catalysis of L-lactate oxidation by the rather cheap L-lactate dehydrogenase plus electrochemical regeneration of NAD^+ at the anode and electrochemical reduction of pyruvate at the cathode. Such an approach can be extended to mere deracemization or complete inversion of all types of chiral α-alcohol–acids provided that the dehydrogenase related to the isomer to be inverted is available. Efficiency was not limited by enzyme or coenzyme deactivations.

The abilities of enzymes to discriminate between enantiomers of racemic substrates are outstanding, and multiple ways of exploiting these enantiomeric specificities have been investigated with the purpose of developing processes for the preparation of chiral compounds.[3]

Resolution of racemic mixtures via acylase[4] or lipase[5] catalyses of α-functionalized carboxylates are well documented. Acylase mediated resolutions of *N*-acyl amino acids are industrially important.[4b] They proceed according to the following reaction sequence: first, the L-enantiomer is catalytically transformed into the L-amino acid; second, the unhydrolyzed *N*-acyl D-amino acid is recycled via chemically induced racemization.

Dehydrogenases are also potentially useful catalysts in chiral synthesis.[6,7] Oxidation of a chiral alcohol or amine is stereospecific, but the net result of such a transformation is most often the loss of chirality since it produces a carbonyl compound with concomitant disappearance of the initial asymmetry of the carbon atom bearing the hydroxy or amino substituent. There are only few examples in which the production of the carbonyl group can create a new asymmetry as may occur when preparing D-glyceraldehyde by means of enzymatic (glycerol dehydrogenase, E.C. 1.1.1.72. plus $NADP^+$) oxidation of glycerol. Therefore, the reductive transformation of a pro-chiral carbonyl compound into an alcohol or an amine is of much greater interest as long as the aim is the production of chiral species and, generally, it is a thermodynamically favorable reaction. Most of the dehydrogenases are NAD(P)(H)-requiring oxidoreductases[8] although there exist some important exceptions.[8b,9] Enzymatic regeneration of NAD(P)H from $NAD(P)^+$ is now relatively straightforward.[7,8,10] Chemical redox mediation of the electrochemical regeneration is also possible.[11] Inherently, these two methods imply either the introduction in the medium of a second enzyme and a second substrate, or at least a chemical redox mediator. Moreover, the enzyme-catalyzed reduction of a prochiral carbonyl derivative on a preparative scale has a built-in disadvantage when aiming at the production of the less naturally occurring enantiomer since the related enzyme, if available, is usually rather expensive.

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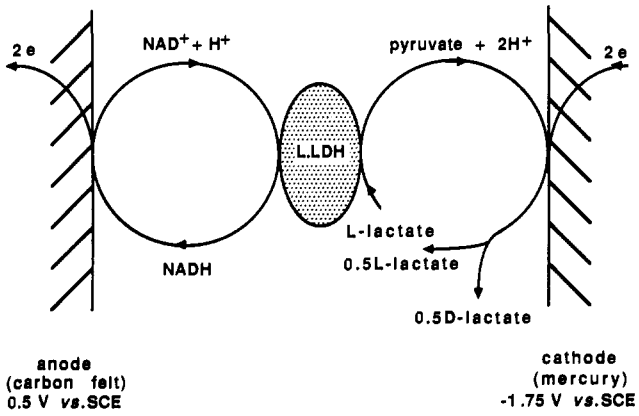

Figure 1. Scheme of the process. The starting material introduced into the system in order to produce D-lactate can be either L-lactate, racemic D,L-lactate, or pyruvate.

The intrinsic advantage of direct electrochemical regeneration of coenzymes consists in performing the regeneration without introducing additional reactants, catalysts, and products into the medium. In previous works we ascertained that the direct electrochemical regeneration of NAD^+ can be carried out with a yield better than 99.99%.[12] Unfortunately, there is presently no way of achieving the direct electrochemical reduction of NAD^+ to NADH with a good yield,[13,14] although the by-products might be recycled anodically with some ingenuity.[15]

The purpose of the present paper is to show that one enantiomer, namely, L-lactate, can be totally transformed into the other, i.e., D-lactate, by coupling the stereospecificity of the L-lactate dehydrogenase (L-LDH, E.C. 1.1.1.27.) catalyzed oxidation of L-lactate by electrochemically regenerated NAD^+, on the one hand, and electrochemical reduction of the oxidized form of the substrate, i.e., pyruvate, which yields the racemic reduced form of the substrate (D,L-lactate), on the other hand. The scheme presented in Figure 1 provides a clear illustration ofthe process which is driven by the two electrochemical reactions. The flow of 2 mol of electrons through the system should provoke the transformation of half a mole of L-lactate into the same quantity of D-lactate, while 1 mol of NAD^+ undergoes a complete redox cycle.

Results and Discussion

Means of Controlling the Electrochemically Driven Process. The enzyme-catalyzed oxidation of L-lactate:

$$\text{L-CH}_3\text{-C*HOH-COO}^- + \text{NAD}^+ \xrightleftharpoons{\text{L-LDH}} \text{CH}_3\text{-CO-COO}^- + \text{NADH} + \text{H}^+ \quad (1)$$

is a thermodynamically uphill reaction whose equilibrium constant is:[16]

$$K_{eq} = (\text{CH}_3\text{-CO-COO}^-)(\text{NADH})(\text{H}^+)/(\text{L-CH}_3\text{-C*HOH-COO}^-)(\text{NAD}^+) = 2.8 \times 10^{-12}$$

Thermodynamically, the ease of oxidation of L-lactate increases with increasing the ratio $K_{eq}/(H^+)$, i.e., with increasing pH. We chose to operate at pH 9.0 which is an upper limit for the stability of the coenzyme when it exists in its oxidized form NAD^+.[17] Then $K_{eq}/(H^+) = 2.8 \times 10^{-3}$. Theoretically, one can conceive that NAD^+ regeneration may allow a total shift of the enzyme-catalyzed equilibrium (reaction 1). However, we showed that this cannot practically occur when NAD^+ is electrochemically regenerated at the interface anode/solution even when the enzyme is immobilized onto the anode surface[18] essentially due to the inhibiting effect of pyruvate.[18,19] Then it appears that the interest of carrying out the reduction of pyruvate is 2-fold: first, allowing the completion of the shift of the enzyme-catalyzed equilibrium (reaction 1) toward its right hand side, a condition which must be fulfilled in order to achieve the selective oxidation of L-lactate; second, transforming the undesired pyruvate into the racemic D,L-lactate.

Table I. Influence of the Flow Rate Φ on the Productivity of the Oxidation Reactor[a]

Φ, mL h^{-1}	20	40	80	360
i_a,[b] mA	5.7	6.7	8.4	15
C_{pyr},[c] M	5.3×10^{-3}	3.1×10^{-3}	2.0×10^{-3}	0.78×10^{-3}

[a] Anode potential: 0.5 V versus SCE. Composition of the solution introduced in the working electrode compartment of the reactor: 0.02 M L-lactate, 2×10^{-4} M NAD^+, and 0.04 mg mL^{-1} L-LDH in 0.5 M carbonate buffer, pH 9.0. [b] Corrected for background current. [c] Deduced from eq 1 (see text).

It follows from the preceding deductions that ensuring a productive use of reaction 1 implies that both the electrochemical regeneration of NAD^+ and the electrochemical reduction of pyruvate must be performed with great efficiencies. As both are interfacially occurring electrochemical reactions, their efficiencies depend on the electrode potentials and on the solution flow rate since we use flow-through reactors. Incidentally, they may also depend on the nature of the buffer which itself must not inhibit the enzyme activity. Proceeding with two separated reactors and potentiostats (one for the anodic regeneration of NAD^+, the other for the cathodic reduction of pyruvate) enabled us to control and monitor the essential experimental parameters conditioning and reflecting the instantaneous yields of the electrochemical steps.

Enzymatic Oxidation of L-Lactate through the Electrochemical Regeneration of NAD^+. We established previously that the flow-through oxidation reactor with an anode made of carbon felt may allow a quite efficient regeneration of NAD^+ once the controlled anode potential is settled above 0.4 V versus SCE (aqueous KCl saturated calomel electrode).[20] For the presently L-LDH catalyzed oxidation of L-lactate, the dependence of the productivity of such a reactor upon the solution flow rate is illustrated by the data gathered in Table I. The relation existing between the pyruvate concentration C_{pyr} (mol L^{-1} = M) at the exit of the reactor, the solution flow rate Φ (L h^{-1}), and the anodic current intensity i_a (A) is:

$$C_{pyr} = 3600 i_a / 2F\Phi \quad (I)$$

taking into account that the production of 1 mol of pyruvate consumes a quantity of electricity numerically equal to 2 faradays (2 F) since the reaction sequence consists of reaction 1 plus reaction 2:

$$\text{NADH} \rightarrow \text{NAD}^+ + \text{H}^+ + 2\text{e} \quad (2)$$

As can be seen in Table I, i_a increases markedly with increasing Φ within the investigated range of flow rates. Such an increase in i_a implies a proportional increase in the rate of oxidation of L-lactate, i.e., a means of improving the productivity of the system if the processes occurring inside this reactor were limiting after coupling with the reduction of pyruvate. Unfortunately, this is not the case due to the efficiency of the pyruvate reduction in the

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Table II. Effects of Flow Rate Φ and Cathode Potential E_c on the Efficiency of the Electrochemical Reduction of Pyruvate 5.3×10^{-3} M in 0.5 M Carbonate Buffer pH 9.0

E_c, V vs SCE		Φ, mL h^{-1} 20	40	80	360
−1.5	i_c[a]/mA	0.9 ± 0.1	1.3 ± 0.2	1.6 ± 0.3	3.0 ± 0.5
	ρ_I[b]	0.16 ± 0.02	0.11 ± 0.02	0.07 ± 0.02	0.03 ± 0.01
	ρ_P[c]	0.14 ± 0.02	0.11 ± 0.02	0.07 ± 0.02	*f*
	ρ_L[d]	0.09 ± 0.01	0.06 ± 0.01	*f*	*f*
	ρ_D[e]	0.08 ± 0.01	0.05 ± 0.01	*f*	*f*
−1.6	i_c	4.5 ± 0.1	7.7 ± 0.2	12.2 ± 0.4	25 ± 2
	ρ_I	0.79 ± 0.02	0.68 ± 0.02	0.54 ± 0.02	0.24 ± 0.02
	ρ_P	0.81 ± 0.03	0.69 ± 0.02	0.56 ± 0.02	0.27 ± 0.02
	ρ_L	0.42 ± 0.01	0.37 ± 0.01	0.29 ± 0.01	0.14 ± 0.01
	ρ_D	0.38 ± 0.01	0.32 ± 0.01	0.26 ± 0.01	0.13 ± 0.01
−1.75	i_c	5.4 ± 0.1	9.7 ± 0.2	16.8 ± 0.4	58 ± 2
	ρ_I	0.95 ± 0.02	0.85 ± 0.02	0.74 ± 0.02	0.57 ± 0.02
	ρ_P	0.96 ± 0.03	0.88 ± 0.02	0.76 ± 0.02	0.58 ± 0.02
	ρ_L	0.51 ± 0.01	0.44 ± 0.01	0.40 ± 0.01	0.30 ± 0.02
	ρ_D	0.45 ± 0.01	0.40 ± 0.01	0.36 ± 0.01	0.26 ± 0.02

[a] Cathodic current corrected for background current. [b] Yield of the pyruvate reduction given by eq 2 (see text). [c] Yield of the pyruvate reduction determined by means of pyruvate assays. [d] Yield of L-lactate production (enzymatic assays). [e] Yield of D-lactate production (enzymatic assays). [f] Too low to be correctly determined.

present circumstances (see below). Instead, we had to focus our attention on the behavior of the enzymatically catalyzed oxidation of L-lactate at the slowest flow rate. Thus $C_{pyr} = 5.3 \times 10^{-3}$ M at $\Phi = 20$ mL h^{-1}. Pyruvate assay of the corresponding reaction mixture confirmed this calculated C_{pyr} value. Injection of such a mixture at the entrance of the oxidation reactor gave an oxidation current of 0.7 mA (Φ being unchanged), due to the inhibiting effect of pyruvate on the enzyme-catalyzed reaction. At $\Phi = 20$ mL h^{-1}, i_a became enzyme concentration insensitive when the enzyme concentration was greater than 0.02 mg mL^{-1}, the inhibition by pyruvate being again the limiting factor.[18] For the same reason, there existed no simple relation, particularly no proportionality, between i_a and the L-lactate concentration of the solution introduced into the reactor.

Efficiency of the Electrochemical Reduction of Pyruvate. The efficiencies of the reduction reactor with a mercury pool cathode at various Φ and cathode potentials are reported in Table II. Based on the measurement of the intensity i_c of the cathodic current, the yield ρ of the pyruvate reduction is:

$$\rho = 3600 i_c / 2F\Phi C_{pyr} \qquad \text{(II)}$$

The above value of ρ is confirmed by the enzymatic assay of the electrochemically produced L- and D-lactates within an uncertainty of 3%. The enzymatic assays also ascertain that the electrochemical reduction actually transforms each mole of pyruvate into at least 97% of racemic D,L-lactate. The yields appearing in Table II were roughly halved when 0.2 M pyrophosphate buffer was used in place of 0.5 M carbonate buffer. This partial inhibition of the electrochemical reduction probably results from some complexation of the pyruvate by the pyrophosphate since pyruvate is prone to such kind of complexation.[21] As a consequence, we chose to use the carbonate buffer at a relatively high concentration, i.e., 0.5 M, in order to increase the buffer capacity, pH 9.0 being markedly lower than the pK_a (ca. 10.2) of hydrogenocarbonate.

Obviously the rather low efficiency of the reduction reactor does not match that of the oxidation reactor which is very good. Things could be improved through the use of a series of reduction reactors similar to the one described in the Experimental Section, or better through the use of an other type of cathode material allowing a significant increase in the electroactive area per volume ratio, the high value of such a ratio being at the core of the efficiency of the oxidation reactor.[20,22] Alas, reduction of water precedes that of pyruvate, if there is any, at a carbon electrode. In the present work, we did not devote potentially substantial efforts to optimize this parameter. Therefore, knowing that ρ would be the limiting factor under our experimental conditions, 20 mL h^{-1} appeared the best suited value for Φ.

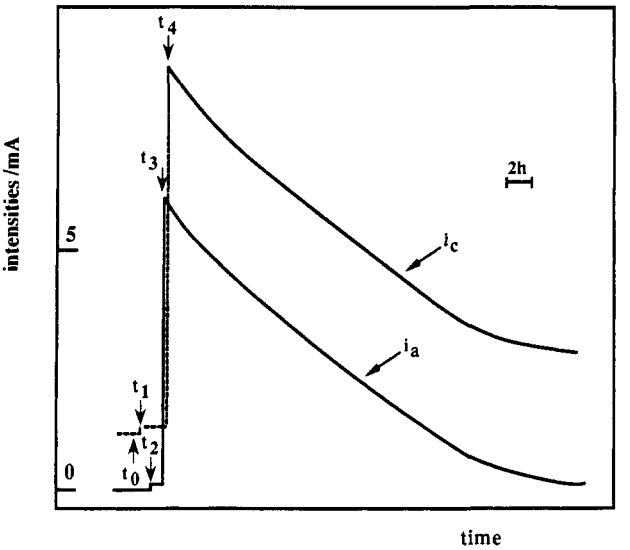

Figure 2. Experimental anodic and cathodic currents i_a and i_c versus time curves (same conditions as in Table III).

In the presence of enzyme (0.04 mg mL^{-1}), the cathodic background current increased noticeably, from 1.2 to ca. 3 mA at −1.75 V versus SCE and $\Phi = 20$ mL h^{-1} and, after an hour, gas bubbles evolved. The enzyme undoubtedly adsorbs at the mercury/water interface and catalyzes the proton discharge.[23] Fortunately this phenomenon does not affect the yield of the pyruvate reduction, but it may interfere with our global purpose in two ways. First, the proton discharge provokes a pH increase, a potential killer for both the enzyme and NAD^+.[17] Second, a loss of catalytic activity of the adsorbed enzyme is likely. However, the amount of adsorbed enzyme should be limited by the saturation of the cathode surface, and, consequently, the effective enzymatic activity of the system should not be modified provided that the enzyme concentration is in plain excess compared with what is needed for an optimum efficiency of the oxidation reactor.

Coupling of the Two Reactors. Inversion of L-Lactate into D-Lactate. We proceeded as follows, the i_a versus t (time) and i_c versus t curves providing the best means of control (Figure 2). The currents in the two reactors were allowed to stabilize, the solution circulating through the working electrode compartments of each reactor containing the 0.5 M carbonate buffer and 0.02

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Table III. Conversion of L-lactate into D-lactate: A Typical Experiment[a]

	working electrode compartments		auxiliary electrode compartments	
	initial conditions ($t = t_3$)[b]	end of the experiment ($t = t_3 = 32$ h)[b]	initial conditions	end of the experiment
volume	29 mL	47 mL	43 mL	25 mL
pH[c]	9.0	9.1	9.0	9.0
L-lactate[d]	2.00×10^{-2} M (580 μmol)	$(1.5 \pm 1) \times 10^{-4}$ M[e] (7 ± 5 μmol)	0	$(1 \pm 1) \times 10^{-4}$ M[e] (2.5 ± 2.5 μmol)
D-lactate[f]	0	$(1.18 \pm 0.02) \times 10^{-2}$ M[e] (555 ± 10 μmol)	0	$(7 \pm 1) \times 10^{-4}$ M[e] (17.5 ± 2.5 μmol)
pyruvate	0	$<10^{-4}$ M	0	$<10^{-4}$ M
NAD^+	$(2.0 \pm 0.1) \times 10^{-4}$ M	$(0.7 \pm 0.1) \times 10^{-4}$ M	0	$<10^{-5}$ M

[a] Initial enzyme concentration: 0.04 mg mL^{-1}. [b] See Figure 2. [c] 0.5 M carbonate buffer. [d] Conversion coefficient: $(580 - 7 \pm 5 - 2.5 \pm 2.5)/580 = 0.985 \pm 0.015$. [e] Means and standard deviations (five experiments). [f] Production yield: $(55 \pm 10 + 17.5 \pm 2.5)/580 = 0.99 \pm 0.02$.

M L-lactate, while only the buffer circulated through the auxiliary electrode compartments. The flow rate Φ was 20 mL h^{-1}, and the anode and cathode potentials were 0.5 and −1.75 V versus SCE, respectively. Then at time t_0 (see Figure 2), NAD^+ was introduced in the reservoir supplying the pump so as to obtain a bulk concentration of 2×10^{-4} M (i.e., 100 times less than L-lactate) in the solution flowing through the working electrode compartments. When NAD^+ reached the reduction reactor (time t_1 in Figure 2) i_c increased by ca. 0.15 mA above its background value. This was due to the reduction of NAD^+ at the mercury cathode held at a potential of −1.75 V versus SCE, such a reduction producing dimers $(NAD)_2$ (reaction 3) and NADH isomers (reaction 4).[13]

$$2NAD^+ + 2e \rightarrow (NAD)_2 \quad (3)$$

$$NAD^+ + H^+ + 2e \rightarrow NADH \quad (4)$$

At time t_2, after a full cycle of the solution circulating through the circuit consisting of the two working electrode compartments, the dimers and the NADH isomers were reoxidized into NAD^+,[13,15] thus regenerating the enzymatically active form of the coenzyme involved in the productive use of reaction 1 and increasing i_a above its background value by the same amount as the one detected for i_c at time t_1. Therefore the latter anodic recycling of the products of reactions 3 and 4 back to NAD^+ prevents the net destruction of the coenzyme.

Once i_a and i_c were again stabilized, the enzyme was introduced at time t_3 in the working electrode compartments reservoir so as to obtain a bulk concentration of 0.04 mg mL^{-1}. This addition of enzyme acted as the starter for the processes taking place in the oxidation reactor and i_a rose abruptly as a consequence up to ca. 6 mA as can be seen in Figure 2. The resulting pyruvate reached the cathode after a delay, and its reduction caused the sharp increase in i_c reproduced in Figure 2 at time t_4. The i_c value was appreciably higher than that of i_a since the enzyme reached the mercury cathode simultaneously and provoked the proton discharge already mentioned. Due to the occurrence of the latter, the determination of the faradaic consumption appeared more trustworthy when based on the integration of the i_a versus t curve than when based on the i_c versus t curve. The experiment was allowed to progress on its own except for pH measurements of the reaction mixture flowing through the working electrode compartments. The pH measurements were performed every hour and followed by additions of solid $NaHCO_3$ in the reservoir, in amounts calculated so as to decrease the pH back to its initial value of 9.0. As can be seen in Figure 2, both i_a and i_c decayed smoothly and tended asymptotically toward their background levels, that of i_a being better defined. When i_a, corrected for background current, was practically nil, we checked that additions of either L-LDH or NAD^+, or both, did not bring on any appreciable resurgences in both i_a and i_c. Conversely, addition of L-lactate resulted in steep increases in both currents. Those tests ascertained that the i_a and i_c decays were related only to L-lactate exhaustion and did not derive from enzyme and/or coenzyme inactivations. They also proved that the excess of L-LDH initially introduced was large enough to prevent a slackening of the process originating in the thermal inactivation of the enzyme, which was found to be rather slow in blank tests, and in the enzyme adsorption and the mercury/water interface. In order to obtain final solutions containing the remaining coenzyme solely in its oxidized form which can be easily assayed enzymatically, the cathode potential was settled at −0.5 V versus SCE, a potential at which NAD^+ is not reduced,[13] and held at this potential for 1 h, Φ being at the same time accelerated up to 360 mL h^{-1}. Finally, after measurements of the volumes of the solutions circulating in the working electrode and auxiliary electrode compartments, pyruvate, L- and D-lactates, and NAD^+ were assayed as described in the Experimental Section.

The data obtained at the end of a typical experiment are gathered in Table III. First of all, they show that, within experimental uncertainty, L-lactate is completely converted into D-lactate. NAD^+ used for the enzymatic oxidation of L-lactate had to be regenerated more than 200 times and ca. 60% remained enzymatically active in the end. The fact that we succeeded under our conditions also proves that we overcame two potential pitfalls. (i) The efficiency of the pyruvate reduction was sufficient to maintain the pyruvate concentration low enough to avoid a significant effect of destructive side reactions.[24] (ii) Besides its involvement in the enzymatic reaction and ensuing electrochemical regeneration, the coenzyme was alternately reduced and oxidized through the already mentioned direct reactions at both electrodes (cathodic reactions 3 and 4 and anodic recycling back to NAD^+). Therefore, it underwent ca. 25 such cycles based on the value of i_a at times $t_2 < t < t_3$. Undoubtedly it existed, at least partially, in its dimeric form $(NAD)_2$ when going from the cathode to the anode. The dimers are known to decompose rapidly, at pH 7, mostly into NADH and NAD^+ but also into enzymatically inactive species.[25] The observed small inactivation of the coenzyme in the present work ascertains that the dimer is satisfactorily stable at pH 9, a pH at which its rate of degradation has not been examined thoroughly.

According to the stoichiometries of the reactions taking place in the scheme given in Figure 1, the conversion of one molecule of L-lactate into one molecule of D-lactate involves 4e per molecule of converted lactate. In the typical experiment to which the data reported in Table III and the i–t curves reproduced in Figure 2 are related, 0.58 mmol of L-lactate was initially introduced in the system. Therefore, the amount of electricity theoretically needed for the exhaustive conversion is $0.58 \times 10^{-3} \times 4F$, i.e., ca. 225 C. Integration of the i_a–t curve beginning at time t_3 gives 265 C. From the latter quantity, we must substract the integration of the background current (ca. 0.1 mA) and the integration of the current due to the direct redox cycling of the coenzyme between the cathode and the anode (ca. 0.15 mA) over the whole duration of the experiment at times $t > t_3$ (ca. 32 h), i.e., ca. 29 C. The fairly good agreement between the theoretically predicted and measured electric consumptions brings on supplementary confirmations of the great efficiency of the process and absence

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of significant degradations through side reactions.

Conclusion

The results presented above show that the complete conversion of the L isomer of an α-alcohol–acid (L-lactate) can be performed with an efficiency of, at least, 97% by taking advantage of the stereospecificity of the rather cheap L-dehydrogenase and driving the system through the electrochemical regeneration of NAD^+ and the electrochemical reduction of the α-keto-acid (pyruvate). The whole process was kinetically controlled by the rate of the α-keto-acid reduction, i.e., neither by enzyme nor coenzyme deactivations. The demonstration of the feasibility of such a type of chiral inversion was our purpose. However, our study also points out a means of enhancing the productivity. Obviously, improvement implies the use of a cathode material allowing an electroactive area per volume ratio approaching that of the carbon felt. Then it would be practically conceivable to perform the anodic and cathodic reactions in the compartments of a sole reactor with configuration and size optimizations.

Experimental Section

Materials. Enzymes and biochemicals, including the lithium salts of L-lactic and D,L-lactic acids (highest purity grades available) were purchased from Sigma and used without further purification. The lyophilized L-LDH and the emulsion of D-LDH (E.C. 1.1.1.28.) were from rabbit muscle and *Lactobacillus leichmanni*, respectively. All other chemicals and the Nafion perfluorinated membranes separating the working electrode and auxiliary electrode compartments inside each reactor were from Aldrich. The felt of carbon fibers was obtained from Carbone-Lorraine.

Apparatus. The NADH concentration was determined by measuring the absorbances of solutions at 340 nm (ϵ = 6320 M^{-1} cm^{-1}) with a Perkin-Elmer 550 SE spectrophotometer. The potential of the working electrode (graphite felt) of the reactor in which NAD^+ is regenerated was controlled with a Tacussel PJT 24-1 potentiostat. The mercury pool cathode potential was controlled with a Tacussel PRT 30 01 potentiostat. The flow rates of the circulating solutions were controlled with a Gibson Minipuls 2 peristaltic pump equipped with a 4-channel head.

Reactors. The reactor in which NAD^+ was regenerated (effective area of the carbon felt anode: ca. 4500 cm^2) was as previously described[20] and was used in the vertical position, the solutions flowing from bottom to top. The reduction reactor was identical but used in the horizontal position, the carbon felt being replaced by the mercury pool (effective area ca. 15 cm^2). The two reference electrodes were connected to the exit and the entrance of the working electrode compartments of the oxidation and reduction reactors, respectively. Two channels of the peristaltic pump were used and fed with two reservoirs maintained under nitrogen atmospheres. Once the two liquid circuits of the working electrode compartments, on the one hand, and the auxiliary electrode compartments, on the other hand, were filled completely, the reservoir for the working electrode compartments initially contained always less than 2 mL. The two solutions flew, in the following order, through the oxidation reactor and through the production reactor and back to their respective reservoirs.

Procedures. In order to determine the volume of solution entrapped within the carbon felt at the end of the experiment, we compared its weight at that time to the weight of dry carbon felt introduced initially into the oxidation reactor. The data reported in Table III bear witness to the occurrence of osmosis between the two liquid circuits. The L- and D-lactate enzymatic assays were adapted from the literature.[26] They involved catalyzed oxidations in the presence of excesses of NAD^+ and hydrazine and spectrophotometric determinations of the amounts of produced NADH. The same combination of reactions was used for the assay of L-LDH enzymatically active NAD^+, excesses of L-lactate and hydrazine being then introduced. The pyruvate concentration was deduced from the increase in absorbance at 260 nm which results from the reaction of pyruvate with an excess of hydrazine. All these assays were carried out in 0.5 M carbonate buffer at pH 9.0.

Registry No. NAD, 53-84-9; NADH, 58-68-4; L-lactate, 79-33-4; D-lactic acid, 10326-41-7; L-lactate dehydrogenase, 9001-60-9; pyruvic acid, 127-17-3.

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Laser Flash Photolysis of 9-Diazofluorene in Low-Temperature Glasses

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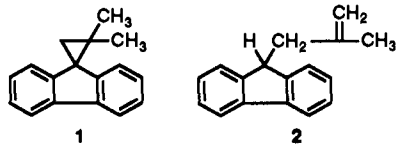
Abstract: The laser flash photolysis of 9-diazofluorene was investigated in several viscous organic glasses at low temperature. The data indicate that triplet fluorenylidene reacts with "soft warm" glasses by classical H atom abstraction, but the mechanism changes to quantum mechanical tunneling in colder and more rigid matrices.

I. Introduction

Product Studies of Carbenes in Frozen Polycrystals. Over 20 years ago Moss and Dolling[1] discovered that the chemistry of certain carbenes is tremendously sensitive to temperature. Photolysis of 9-diazofluorene (DAF) at 273 K in isobutylene, for example, generates fluorenylidene (FI), a ground-state triplet carbene which reacts with solvent to give high yields of cyclopropane **1** and minor amounts of alkene **2**.[2,3]



1 **2**

The ratio of **1**/**2** changes only slightly between 273 and 173 K; however, at 133 K and at 77 K the product ratio is reversed. In a frozen isobutylene polycrystal the alkene product predominates and the spirocyclopropane is formed in only small quantities. In later studies Tomioka found similar changes in the product distribution in the reaction of arylcarbenes with alcohols and alkanes. These changes occur exactly at the temperature at which there is a change in phase of the solvent from a fluid solution to a frozen polycrystalline solid. Tomioka has also shown that the matrix effect on carbene chemistry applies only to those carbenes

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