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An approach to optically active ibuprofen through whole-cell and enzyme-catalyzed transformations

Mary Ann A. Endoma*, Maria Celeste R. Tria, and Susan D. Arco

Institute of Chemistry
University of the Philippines
Diliman, Quezon City, PHILIPPINES

Synthesis of optically active ibuprofen is attempted using two methodologies: (1) *via* yeast-catalyzed reduction of phenyl acrylic acid or ester **1** and derivatives **2** wherein no reduction products were detected in the biotransformation culture medium; and (2) *via* lipase-catalyzed resolution of racemic phenyl propanol and ibuprofen alcohol. The latter method afforded optically active ibuprofen alcohol as the (–)-*S*-enantiomer.

Keywords: ibuprofen; biotransformation; enzyme-catalyzed reduction; lipase-catalyzed resolution

INTRODUCTION

Enzymatic transformations can be conveniently used for organic synthetic purposes [1, 2] without a detailed knowledge of the characteristics of the enzyme's active sites. A particular enantioselective chemical reaction can be sought from a listing of similar transformations found in published research and review articles [3, 4] in this field. Two widely different enzyme and enzyme source are employed in this report–yeast as source of yeast alcohol dehydrogenase, and lipase as an isolated enzyme.

Recently, there has been an increased interest in the production of enantiomerically pure compounds for use as drugs for medicinal purposes. The pharmaceutical industry has become stricter in quality assurance, and a foremost concern is the production of drugs as pure stereoisomer. This is based on the fact that the unwanted stereoisomer may be either completely inactive or even lethal.

Yeast reduction. Yeast cells are recognized to be a useful chemical reagent [5]. The most common application is in the reduction of ketones and hence preparation of chiral secondary alcohols with enantiomeric excesses (% ee) ranging from 60% to 99% (Fig. 1) [6].

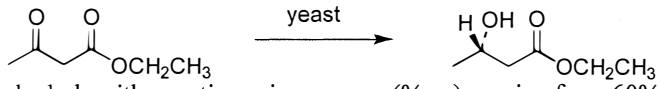


Fig. 1

The methodology is simple to follow and does not require the use of special equipment. The desired transformation in our present work involves an extension of reduction reaction to that of carbon-to-carbon double bond. This transformation is newly recognized, but because of its potential applications, definitely merits investigation. Some preliminary work has been conducted by some researchers in the case of the 1,4-reduction of α,β-unsaturated aldehydes and ketones using fermenting yeast cells [7, 8].

Lipase resolution. Many investigators have confirmed that lipases can efficiently catalyze the hydrolysis of esters in aqueous media and the esterification of alcohols in organic solvents. Lipase-catalyzed resolution of alcohols and esters result in %ee typically in the range of 70–99% (Fig. 2) [9]. They have utilized lipase-mediated resolution as the key step in the total synthesis of a number of natural products [10].

**To whom correspondence should be addressed.*

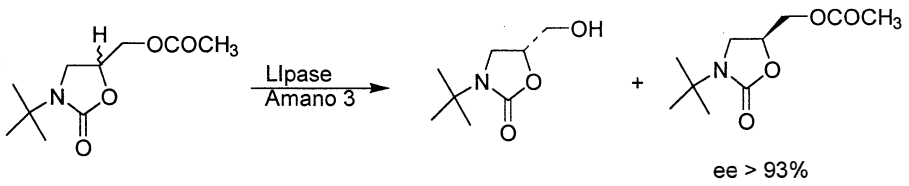


Fig. 2.

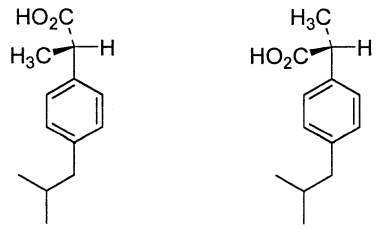


Fig. 3.

The choice of lipase is usually dictated by the results of actual experimentations–by screening a number of lipases and choosing the one that provides the highest %ee [11]. Finally, hydrolysis and esterification and even interesterification processes are alternative ways to conduct a lipase-catalyzed resolution which in some situations can give different results concerning %ee [12, 13].

Ibuprofen. Ibuprofen belongs to a class of compounds called 2-arylpropionic acids, which have a single stereogenic tertiary center and an important class of non-steroidal anti-inflammatory agents. They relieve inflammation by inhibiting cyclo-oxygenase activity thereby regulating the arachidonic acid cascade [14]. The pharmacological activity of the *S*-isomer has been reported to be stronger than the *R*-isomer (Fig. 3) [15]. Because of this, synthesis of *S*-ibuprofen in an enantiospecific manner has received considerable attention [16, 17].

There are a wide variety of methodologies known for the preparation of ibuprofen in enantiomerically pure form. Some are physical, chemical or a combination of both but more recently has been geared towards chemoenzymatic or a combination of enzymes and organic synthesis [18].

EXPERIMENTAL

General procedure

NMR spectra were determined in $CDCl_3$ using a Gemini 300 or VXR 300 spectrometer. Coupling constants (J) are given in Hertz. All solvents that required drying were dried according to standard procedures. Flash column chromatography was performed using Fisher silica gel (grade 60, 200–425 mesh). Optical rotations were measured on a Perkin-Elmer model 341 polarimeter.

2-Phenylacrylic acid (Atropic acid) (1). Tropic acid **9** (10 g, 60.2 mmol) was added to 50% KOH solution (60 mL). The mixture was heated to reflux for 40 mins. The solution was cooled and was extracted with ether (50 mL). The aqueous layer was acidified with 12 M HCl until its pH was 3, then extracted with ether (3 × 50 mL). The combined ether extracts were dried with anhydrous $MgSO_4$ and the solvents were removed by rotary evaporation. Recrystallization of the residue in 95% ethanol afforded the desired acid (7.6 g, 85% yield). ^{1}H NMR ($CDCl_3$) δ 7.40 (m, 5H), 6.58 (s, 1H), 6.25 (s, 1H).

Ethyl 2-phenyl acrylate (Ethyl atropate) (8). Atropic acid (5 g, 30.1 mmol) was dissolved in DCM (100 mL). Ethanol (1 mL) was added dropwise. The solution was cooled to 0°C. DCC (15.5 g, 75.3 mmol) was added in several portions over 15 min followed by a catalytic amount of DMAP. The solution was stirred at room temperature overnight and then filtered through Celite. The filtrate was evaporated using a rotary evaporator. The residue was chromatographed on silica gel using mixtures of hexanes/EtOAc (6:1) as eluent to afford ethyl atropate as a yellow oil (2.2 g, 50% yield).). ^{1}H NMR ($CDCl_3$) δ 7.30 (m, 5H), 6.35 (s, 1H), 5.85 (s, 1H), 4.25 (q, J = 7 Hz, 2H), 1.15 (t, J = 7 Hz, 3H).

Typical procedure for yeast reduction

Dry yeast was purchased from Sigma Chemical Co. A mixture of dry yeast (10 g) and glucose (5 g) in tap water (50 mL) was stirred at room temperature or 10 min. A solution of acetophenone (100 mg) in ethanol (0.2 mL) was added and the mixture was incubated at 30 C with rotary shaking at 150 rpm. After 48 h, the broth was extracted with EtOAc (2 × 50 mL). TLC analysis in mixtures of hexanes/EtOAc with 2,4-dinitrophenylhydrazine as visual indicator for ketone revealed a decrease in its concentration. Co-spotting with a pure sample of product alcohol was performed to ascertain the formation of the reduction product.

2-Phenylpropanol (13). A solution of 2-phenylacetaldehyde (5 g, 37.3 mmol) in MeOH (100 mL) was cooled to 0°C. $NaBH_4$ (2.1 g, 55.9 mmol) was added in three portions. After the evolution of gas ceased, the reaction mixture was quenched with acetone. The solvents were removed by rotary evaporation. The residue was diluted with water (20 mL) and extracted with EtOAc (2 × 100 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated using rotary evaporation. The residue was chromatographed on silica gel

(using 10: 1 hexanes/EtOAc as eluent) to afford (4.6 g, 90% yield) **3** as a clear oil whose ^{1}H NMR data agree with that given in the literature [16].

1-Acetoxy-2-phenylpropane (15). A solution of 2-phenylpropanol **13** (4 g, 29.4 mmol) in DCM (100 mL) was cooled to 0°C. NEt_3 (15.3 mL, 110.4 mmol) was added in one portion followed by the dropwise addition of acetyl chloride (3.9 mL, 55.2 mmol). The solution was allowed to cool down to room temperature. After one hr of stirring at room temperature, the solvents were removed by rotary evaporation. Chromatography of the residue in silica gel using mixtures of hexanes/EtOAc (20:1) as eluent afforded the acetate (3.1 g, 54% yield) as an oil whose ^{1}H NMR data agreed with that published in the literature [19].

Lipase-catalyzed hydrolysis of 1-acetoxy-2-phenylpropane. To a suspension of acetate (500 mg, 2.8 mmol) in a pH 7.0 0.1 M buffer solution of NaH_2PO_4/Na_2HPO_4 (25 mL) was added porcine pancreatic lipase (100 mg). The mixture was incubated at 30°C with shaking at 150 rpm. The pH was checked and adjusted to 7.0 every hour. After two days, the enzyme was filtered and the filtrate was extracted with EtOAc (2 × 50 mL). The combined organic extracts were dried over anhydrous $MgSO_4$ and concentrated using rotary evaporation. Chromatography of the residue over silica gel afforded the hydrolysis product alcohol (170 mg, 48% yield) with a preference for the *S*-enantiomer $\alpha_D = -4.2$ (c 1.2, C_6H_{14}). No attempt to assess %ee was done in this transformation. The work is published elsewhere [20].

2-(4-Isobutylphenyl)-1-propanol (ibuprofen alcohol) (14). Ibuprofen (2 g, 9.7 mmol) was dissolved in THF (50 mL) at 0°C. $LiAlH_4$ (388 mg, 9.7 mmol, 95% pure) was added slowly with stirring. The reaction mixture was allowed to warm up to room temperature after which it was diluted (quenched) with water (25 mL). The suspension was filtered through Celite and the filtrate was extracted with EtOAc (3 × 50 mL). The combined organic extracts were dried using anhydrous $MgSO_4$, evaporated using a rotary evaporator and passed through a short column of silica gel to afford the desired alcohol **14** as a colorless oil (1.3 g, 70% yield). ^{1}H NMR ($CDCl_3$) δ 7.15 (m, 2H), 7.10 (m, 2H), 3.69 (d, J = 6.8 Hz, 2H), 2.92 (m, 1H), 2.45 (d, J = 7.3 Hz, 2H), 1.85 (m, 1H), 1.26 (d, J = 7.1 Hz, 3H), 0.90 (d, J = 6.6 Hz, 6H).

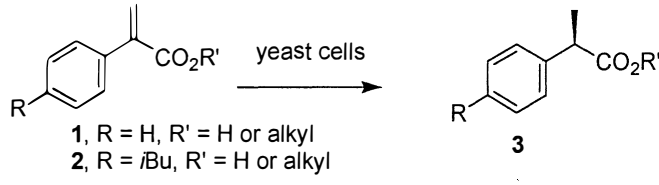
2-(4-Isobutylphenyl)-1-acetoxypropane (ibuprofen alcohol acetate) (16). A solution of ibuprofen alcohol (500 mg, 2.6 mmol) in DCM (100 mL) was cooled to 0 °C. NEt_3 (15.3 mL, 110.4 mmol) was added in one portion followed by the dropwise addition of acetyl chloride (3.9 mL, 55.2 mmol). The solution was allowed to cool down to room temperature. After one hr of stirring at room temperature, the solvents were removed by rotary evaporation. Chromatography of the residue in silica gel using mixtures of hexanes/EtOAc (20:1) as eluent afforded the acetate (380 mg, 62% yield) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.13 (d, J = 8.3 Hz, 2H), 7.08 (d, J = 8.3 Hz, 2H), 4.19 (dd, J = 7.4, 10.8 Hz, 1H), 4.09 (dd, J = 6.8, 10.8 Hz, 1H), 3.06 (m, 1H), 2.44 (d, J = 7.1 Hz, 2H), 2.02 (s, 3H), 1.85 (m, 1H), 1.29 (d, J = 6.8 Hz, 3H), 0.89 (d, J = 6.8, 6H).

Lipase-catalyzed hydrolysis of ibuprofen alcohol acetate. The same protocol as in phenyl propanol was used, the yield of alcohol was 50% and with a %ee of 58%.

Lipase-catalyzed esterification of ibuprofen alcohol to the acetate. To a solution of ibuprofen alcohol (1.0 g, 5.2 mmol) and vinyl acetate (1.6 g, 18.5 mmol) in water-saturated hexane (10 mL) was added porcine pancreatic lipase (500 mg). The reaction was incubated at 30°C with shaking at 150 rpm for two days. The enzyme was filtered, and the filtrate was extracted with EtOAc (3 × 20 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered and the filtrate was concentrated using rotary evaporation. The residue was chromatographed on silica gel using hexanes/EtOAc (5:1) as eluent to afford the enantiomerically pure acetate (206 mg, 17% yield) as a yellow oil whose spectral properties were identical to the racemic sample prepared previously. $\alpha_D = -4.1°$ (c, $CHCl_3$).

RESULTS AND DISCUSSION

The project was originally conceptualized according to scheme 1. The first step was the synthesis of the substrates phenyl acrylic acid **1** (R = H) and ibuprofen derivatives **2** (R = *i*Bu). Once the substrate was obtained, a yeast reduction reaction was expected to result in the saturated compound **3**. It was not particularly important at this point whether **3** is produced or its enantiomer.

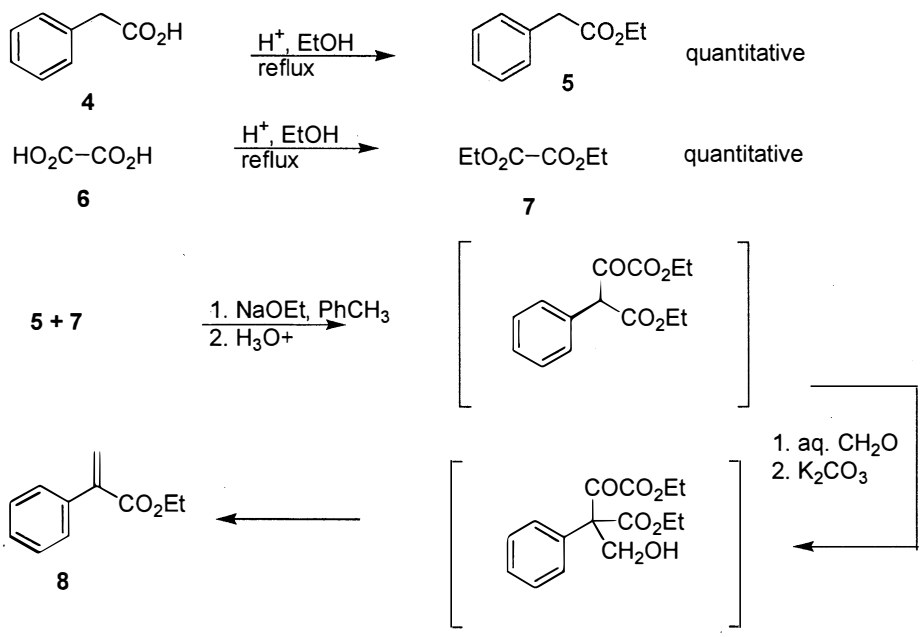


1, R = H, R' = H or alkyl
2, R = *i*Bu, R' = H or alkyl
3

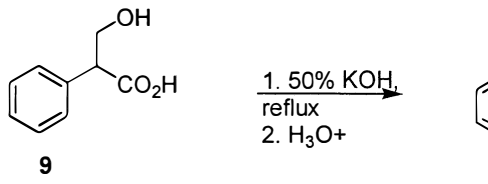
Scheme 1

If the biocatalytic step worked, it was a good way to obtain chiral phenyl acetic acid and derivatives otherwise obtained by the asymmetric hydrogenation of phenyl acrylic acid using expensive catalysts.

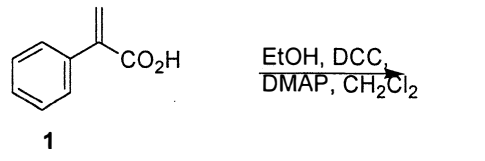
Since compounds **1** and **2** were not commercially available, literature procedures [21, 22] were followed thus (Scheme 2). The above synthesis was lengthy because of the unavailability of ethyl phenyl acetate and diethyl oxalate. It was not a big concern because these reactions were essentially quantitative. The next few steps were convenient because there were no isolation of intermediates involved. The final product, however,



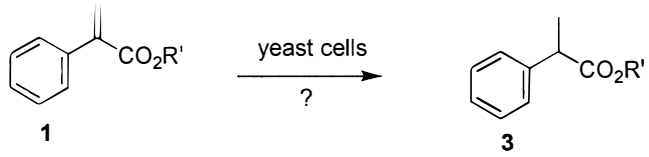
Scheme 2



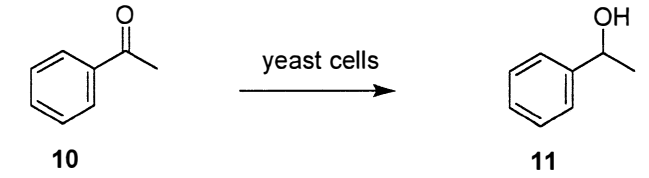
Scheme 3



Scheme 4



Scheme 5



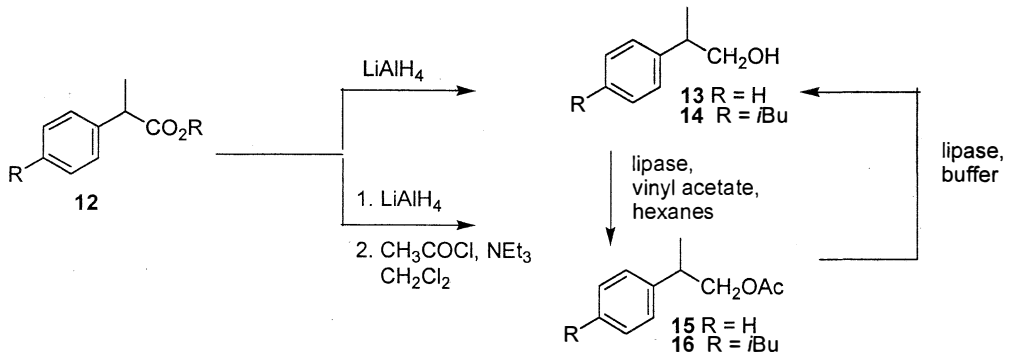
Scheme 6

was heavily contaminated with phenyl acetate rendering this synthesis impractical for our purposes. It is claimed in the literature that the authors [12] used this route for their synthesis. The difference lies in the fact that the desired phenyl acrylic acid was not their final product. It was further modified to a substance amenable to a different method of purification. Ethyl phenyl acetate and ethyl phenyl acrylic ester boil closely at about 5 °C difference. Purity of the fractions was assayed using ^{1}H NMR. We tried several times to employ fractional distillation for separation with no success. In some cases separations of this sort can be accomplished after careful modification of the distillation set-up or the use of longer distillation columns. This approach is impractical for our purpose so we decided to take another synthetic route that is clearly more straightforward. The next synthetic route was tried (Scheme 3).

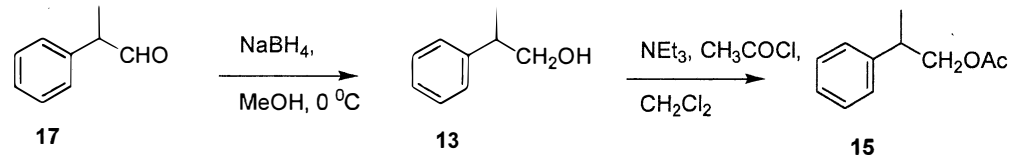
Using tropic acid **9** as the starting material, this method led to the desired acrylic acid derivative **1** (R = H, R' = H) as described in the literature [22]. ^{1}H NMR of the product matched that reported spectra of **1**.

The ethyl ester was synthesized from the acid *via* DCC (dicyclohexyl carbodiimide) coupling (Scheme 4).

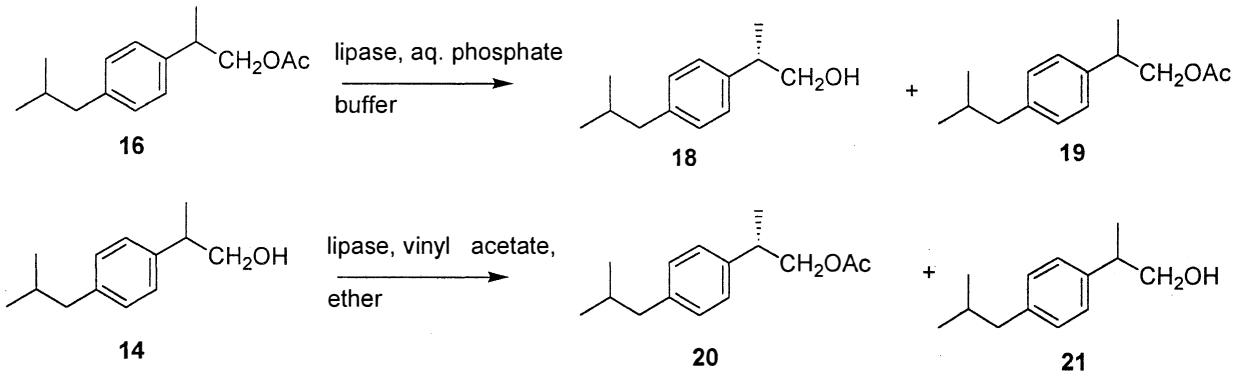
The formation of the desired product **8** was originally monitored by the appearance of a less polar compound on TLC (silica gel, hexanes/EtOAc as eluent) and the presence of signals in the ^{1}H NMR spectrum corresponding to that of the ethyl group. Compound **8** was isolated after DCC reaction using flash column chromatography on silica gel.



Scheme 7



Scheme 8



Scheme 7

At this point what remained to be done was to subject these two substrates **1** and **8** to yeast-mediated biotransformation to hopefully, afford **3** (R = H, R' = H or Et) (Scheme 5).

The enzymatic activity of yeast was tested with a standard ketone acetophenone. The product was formed as expected (Scheme 6).

Co-spotting of the TLC plates with a standard sample of phenyl ethanol **11** confirmed the production of the alcohol, which is very polar, compared with the starting material.

When the phenyl acrylic acid **1** (R' = H) and ester **1** (R' = Et) were subjected to biotransformation reaction using yeast, reaction monitoring proved difficult. A standard sample of the desired reduction products and starting phenyl acrylic acid cannot in any solvent system studied be separated on silica gel. The only fast yet sensitive method to use was *via* monitoring of reaction products using ^{1}H NMR. Aliquots were taken at frequent intervals to check for the formation of reduction products by the disappearance of vinyl signal at δ of around 6.5 ppm and the appearance of the doublet methyl and quartet methine. Even after extended periods of biotransformation under different conditions (with glucose, without glucose, etc.), there were no traces of reduction products. It was very frustrating but this situation is not uncommon in the field of biocatalysis that some substrates fall under the category "non-substrates" or "not metabolized."

The idea to carry out a yeast reduction of this sort is not completely precedented. Inspection of the substrates listed under carbon-carbon reduction did not have a terminal or vinylic methylene [4]. This negative result can be viewed not as a failure but that acrylic acids of this structure are non-substrates. Having observed that phenyl acrylates are not converted to phenyl acetic acid by the use of yeast, we next focused our attention to another chemoenzymatic approach (Scheme 7).

The above substrates were subjected to both lipase-catalyzed hydrolysis (acetate **14**) and esterification (alcohol **13**). If a chiral alcohol would be obtained, for **16** (when R = *i*Bu), a formal synthesis of chiral ibuprofen alcohol would be accomplished since several authors have successfully converted this alcohol to ibuprofen *via* Jones oxidation [23].

In the present work, the primary alcohol acetate **15** (phenyl ethyl alcohol acetate) was obtained as follows (Scheme 8).

This substrate was subjected first to lipase-catalyzed hydrolysis to check for the activity of the lipase. As reported in the literature [20], hydrolysis proceeded smoothly with a preference for the *S*-enantiomer. No further investigations on the exact %ee were performed as this was already reported previously by several workers [20]. The process served to determine the activity of the lipase preparation. We proceeded to check if ibuprofen alcohol **14** would be resolved similarly (Scheme 9).

The resolution of ibuprofen alcohol acetate was performed using the same protocol as that for phenyl ethyl alcohol. At 50% conversion, the product alcohol was found to be with %ee of 58%. The enantioselectivity obtained in this case was moderate. It is known that enantioselectivity can be enhanced by the use of esterification reaction as an alternative to hydrolysis. We decided to run the reaction in this direction. The reaction proved to be very slow. Even after two days, the % conversion was at 17% with a %ee of >98%. The observed optical purity matched that which is given in the literature. The uncertainty in measurements due to a very low value of optical rotation ($\alpha\square_D$ = -3.8°, $CHCl_3$), however, makes the conclusion less reliable. Most authors use this as a basis for their %ee determinations nevertheless.

This work could be made more conclusive if several derivatives (Mosher ester, lanthanide-shift) can be prepared to assess %ee of ibuprofen alcohol acetate **20**. The ibuprofen alcohol acetate **20** prepared from lipase-catalyzed esterification could be converted to *S*-ibuprofen for comparison.

CONCLUSION

From the two phases of this project, the following are concluded: (1) Phenyl acrylic acids **1** and esters **8** of the structure shown in scheme 4 are not substrates for yeast-mediated hydrogenation reaction; (2) Lipase-catalyzed esterification of ibuprofen alcohol **14** in organic media afforded enantiomerically pure ibuprofen alcohol acetate **20**; and, (3) Lipase-catalyzed hydrolysis of ibuprofen acetate in phosphate buffer afforded enantiomerically enriched ibuprofen alcohol **18**.

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