PREPARATION OF ENANTIOMERICALLY-ACTIVE DEUTERIUM-LABELLED IBUPROFEN

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SUMMARY The chemoenzymatic preparations of optically-active [3,3,3-$^2H_3$]-ibuprofen and [2-^2H]-ibuprofen are described.

Key Words: optically-active [3,3,3-$^2H_3$]-ibuprofen, optically-active [2-^2H]-ibuprofen.

INTRODUCTION

Ibuprofen is one of the nonsteroidal antiinflammatory drugs (NSAIDs) which are commonly used in the initial therapy of rheumatoid arthritis [1]. Its activity in inhibiting cyclo-oxygenase is mainly effected by the S-enantiomer; whereas the R isomer is virtually inactive. However, the *in vivo* anti-inflammatory activity ratio of the S- to R-enantiomer was shown to be much lower than that *in vitro* (1.6 vs. 160) [2]. Such a discrepancy has been ascribed to the metabolic conversion of the R antipode to the corresponding S counterpart in the body [3]. Several lines of evidence have indicated that this bioconversion proceeds via a thioester carbanion intermediate [4]. Evidently, this *in vivo* bioactivation process leads to enhanced therapeutic/toxic effects of the drug, and warrants special considerations. In view of the kinetic and mechanistic studies on this unique metabolism, we thus developed efficient chemoenzymatic routes to optically active [3,3,3-$^2H_3$]-ibuprofen (**1**) and [2-^2H]-ibuprofen (**2**).

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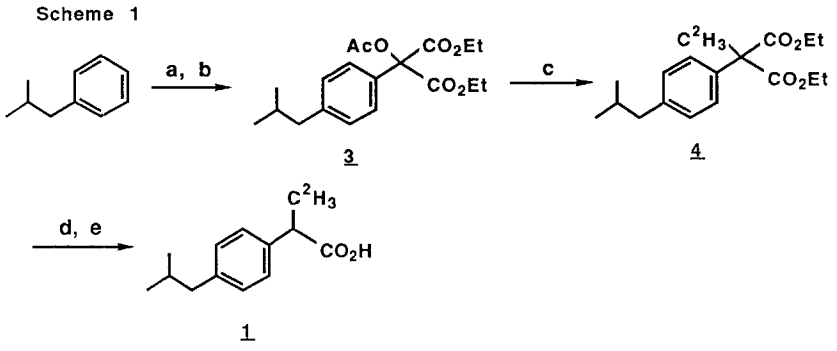
RESULTS AND DISCUSSION

Synthesis of Racemic [3,3,3-$^2H_3$]-Ibuprofen (1)

Scheme 1



a) $CO(CO_2Et)_2$, $SnCl_4$; b) Ac_2O, Et_3N, DMAP; c) Sodium α-(dimethylamino)naphthalenide, C^2H_3I; d) KOH, reflux; e) H^+, reflux.

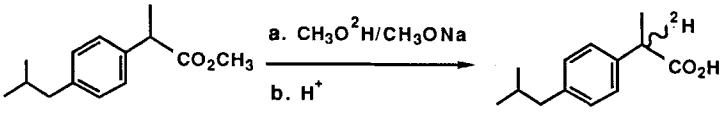
As shown in Scheme 1, reductive deacetoxylation of 3 by sodium α-(dimethylamino)naphthalenide, followed by alkylation with C^2H_3I, afforded a direct access to the target molecule in satisfactory yield. Salomon and his coworkers first reported the naphthalenide reductive deoxygenation of α-acetoxymalonates, from which the ester enolates generated provided an efficient route to various α-aryl alkanoic acids [5]. The key intermediate 3 was prepared from isobutylbenzene and diethyl ketomalonate by $SnCl_4$-catalyzed electrophilic substitution, followed by acetylation of the alcohol [5]. The overall yield was 57% based on isobutylbenzene. The location of the labelled substituent was confirmed by both ^{1}H-NMR and mass spectral analyses. Essentially, radiolabelling at any other positions on the molecule can also be accomplished accordingly.

Synthesis of Racemic [2-^{2}H]-Ibuprofen (2)

By taking advantage of the acidity of the α-methine proton of ibuprofen methyl ester, the α-methine deuterated molecule was readily obtained by the proton/deuterium exchange of the ester with

an excess of CH_3O^2H in the presence of sodium methoxide, followed by acid hydrolysis and subsequent recrystallization from petroleum ether (Scheme 2). The proton NMR spectrum indicated the disappearance of the α-methine proton signal at 3.75 ppm and the switch of the α-methyl proton signal from a doublet to a singlet at 1.50 ppm, which confirmed the complete displacement of the α-methine proton with a deuterium atom.

Scheme 2



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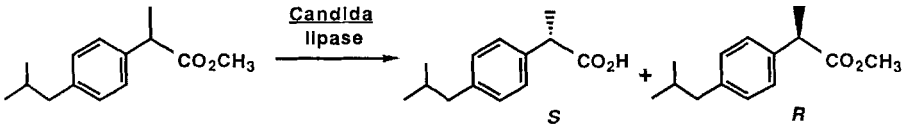
Enzymatic Resolution of Racemic Ibuprofen

Conventionally, access to optically active ibuprofen has relied upon chemical procedures using chiral amines as resolving agents [6], which proved to be cumbersome and time-consuming. Recently, Sih et al reported that Candida cylindracea lipase catalyzed enantiospecific hydrolysis of the 2-arylpropionic esters of which the S enantiomers were preferentially cleaved [7]. Therefore, this enzymatic procedure allowed us to prepare both R- and S-ibuprofen in multigram quantities (Table 1).

Although this enzymatic process was not absolutely enantioselective, it is conceivable that in kinetic resolution, the optical purity of the remaining substrate can be enhanced by extending the reaction beyond 50% conversion [9]. Therefore, R-ibuprofen can be obtained with high optical purity (98 e.e.) by the acid hydrolysis of the ester fraction which was recovered after a prolonged incubation. On the other hand, the S-acid fraction (e.e. = 0.71) was remethylated and exposed to the lipase again under the same conditions, which gave rise to S-ibuprofen with satisfactory optical purity (e.e. = 0.97). Optically active deuterated ibuprofen have thus been prepared according to the

Table 1. Enantiospecific hydrolysis of racemic ibuprofen methyl ester by Candida cylindracea lipase



Incubation Time (h)	% Extent of Conversion	Enantiomeric Excess [8] Product	Remaining Substrate	E [9]
120	46	0.86	0.74	29
192	58	0.71	0.98	26

(Experimental conditions are described under "Materials and Methods")

procedure. Kinetic and mechanistic investigations of the metabolic inversion of ibuprofen using these labelled compounds are currently undergoing in this laboratory.

MATERIALS AND METHODS

C^2H_3I, 99.5+ atom %, and CH_3O^2H, 99.5+ atom %, were obtained from the Aldrich Chemical Co., Milwaukee. Racemic ibuprofen was prepared from commercial Advil tablets, and further purified by recrystallization from petroleum ether. Crude Candida cylindracea lipase powder was purchased from Sigma Chemical Co. All other chemical and biochemical reagents and solvents (HPLC grade) were purchased from commercially available sources, and used without further purification. Proton NMR spectroscopy was carried out on a Varian EM-390 spectrometer in deuteriochloroform with tetramethylsilane as the internal standard. ^{13}C and Deuterium NMR spectroscopy were performed on a Bruker AM-300 spectrometer. Low resolution electron impact (EI) and chemical ionization (CI) mass spectra were obtained on a Kratos MS-80RFA spectrometer, Chemical Instrument Center, Yale University.

Diethyl Acetoxy(4-isobutylphenyl)propanedioate (3) was prepared from isobutylbenzene and diethyl ketomalonate according to the

procedure described by Salomon et al [5]. However, the intermediate prior to acetylation was used without purification after workup, and the resulting product 3 was purified by silica gel chromatography instead of fractional distillation. The combined yield of these two steps was 78%.

Diethyl (4-isobutylphenyl)-($^2H_3$-methyl)-propanedioate (4). A mixture of sodium (1.0 g, 42.6 mmol) and α-(dimethylamino)-naphthalene (6.1 g, 35.5 mmol) in hexamethylphosphoramide (35 ml) was stirred under argon for 12 h at 25°. This solution was then added dropwise under argon to a solution of 3 (6 g, 17.2 mmol) in dry benzene (60 ml) until the green color persisted more than 10 s. C^2H_3I (5 g, 34.5 mmol) was added dropwise to the solution in a ice bath which was removed after the addition. After stirring the mixture at 25°C for 30 min, the solution was poured into cold 10% HCl (100 ml), and was extracted with 100 ml of ethyl ether three times. The ether extracts were combined, and washed with 10% HCl and brine solution. The organic solution was dried over sodium sulfate, and removed in vacuo. Purification of the oily residue (5.6 g) on a silica gel column (120 g) afforded pure 4 as a colorless liquid (4.5 g; 85% yield). δ_H (90 MHz; $CDCl_3$) 0.90 (d, 6H. J = 6.5 Hz), 1.25 (t, 6H, J = 7.0 Hz), 2.48 (d, 2H, J = 6.5 Hz), 4.26 (q, 4H, J = 7.0 Hz), 7.06 (ABq, 4H).

[3,3,3-$^2H_3$]-Ibuprofen (1). The diester 4 (5 g, 16.2 mmol) was hydrolyzed by refluxing with a solution of KOH (9 g, 162 mmol) in water (35 ml) for 12 h. The solution, after cooled down, was acidified with concentrated HCl (21 ml) and heated under reflux for 6 h. The resulting mixture was saturated with sodium chloride, and extracted with 50 ml of ethyl ether twice. The combined organic layer was dried over sodium sulfate, and removed in vacuo. The resulting residue was then subjected to silica gel chromatography to give pure 1 (2.9 g, 86% yield). ^1H NMR ($CDCl_3$) δ 0.95 (d, 6H, J = 7 Hz), 1.45 - 2.0 (m, 1H), 2.4 (d, 2H, J = 7Hz), 3.75 (s,

1H), 7.05 (ABq, 4H, J = 9Hz), 10.7 - 11.3 (br., 1H). ^{13}C NMR ($CDCl_3$) δ 17.26 (quintet, J_{C-D} = 19.34Hz), 22.39, 30.10, 44.77, 45.05, 127.26, 129.22, 136.97, 140.36, 181.00. ^{2}H NMR ($CDCl_3$) δ 1.5.

[2-^{2}H]-Ibuprofen (2). Ibuprofen methyl ester (6.7 g, 30 mmol), prepared by the BF_3 etherate-catalyzed methylation, was added to a solution of CH_3O^2H (50 ml, 1.5 mol) containing sodium methoxide (0.8 g, 15 mmol). The resulting mixture was stirred under reflux for 12 h. Aliquot of the solution (2 ml) was taken, and the reaction was quenched by adding 60 μl of 10 N ^{2}HCl solution. The solvent was then removed in vacuo, and the residue was dissolved in 3 ml of ethyl ether. The organic solution was then washed with brine solution, dried over sodium sulfate, and removed in vacuo. The NMR spectrum of the crude residue showed the disappearance of the signal at 3.75 ppm, and the emergence of a singlet instead of a doublet at 1.50 ppm, indicating the completion of the substitution. The remaining solution was then subjected to the same workup procedure as described above. The resulting residue was treated with 6N HCl under reflux to afford crude 2. The oily compound was purified by a silica gel column to give pure 2 (5.7g, 90 %). ^{1}H NMR ($CDCl_3$) δ 0.9 (d, 6H, J = 6 Hz), 1.5 (s, 3H), 1.6 - 2.0 (m, 1H), 2.45 (d, 2H, J = 7Hz), 7.1 (ABq, 4H, J = 9Hz), 10.8 - 11.2 (br., 1H). ^{13}C NMR ($CDCl_3$) δ 18.18, 22.59, 30.28, 44.78 (t, J_{C-D} = 19.96Hz), 45.22, 127.38, 129.40, 137.03, 140.62, 181.36. ^{2}H NMR ($CDCl_3$) δ 3.63.

Enzymatic Resolution of Racemic Ibuprofen. A general procedure for the preparation of optically active ibuprofen by lipase-mediated enantiospecific hydrolysis is illustrated as follows. Racemic ibuprofen methyl ester (10 g) was suspended in 400 ml of 0.1 M potassium phosphate buffer (pH 7.2) containing 4 g of crude Candida cylindracea lipase. The suspension was incubated at 30°C on a rotary shaker (250 rpm) for 192 h. TLC analysis showed that

the product/substrate ratio was approx. 3:2. The aqueous solution was acidified with 6N HCl to pH 2, saturated with sodium chloride, and extracted with an equal volume of ethyl acetate twice. The organic extract was dried over sodium sulfate, and removed *in vacuo*. Silica gel chromatography of the crude mixture afforded *S*-ibuprofen (5.2 g, 71% *e.e.*) and the methyl ester of *R*-ibuprofen (3.9 g, 98% *e.e.*). The extent of conversion (c) was calculated according to the equation: c = ee(S) / [ee(S) + ee(P)] (S and P represent substrate and product, respectively) [9]. The kinetic parameter, E, which controls the enantioselectivity of an enzymatic resolution was determined according to the equation derived by Sih *et al* [9]:

$$E = \frac{1 - c\,[1 + ee(P)]}{1 - c\,[1 - ee(P)]}$$

The ester obtained was treated with 6N HCl under reflux to yield *R*-ibuprofen ($[\alpha]_D^{25}$ = -56.3°, c = 1.0, EtOH). In parallel, the *S*-acid fraction was remethylated with diazomethane, and subjected to enzyme hydrolysis under the same condition. After 96 h, *S*-ibuprofen (3.5 g, $[\alpha]_D^{25}$ = +56°) was obtained with optical purity of 97% *e.e.* after the same workup procedure.

Determination of Enantiomeric Purity. Two drops of thionyl chloride were added to approx. 3 mg of ibuprofen in a test tube. The mixture was incubated at 60°C for 30 min, and the remaining thionyl chloride was removed *in vacuo*. (1*R*,2*S*,5*R*)-(-)-Menthol (5 mg) (Aldrich Chemical Co.), dissolved in 150 µl of methylene chloride, and one drop of pyridine were then added. The solution stood at room temperature for 15 min, and was passed through silica gel (1 g) fitted in a pasteur pipette. The column was eluted with 6 ml of hexane/ether (20:1, v/v), and the collected solvent was dried under a stream of nitrogen. The diastereomeric *l*-menthyl esters thus formed were analyzed by HPLC using a Model 501 pump (Water Associates) equipped with a Rheodyne injector, two Whatman

Partisil (10μm) columns (2 x 4.6 mm x 25 cm) in tandem, and a Model 481 UV/Vis detector (Water Associates). The column was eluted with a solvent mixture consisting of hexane/ether (100:1) at a flow rate of 1 ml/min. The retention times for the l-menthyl esters of R- and S-ibuprofen were 21 min, and 22 min 20 s, respectively. The enantiomeric purity was calculated according to the equation e.e. = R - S / R + S.

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REFERENCES

1. Ehrlich, G.E. - Am. J. Med. 79 (suppl. 4B): 2 (1985).

2. Adams, S.S., Bresloff, P. and Mason, C.G. - J. Pharm. Pharmacol. 28: 256 (1976).

3. Caldwell, J., Hutt, A.J. and Fournel-Gigleux, S. - Biochem. Pharmacol. 37: 105 (1988), and references therein.

4. a) Nakamura, Y., Yamaguchi, T., Takahashi, S., Hashimoto, S., Iwatani, K. and Nakagawa, Y. - J. Pharmacobiol. Dynamics 4: s-1 (1981); b) Baillie, T.A., Adams, W.J., Kaiser, D.G., Olanoff, L.S., Halstead, G.W., Harpootlian, H. and VanGissen, G.J. - J. Pharmacol. Exp. Ther. 249: 517 (1989); c) Chen, C.-S., Chen, T. and Shieh, W.-R. - Biochim. Biophys. Acta (1989) in press.

5. Ghosh, S., Pardo, S.N. and Salomon, R.G. - J. Org. Chem. 47: 4692 (1982).

6. Newman, P. - "Optical Resolution Procedures for Organic Compounds," Vol 2, Part II, Mahattan College, New York (1981).

7. Gu, Q.M., Chen, C.-S. and Sih, C.J. - Tet. Lett. 27: 1763 (1986).

8. Enantiomeric excess (e.e.) is defined as R - S / R + S.

9. E (enantiomeric ratio) is defined as the ratio of the specificity constants of the two enantiomers, $(kcat/Km)_S$ / $(kcat/Km)_R$. For a comprehensive treatment of the principles involved in biochemical kinetic resolutions, see: Chen, C.S., Fujimoto, Y., Girdaukas, G. and Sih, C.J. - J. Am. Chem. Soc. 104: 7294 (1982).