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Novel Synthesis of (R)-(+)-γ-Butyrolactone-γ-3-propionates by Fermenting Baker's Yeast and Enantiomeric Purity Determination Using NMR Technique

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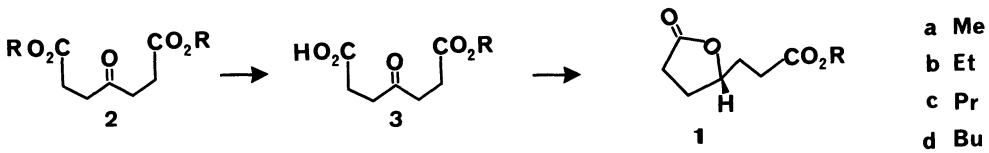
Optically pure (R)-(+)-γ-butyrolactone-γ-3-propionates **1** were prepared by reducing the precourser, 3-ketoheptane-1,5-dicarboxylic acid mononoesters, with fermenting baker's yeast. The optical purity (more than 98%) was determined by means of HPLC analysis and NMR determination.

The optically active (R)-(+)-γ-butyrolactone-γ-3-propionates **1** provides a versatile chiral building block in the synthesis of biologically active natural products. For example, (-)-podorzon, (+)-bursrand, (-)-isostegan, or (-)-steganone as an antileukemic lignan,[1] candesolide as an antifungal metabolite,[1] 2-norleukotriene analogues as a leukotriene D_4 antagonist,[2] and (R)-4-hexanolide as a pheromone[3] all have a butyrolactone skeleton in molecule. Therefore, synthetic procedures for those have been extensively investigated.[4a,b] They were, however, inadequate usually in the production on an industrial scale because of the delicacy of the reaction conditions as well as relatively low optical purity of the products.

In this work, we would like to report an improved methodology for preparing the optically active (R)-(+)-enantiomers, **1a-d**, on a large scale. This mehtod involves regiospecific reduction of 3-ketoheptane-1,5-dicarboxylic acid monoesters, **3**, as shown in Scheme 1. We describe also a convenient method to determine the optical purity of the product, **1**, by NMR and HPLC analyses.



Scheme 1.

The baker's yeast mediated reduction of ketones which bear various functional groups is one of useful methods to obtain optically active secondary alcohols. This method has been widely employed as a chiral building block preparation in the natural product syntheses.[5,6] As the stereoselectivity in the baker's yeast reduction is generally predicted by Prelog rule,[7] the reduction of α, β, γ, or δ -keto acids or their esters gives highly optically pure α or β-hydroxy acids or their esters,[3] and γ or δ -lactones.[8] For controlling the stereoselectivity in

the yeast reduction, several methods such as the manipulation of the size of the ester group,[9] the substitution of a hydrophilic group,[10] the immobilization of the yeast,[11] and the introduction of an electronegative group at the α-position of the ketones[12] have been developed. Meantime, in order to obtain chiral monoesters by asymmetric hydrolysis of prochiral diesters using an esterase, several attempts have been made also.[13,14] However, the asymmetric reduction or hydrolysis with enzymes has a limitation, due to the high substrate specificity as requested by the enzyme,[6,14] and subsquently, we have been unable to find any esterase for this purpose. First of all, therefore, we looked for an appropriate microbe to work well for this purpose. Among approximately 200 microbes, we selected *Psedomonas diminuta* IFO 13181 as the best one for the partial hydrolysis of **2** to give its half ester, **3**. In addition, the half ester was found to be suitable precurser to obtain optically active **1** with fermenting Baker's yeast.

A typical procedure of the baker's yeast reduction is the following. The baker's yeast (500.0 g, Oriental Yeast Co., Ltd.,), was suspended in a 5000 ml aqueous solution containing sucrose (800.0 g), KH_2PO_4 (10.0 g), $MgSO_4$ (5.0 g), $CaCO_3$ (25.0 g), and $(NH_4)_2SO_4$ (10.0 g) and stirred at 30 °C for 30 min. To the fermenting mixture so obtained was added **3b** (50.0 g, 0.25 mol) dissolved in 500 ml of water and pH was adjused to 6.8 with 2 mol dm^{-3} KOH. After stirring the mixture for a day at 30 °C, the reaction mixture was centrifuged for 20 min at 4800 x g at 4 °C. The supernatant was acidified to pH 2.0 with 5 mol dm^{-3} HCl and organic products were extracted three times with 1000 ml of ethyl acetate. The organic solvent layer combined was dried over Na_2SO_4 and evaporated, which gave a crude oilly product (32.0 g). It was purified by silicagel column chromatography ($CHCl_3$: AcOEt= 8:2 by vol.) to give 25.1 g (54.5%) of (R)-(+)-**1b** as colorless oil, $[\alpha]_D^{25}$ +59.62°(c 1.04, $CHCl_3$)(lit.[4b] $[\alpha]_D^{25}$ +53.8°(c 1.0, $CHCl_3$)). Yeast cells could be employed repeatedly at least six times for this reduction. In a similar manner, the fermenting baker's yeast reduction of monoesters (**3a**, **c**, and **d**) afforded the corresponding (R)-(+)-γ-butyrolactone-γ-3-propionates as shown in Table 1.

Table 1. Partial hydrolysis of **2** by *P. diminuta* and asymmetric reduction of **3** by Baker's yeast

R	Yield/%		$[\alpha]_D^{25}$/°	ee (%) of **1**	
	2 → **3**	**3** → **1**		by HPLC	by NMR
Me	55.2	25.4	+61.98	-	98
Et	85.2	54.0	+59.62	98	98
Pr	69.3	45.1	+53.31	-	98
Bu	65.4	43.2	+49.76	-	98

The optical purity of γ-butyrolactones was determined generally by Jones' method; namely, the NMR measurment of the diols as obtained by the reduction of lactones with methyllithium, in the presence of a shift reagent.[15] Unfortunately, the reduction of our lactones with methyllithium gives symmetric triol. Therefore, the Jones' method was inapplicable to our case. However, the chiral solvating reagent, (R)-(-)-2,2,2-trifuluoro-1-(9-anthryl)ethanol, **4**, was found to be useful for NMR determination of **1**. According to Pirkle's suggestion,[16] nonequivalency on the methylene signal caused by the addition of the chiral reagent was carefully examined. For example, the ^{1}H-NMR (300 MHz NMR, Varian VXR 300) of a mixture of (±)-**1b**, which was obtained by chemical reduction of **2b** with $NaBH_4$ in ethanol, and the baker's yeast reduction product (+)-**1b** was measured in the presence of **4** (38.6 mg, 0.14 mmol). The methylene signals of ethyl ester group of (±)-**1b** (11.4 mg, 0.061 mmol) was found to be double quartet signals at 4.015 ppm (q, J=7.0 Hz, due to (+)-**1b**) and 4.024 ppm (q, J=7.0 Hz, due to (-)-**1b**) in CCl_4: $CDCl_3$ (3:1 v/v) as shown in Fig. 1a. On the other hand, the baker's yeast reductant, **1b** (11.1mg, 0.06 mmol), in the same solvent with 4 (39.2 mg, 0.14 mmol) shows almost single quartet signal at 4.015 ppm (q, J=7.0 Hz, due to (+)-**1b**)(Fig. 1b). These results indicate that the baker's yeast reduction product, **1b**, is the (R)-(+)-form and it's optical purity is more than 98%. In order to obtain higher accuracy, NMR of a mixture of the chemical reduction product, (±)-**1b**, (10.7 mg, 0.057 mmol [17]) and the baker's yeast reduction product (+)-**1b**, (103.4 mg, 0.56 mmol), of which optical purity was caluculated to be 92.3%, was measured in a similar manner (Fig. 1c). Furthermoer, upon the addition of the probe **4**, the triplet due to terminal methyl protons of (±)-**1b** also splits into a couple of double triplets similarly to the methylene signal. The magnitude in the separation of the methyl proton signal was, however, not necessarry larger than that of the methylene proton signal.

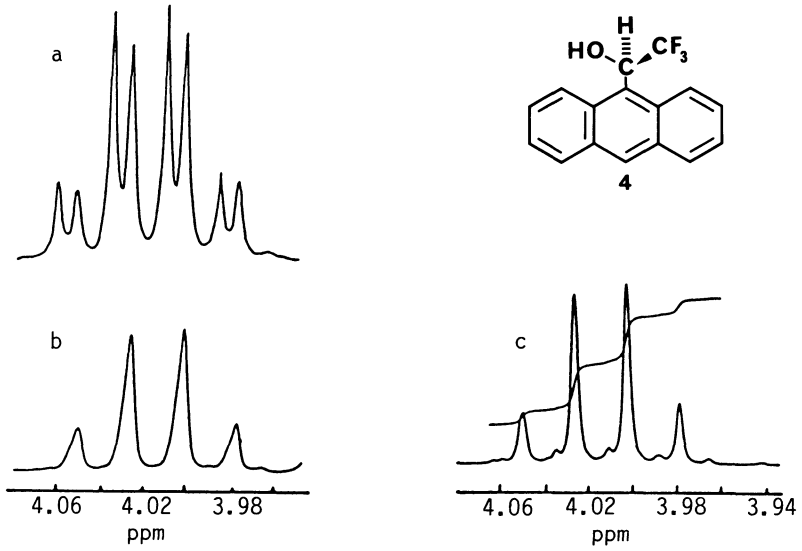


Fig.1. ^{1}H-NMR Spectra of methylene signal of ethyl ester group (±)-**1b** (a), (+)-**1b** (±), and a mixture of (±)-**1b** and (+)-**1b** (1:9.7 by wt) (c) in the presence of 4 (3:1 v/v) in CCl_4: $CDCl_3$ (3:1 v/v).

The optical purity of the product **1** was also determined by HPLC on a Waters M 600 system with a chiral column (Chiralcel OA, 4.61D x 250 mm, Daicel Chemical Industries, Ltd) with a mixture of hexane and 2-propanol (9:1, v/v) as the mobile phase at the flow rate of 1.5 ml/min. The reaction product **1b**, which was photometically detectable at 230 nm, gave a single peak at R_t 6.8 (min). The mixture of (+)-**1b** and (-)-**1b** gave two peaks at R_t 6.8 and 7.8 (min). The optical purity of the reaction product estimated by HPLC analysis was almost identical to that obtained in the NMR measurement.

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17) The compound (±)-**1b** consists of 58.8% of (+)-**1b** and 41.2% of (-)-**1b**, which is determined by HPLC analysis with chiral column.

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