Enantiomerically pure epoxides are valuable synthetic intermediates. These compounds are now usually prepared from natural products or by asymmetric epoxidation. This paper describes highly enantioselective enzymatic syntheses of (S)- and (R)-chlorolactic acids and the conversion of these compounds into the corresponding epoxides (R)- and (S)-glycidic acid, epoxycrylic acid) (Figure 1).

**Table I. Michaelis-Menten Constants ($K_m$) and Relative Rates under Saturating Conditions ($v_{max}^{rel}$) for Substrates $XO_2CO_2H$**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>L-LDH $K_m$ (mM)</th>
<th>$v_{max}^{rel}$</th>
<th>D-LDH $K_m$ (mM)</th>
<th>$v_{max}^{rel}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>0.2</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Cl</td>
<td>4</td>
<td>0.4</td>
<td>50</td>
<td>0.8</td>
</tr>
<tr>
<td>Br</td>
<td>4</td>
<td>0.5</td>
<td>13</td>
<td>0.4</td>
</tr>
</tbody>
</table>

$^a$ These parameters, determined with 0.31 mM NADH in 0.6 M Hepes, pH 7.6, at 26°C, are approximate due to irreversible enzyme inactivation at high halopyruvic acid concentrations. $^b$ $v_{max}^{rel}$ is the estimated value of $v_{max}$, relative to $v_{max}$ for pyruvic acid itself.

**Table II. Synthesis of D- and L-Chlorolactic Acids and of Potassium D- and L-Glycidate**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>D-LDH</th>
<th>L-LDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ba-6-P-gluconate</td>
<td>85%</td>
<td>85%</td>
</tr>
<tr>
<td>K glycidate</td>
<td>&gt;97</td>
<td>&gt;97</td>
</tr>
<tr>
<td>turnover numbers</td>
<td>2 x 10^4</td>
<td>5 x 10^4</td>
</tr>
</tbody>
</table>

$^a$ Potassium glycidates were produced from chlorolactic acids on an 8-nmol scale. $^b$ Immobilization yield = 100 x (immobilized activity/activity in solution before immobilization). $^c$ These percentages of the starting activities were recovered after a single use of the enzymes in the preparations summarized in this table. $^d$ Isolated yields. The moles % reported for Ba-6-P-glucurone are based on enzymatic assays; the isolated samples were ~ 76% pure with the major impurities being water of hydration and Ba$^+(PO_4)_2$. $^e$ The same values of ee were obtained by using crude (noncrystallized) β-chlorolactic acid and material which had been isolated by crystallization. $^f$ Total turnover number (TTN) = mol of product/mol of enzyme (cofactor).


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Synthesis of Chlorolactic Acids

Figure 1. Synthesis of chlorolactic acids and potassium glycidades (G-6-PDH = glucose 6-phosphate dehydrogenase; LDH = lactate dehydrogenase).

Figure 2. (R)-(+)-MTPA derivatives prepared from chlorolactic and glycidic acids.

β-Chlorolactic acid was isolated as the free acid. It is a stable and nonhygroscopic white crystalline solid. Conversion of β-chlorolactic acid to glycidic acid was accomplished by treatment with base and the glycidic acid isolated as its potassium salt.7 The temperature of the reaction mixture and the amount of potassium hydroxide added (2 equiv) were controlled carefully to prevent undesired hydroxide attack on the epoxide ring (forming glyceric acid).

The enantiomeric purity of the products was determined by examination of the 1H NMR spectra of (R)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl ((R)-(+)-MTPA) derivatives (Figure 2).8 β-Chlorolactic acid was converted first into its methyl ester. Glycidic acid was treated first with benzenethiol (to open the epoxide ring, presumably with retention of configuration at C2) and then converted to its methyl ester. The intensities of the resonances due to both the methyl ether protons (Hα, a quartet) and the methyl ester protons (Hβ, a singlet) of the diasteriomers produced from racemic chlorolactic and glycidic acids and of the diasteriomers derived from the two enzymatic reactions9 were compared. By this criterion both the chlorolactic acids and the glycidic acids showed no enantiomeric contamination. The wrong isomer would not have been detected at values of the enantiomeric excess greater than 97%.

Conclusions

The syntheses described here provide practical routes to both enantiomers of chlorolactic and glycidic acid. As described, the procedures should be capable of generating quantities up to several moles. For larger quantities, it would be worthwhile to increase procedures should be capable of generating quantities up to several moles. For larger quantities, it would be worthwhile to increase


(10) The volume of gel containing the immobilized enzymes was less than 10% of the total volume in each of these syntheses; the use of larger quantities of enzyme (and gel) should not be problematic. Careful optimization of the enzyme immobilization yields would also prove advantageous for large-scale syntheses.

(11) Glucose 6-phosphate of higher purity can be prepared by enzymatic phosphorylation of glucose using an ATP regeneration system involving phosphoenolpyruvic acid (PEP) and pyruvate kinase. Hirschbein, B. L.; Mazenod, F. P.; Whitesides, G. M. J. Org. Chem., in press.

(12) Enzymatic reduction of hydroxypropionic acid (which is comparable to chloropyruvic acid as a substrate for both β- and γ-LDH) is not expected to inactivate these enzymes followed by two-step conversion to glycidic acid (via the monotosylate) may provide a more economical large-scale route to these epoxides.


(16) Bergmeyer, H. U. "Methods of Enzymatic Analysis"; Verlag Chemie: Weinheim, Academic Press: New York, 1974: (a) 458; (b) 1238; (c) 1248; (d) 1446; (e) 481; (f) 2050; (g) 2053.
was wased about 15 times. A linear response was obtained for the change of absorbance with time.

Immobilization of Enzymes. t-LDH, d-LDH, and G-6-PDH were immobilized separately in cross-linked PAN gel with a loading of ca. 2.5 mg protein/g of PAN-500. The reaction mixtures contained enzyme substrates to protect the active sites during the immobilization procedure. The concentration of enzyme (activity before immobilization) and substrate concentrations for the other immobilizations were as follows: t-LDH (27%, 8 mM pyruvate, 1.0 mM NADH); G-6-PDH (14%, 6.0 mM G-6-P, 0.6 mM NADP). Commercially available suspension of ca. 20 mg of t-LDH in 4 mL of 3.2 M (NH₄)₂SO₄ was centrifuged at 4 °C at 15,000g for 10 min. The precipitate was dissolved in 3 mL of 0.3 M Hepes buffer (pH 7.5). This solution was dialyzed against 1 L of a 50 mM Hepes buffer (pH 7.5, deoxygenated with a stream of argon) to decrease the concentration of (NH₄)₂SO₄. The resulting solution contained 1160 units of chlorotetracycline dehydrogenase activity (using 5 mM chloropyruvate, pH 7.6, 25 °C). To PAN 500 (130 g) in a 500-mL beaker was added 42 mL of 0.3 M Hepes buffer (pH 7.5, 0.05 M MgCl₂) containing 50 mg of sodium pyruvate and 50 mg of NADH. The mixture was stirred vigorously. After 1 min, 650 μL of a dithiothreitol solution (0.50 M) were added. One minute later, the t-LDH-containing solution was added. The mixture gelled after ca. two additional minutes of stirring. The gel was kept at room temperature for 1 h, ca. 200 mL of 0.005 M Hepes buffer (pH 7.5) containing 50 mM ammonium sulfate was added, and the gels were dissolved in a Waring blender at low speed for 3 min and then at high speed for 30 s. The gel particles were separated by centrifugation, washed with 200 mL of 50 mM Hepes buffer (pH 7.5), and again separated by centrifugation. The gel particles were suspended in H₂O to produce 20 mL of a 0.025 M suspension containing 405 units of chlorotetraacycline dehydrogenase activity (35% immobilization yield).

β-Chlorolactic Acid. A 2.5-L aqueous solution containing dithiothreitol (3.6 mM), EDTA (1.4 mM), MgCl₂ (7.0 mM), and glucose 6-phosphate (0.51 mol, 0.20 M) was adjusted to pH 7.6 with KOH. The reaction mixture was stirred at room temperature for 48 h, then the methanol was evaporated, and the resulting solid filtered and concentrated by rotary evaporation. The resulting oil was purified by preparative TLC on silica gel using 1:1 CHCl₃/pentane as solvent.

Potassium Glycicates. A 10 mL round-bottom flask containing a solution of 1.05 g of KOH in anhydrous methanol was equilibrated in an ice bath. To this solution was added this substrate 0.49 mmol of chloropyruvic acid and 1.27 mmol of NAD. The reaction was carried out under an argon atmosphere (ca. 20 °C), and the pH was maintained at pH 7.4-8.0 by adding 0.05-0.20 mM concentration of NADH in the reaction mixture. After 172 h the reaction mixture was stirred an additional hour at room temperature and kept overnight at 5 °C. Potassium chloride was removed by filtration and washed with cold methanol. The combined filtrate and wash solution was added dry ether to precipitate the potassium glycicate completely. The product was dried under reduced pressure: di-potassium glycitate (0.89 g, 88% yield); t-potassium glycitate (0.79 g, 78% yield); l-potassium glycitate (0.83 g, 85% yield).

Registry No. n-p-Chlorolactic acid, 82079-44-5; t-β-chlorolactic acid, 61505-41-7; p-β-chlorolactic acid, 82044-23-3; l-β-chlorolactic acid, 82079-45-6; pyruvic acid, 127-17-3; chloropyruvic acid, 3681-17-2; bromopyruvic acid, 1113-59-3; methyl β-chlorolactate MTPA, 82044-24-4; methyl β-(thiophenoxo)lactate MTPA, 82044-25-5; methyl β-chlorolactate, 32777-04-1; t-LDH, 9028-36-8; l-LDH, 9001-60-9; G-6-PDH, 9001-40-5.