Short Communication

Novel Chemoenzymatic Synthesis of D(-)-Pantoyl Lactone

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Received September 11, 1986

D(-)-Pantoyl lactone (*R*(-)-dihydro-3-hydroxy-4,4-dimethyl-2(3*H*)-furanone, **D-1**) is an important starting compound for the chemical synthesis of D(+)-pantothenic acid. The conventional synthetic process for **D-1** involves synthesis of racemic **1** from isobutyraldehyde, formaldehyde, and cyanide, and then optical resolution of the racemic **1**. A disadvantage of this process, apart from the use of cyanide, is the troublesome resolution of the racemic **1**. To omit the resolution-reracemization step, the use of ketopantoyl lactone (**2**) as a starting substrate has been suggested.^{1~4)} No efficient method for its practical preparation have been reported and its reduction to **D-1** is still required for improvement. Here we report an efficient chemoenzymatic synthesis method which involves one-pot synthesis of **2** followed by asymmetric reduction to **D-1** using microbial cells as a catalyst. Since this synthetic route involves neither optical resolution nor reracemization processes, it is highly advantageous for practical synthesis.

The key intermediate in this synthesis, **2**, is prepared from isobutyraldehyde, diethyl oxalate, sodium methoxide, and formalin. The reaction is done in one step at room temperature. To a mixture of 77.1 g (0.40 mol) of 28% sodium methoxide in methanol and 58.4 g (0.40 mol) diethyl oxalate was added 34.6 g (0.48 mol) isobutyraldehyde over a period of

5 min at 10°C, and then the mixture was stirred for a further 20 min at the same temperature. After the temperature of the reaction mixture had been raised to 40°C, 41.1 g (0.84 mol) of 35% formalin was added over a period of 5 min, and then stirring was continued for a further 20 min at 40°C. Addition of 44.0 g (0.44 mol) of sodium hydroxide aqueous solution and stirring for 20 min at 40°C gave **2** in a 81.0% yield (based on diethyl oxalate) on analysis by gas liquid chromatography (GLC).⁴⁾ **1** and 5-isopropylketopantoyl lactone (**3**) were formed as by-products in 3.2% and 3.0% analytical yields, respectively. This reaction mixture was extracted with ethylene chloride (360 ml) after adding conc. HCl (35 ml). Distillation of the extract gave 51.7 g fraction at 85~90°C/4 Torr. Recrystallization of the fraction with CCl₄ (200 ml) gave 34.8 g **2** (elemental analysis, C; 56.2%; 6.3%; ¹H NMR (CDCl₃, δ), 1.03 (s, 6H), 4.27 (s, 2H) ppm; IR max (KBr disk), 1770, 1455, 1390, 1275, 1170, 985 cm⁻¹; mass spectrum, *m/z*, 128 (M⁺); mp 64~65°C). The purity was 96.4% and the yield was 65.5% based on diethyl oxalate.

The reaction pathway is shown in Scheme 1. Diethyl oxalate was converted rapidly to dimethyl oxalate through the action of sodium methoxide. **3** in a 98% isolated yield was obtained when isobutyraldehyde (2 mol), diethyl oxalate (1 mol), and sodium methoxide (1 mol) were allowed to react (¹H NMR (CDCl₃, δ), 0.98, 1.07 (d, *J*=6.4 Hz, each 3H), 1.20 (s, 3H), 1.31 (s, 3H), 2.01 (septet, *J*=6.4 Hz, 1H), 4.17 (d, *J*=6.4 Hz, 1H) ppm).

SCHEME 1. The Proposed Reaction Pathway for the Synthesis of D(-)-Pantoyl Lactone.

When isobutyraldol (1 mol) was used in place of isobutyraldehyde, essentially the same result was obtained. Corresponding amounts of methyl formate were found on analysis by GLC in both of the reaction mixtures. **3** was in equilibrium with isobutyraldehyde and dimethylpyruvic acid (**4**). The consumption of **4** on addition of formalin lead to a decrease in **3** with simultaneous recycling of the isobutyraldehyde formed. When 5% sodium hydroxide aqueous solution (1.2 mol) was added to the reaction mixture derived from isobutyraldehyde (1.2 mol), diethyl oxalate (1 mol) and sodium methoxide (1 mol), **4** in a 90.9% yield on analysis by GLC⁴⁾ was formed (¹H NMR (CDCl₃, δ), 1.21 (d, $J=6.9$ Hz, 6H), 3.41 (septet, $J=6.9$ Hz, 1H), 10.06 (broad s, 1H) ppm). However, the yield of **2** from **4** and formalin was only 66.8%, and **1** was also formed as a by-product in a 11% yield, even though the reaction conditions were optimized. Thus, our one-pot reaction for preparing **2** is superior.

Asymmetric reduction of **2** to D-**1** was done with washed cells of *Rhodotorula minuta* IFO 0920⁴⁾ as a catalyst and glucose as an energy source. *R. minuta* was cultivated with 500 ml of medium in a 2 liter flask for 3 days at 28°C with shaking. The medium contained 25 g of glucose and 25 g of corn steep liquor, pH 6.0. The cells were harvested by centrifugation and washed with the same volume of water as broth. The reaction mixture (100 ml), containing 1.5 g of cells (dry weight) and 5 g of glucose, was shaken on a rotary shaker at 28°C. As shown in Fig. 1, when 1 g of **2** was added in portions with 12 hr intervals 5 times and the reaction was continued for a further 1 day, 99.1% of the added **2** was converted to D-**1** with 94.4% *e.e.* on analysis by diastereomer conversion with L-menthylchloroformate.⁴⁾ The reaction was stopped by removing the cells by centrifugation. To the supernatant (100 ml) containing 4.90 g D-**1**, 0.14 g L-**1** and 0.05 g **2** was added 30 g Na₂SO₄. The lacton-

FIG. 1. Stereoselective Conversion of Ketopantoyl Lactone to D(-)-Pantoyl Lactone by *R. minuta* Cells.

See the text for details. ●, D(-)-pantoyl lactone; ○, ketopantoyl lactone.

ized compounds were extracted with methyl isobutyl ketone (100 ml). The organic layer containing 4.38 g D-**1**, 0.10 g L-**1** and 0.02 g **2** was evaporated *in vacuo*. The crude product (4.6 g) was recrystallized from toluene (5 ml): yield, 4.10 g; molar yield, 80.3%; purity as **1** based on weight, 99.7%; optical purity, 99.7% *e.e.*; mp 91~92°C; ¹H NMR (DMSO-*d*₆, δ), 1.00 (s, 6H), 3.82 (s, 2H), 3.96 (d, $J=6$ Hz, 1H), 5.78 (d, $J=6$ Hz, 1H) ppm; mass spectrum, *m/z*, 71, 57, 55, 53, 45, 43, 41, 39, 31, 29, 27.

The simplicity and high stereospecificity of this system make it very convenient and practical for large-scale synthesis.

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