# Resolution of Etodolac and Antiinflammatory and Prostaglandin Synthetase Inhibiting Properties of the Enantiomers

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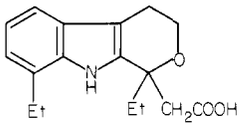
Etodolac, 1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic acid, a clinically effective analgesic and antiinflammatory agent, has been resolved via a chromatographic separation of its diastereoisomeric esters with (−)-borneol. The effects of the enantiomers were studied in vitro on prostaglandin synthetase and on adjuvant-induced arthritis in rats. The biochemical and pharmacological results show that virtually all of the effects of etodolac are due to the (+) enantiomer.

Etodolac (Ultradol, I), 1,8-diethyl-1,3,4,9-tetrahydro-

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pyrano[3,4-*b*]indole-1-acetic acid, is a clinically effective analgesic and antiinflammatory agent[1-8] that does not cause gastrointestinal microbleeding at doses in excess of those determined to be clinically effective.[9,10] Its synthesis,[11] metabolic disposition,[12,13] animal pharmacology,[14,15] and prostaglandin synthetase inhibiting properties[13] have been previously described. In the present report, the resolution of etodolac and the effects of the enantiomers on prostaglandin synthetase and on adjuvant arthritis are described.

## Results

**Chemistry**. Etodolac was converted to its diastereoisomeric esters with (−)-borneol. Preparative HPLC gave the pure, crystalline (−) and (+) diastereoisomers. Hydrolysis of each diastereoisomer with potassium hydroxide in methanol gave the (+)- and (−)-etodolac enantiomers. Enantiomeric purity was assessed by HPLC analysis of the methyl ester derivatives using a Bakerbond DNBPG [(*R*)-*N*-(3,5-dinitrobenzoyl)-1-phenylglycine] chiral column. The etodolac methyl ester enantiomers were separated with this column upon injection of the racemate, and the enantiomeric purities of (−)- and (+)-etodolac were found to be in excess of 99.9%.

**Pharmacology**. The antiinflammatory effects of the etodolac racemate and the enantiomers were assessed in rats with established adjuvant-induced polyarthritis after daily oral treatment with drugs for 8 days.

The (+)-etodolac was found to be 2.6 times (95% confidence limits 1.5 to 5.3) more potent than the racemate. In contrast, at the active dose of (+)-etodolac (0.5 to 4 mg/kg), (−)-etodolac was inactive. At a dose of 50 mg/kg, (−)-etodolac produced an antiinflammatory effect that was about equivalent to that produced by 0.5 mg/kg of (+)-etodolac in the model used (Figure 1).

**Biochemistry**. (±)-Etodolac and the etodolac enantiomers were investigated in vitro for their effects on the biosynthesis of prostaglandins from tritiated arachidonic acid. The results are shown in Figure 2 in terms of the percent of unreacted arachidonic acid remaining after various concentrations of the drugs. It was derived from these graphs that the concentration producing a 50% inhibition of arachidonic acid utilization by (±)-etodolac was 240 μM, while that for (+)-etodolac was 120 μM. (−)-Etodolac had no effect at 400 μM, the highest concentration tested.

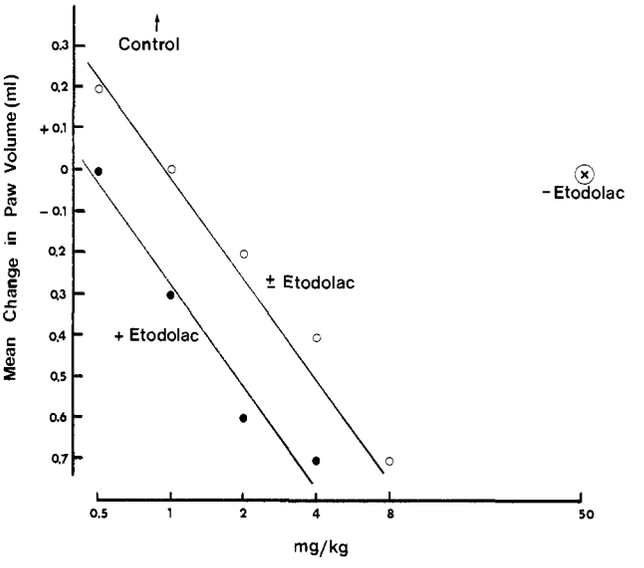


**Figure 1.** Effects of etodolac and enantiomers on adjuvant arthritis.

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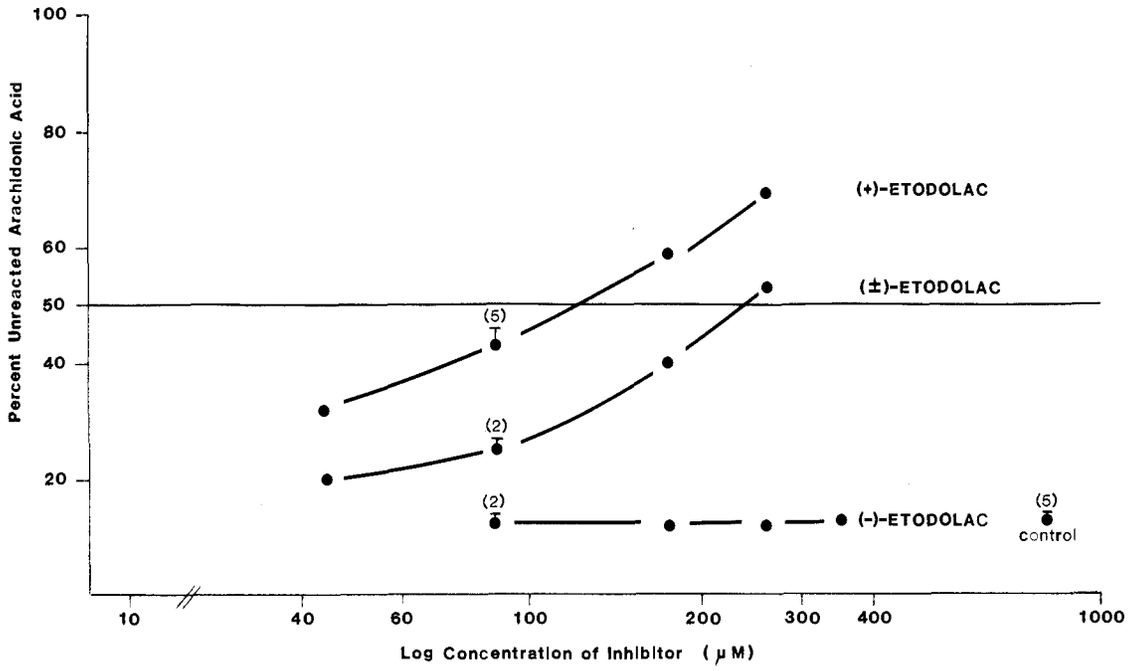


**Figure 2.** Effects of etodolac and enantiomers on prostaglandin biosynthesis.

## Discussion

The pharmacological and biochemical results generated with racemic etodolac and with the optically pure enantiomers show that most, if not all, of the antiinflammatory activity of etodolac is due to the (+) enantiomer. Similar results have been obtained with other nonsteroidal antiinflammatory agents, which contain chiral centers of known absolute configuration. Thus, (*R*)-(-)-ibuprofen has been shown to be 165 times more active than the (*S*)-(+) enantiomer as an inhibitor of prostaglandin biosynthesis[16] while virtually all of the activity of carprofen in an adjuvant arthritis model and as a prostaglandin synthetase inhibitor resides in the (*S*)-(+) enantiomer.[17]

A detailed study of the molecular shape of these chiral prostaglandin synthetase inhibitors may provide some insight regarding a putative common binding site on prostaglandin cyclooxygenase. To this end, studies are under way to determine the absolute configuration of (+)-etodolac.

## Experimental Section

**High-Performance Liquid Chromatography.** The chromatographic system consisted of a Dupont Model 850 pump, a Dupont Model 800 microprocessor, and a LDC Spectromonitor III UV detector. The chiral column used was a 250 × 4.6 mm Bakerbond-DNBPG 07651-2-20 column (J. T. Baker Co.), and the mobile phase consisted of hexane and 2-propanol in the ratio of 99:1. The flow rate was maintained at 1 mL/min, at a back pressure of 19–20 bars, and the procedure was carried out at ambient temperature. Ten microliters of the sample solution (concentration: ca. 3 μg/10 μL) was injected onto the column in 20% 2-propanol in hexane, and the effluent was monitored at 274 nm.

**Resolution of (±)-Etodolac. (a) Formation of (1*S*)- and (1*R*)-Etodolac Esters with (*S*)-(-)-Borneol.** A mixture consisting of (±)-etodolac (100 g, 0.348 mol), (-)-borneol (64.46 g, 0.418 mol), 4-(dimethylamino)pyridine (5.09 g, 0.0417 mol), and *N*,*N*′-dicyclohexylcarbodiimide (86.24 g, 0.418 mol) in 1.5 L of diethyl ether was stirred at 22 °C for 18 h. The reaction was cooled in an ice–water bath and filtered. The filtrate was washed once with 5% aqueous sodium hydroxide, twice with 5% HCl, and twice with water. After the solution was dried over $MgSO_4$ and the solvent was evaporated 160.3 g of semisolid was obtained. A TLC on silica gel using 10% EtOAc in hexane revealed two products. Filtration through 1.5 kg of silica gel using 10% ethyl acetate in hexane as eluant afforded 119.3 g of the mixture as a solid.

Preparative HPLC (20–25-g batches) using Prepak 500 silica gel cartridges and 3% ethyl acetate in hexane as eluant gave (+)- and (-)-*endo*-(1*S*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl 1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetates.

The (+) diastereoisomer was crystallized from hexane to afford 52.33 g of material (35.5% yield): mp 142–143 °C; $[\alpha]_D$ +47.4° (*c* 1, ethanol). Anal. ($C_{27}H_{37}NO_3$) C, H, N.

The (-) diastereoisomer (53.33 g, 36.2% yield) was a solid: mp 93–96 °C; $[\alpha]_D$ -61.4° (*c* 1, ethanol). Anal. ($C_{27}H_{37}NO_3$) C, H, N.

**(b) Hydrolyses of Borneol Esters.** The (+) and (-) diastereoisomeric esters obtained above were dissolved in methanol (1 L) containing potassium hydroxide (34.8 g) and water 260 mL. The mixture was refluxed while stirring for 3 h. Most of the methanol was distilled off, water (500 mL) was added, and the mixture was extracted with toluene. The aqueous phase was acidified with 6 N hydrochloric acid and extracted with chloroform. The chloroform extracts were washed with water and dried, and the solvent was removed to afford crude (+)-etodolac (32.5 g) and (-)-etodolac (35.9 g). Each one was purified by chromatography on 1 kg of silica gel impregnated with phosphoric acid by stirring the silica gel with a 1% solution of phosphoric acid in methanol, followed by air-drying. Elution with 10% acetone in toluene gave the etodolac enantiomers. They were obtained as solids by dissolving in benzene (100 mL) and pouring into cold petroleum ether (bp 30–60 °C, 1.2 L) with stirring. Subsequent crystallization from benzene–petroleum ether (bp 30–60 °C) gave the pure enantiomers.

(+)-Etodolac (24.02 g), obtained in 66.4% yield from the (-) diastereoisomer, had mp 138–140 °C and $[\alpha]_D$ +25.2° (*c* 3, ethanol). Anal. ($C_{17}H_{21}NO_3$) C, H, N. It had a $t_R$ of 6.65 min, and the HPLC scan indicated an enantiomeric purity in excess of 99.9%.

(-)-Etodolac (21.46 g), obtained in 60.4% yield from the (+) diastereoisomer, had mp 139–141 °C and $[\alpha]_D$ -25.6° (*c* 3, ethanol). It had a $t_R$ of 6.29 min, and the HPLC scan indicated an enantiomeric purity in excess of 99.9%.

**Effect on Established Adjuvant Arthritis.** Male inbred Wistar Lewis rats (180–200 g) obtained from Charles River Breeding Laboratories, Boston, MA, were injected intradermally

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in the distal third of the tail with 0.1 mL of Freund's complete adjuvant (FCA) composed of a fine suspension of killed and dried *Mycobacterium butiricum* (Difco) in liquid paraffin at a concentration of 5 mg/mL. The day of FCA injection was designated as day 0 of the experiment. On day 16 after FCA, the volume of both hindpaws was measured by mercury displacement according to the method of Hall and Hallet.[18] Only the rats with well-established arthritis (mean increase in volume of both hindpaws between 1 and 2.5 mL) and comparable edema volumes in the left and right hindpaws were selected for the experiment. Arthritic animals were distributed into experimental groups of six rats so that all groups had comparable mean hindpaw edema volumes. Drug treatment was started on day 16 and administered daily until day 23. On the last day of the experiment, hindpaw volumes were measured, and changes from day 16 readings for the 12 hindlegs of each group were calculated. From these measurements, the relative potency and the 95% confidence limits of the drugs were established by analysis of variance.[19]

**Inhibition of Prostaglandin Synthesis.** The preparation of an acetone and diethyl ether washed powder of sheep vesicular glands has been described previously.[20] The powder was homogenized in 0.05 M $KH_2PO_4$–NaOH buffer (pH 7.4) in the presence of phenol (0.6 mM) as activator. The homogenate was centrifuged at 1000*g* at 2 °C for 5 min, and the resulting low-speed supernatant was used as enzyme source. The assay consisted of 4 mL of enzyme solution (equivalent to 5 mg of acetone powder), test drug (50–400 μg added in 40 μL of acetone), and arachidonic acid (20 μg containing $2 \times 10^6$ dpm $^3H_8$-labeled material) dissolved in 40 μL of acetone. The enzyme solution was preincubated for 1 min at 37 °C with test drug before substrate arachidonic acid was added and further incubated at 37 °C for 10 min. Control experiments consisted of enzyme solution and arachidonic acid in the absence of test drug. The incubation was terminated by the addition of 5 vol of ethanol. The precipitated protein was filtered, and the filtrate was evaporated to complete dryness in vacuo. The residue was dissolved in ethanol and analyzed by TLC (silica gel G; Brinkmann) using as developing solvent chloroform–methanol–acetic acid–water (90:9:1:0.65, v/v). After development, the radioactive zones were located with a Berthold radiochromatogram scanner and scraped with scintillation vials, and after the addition of methanol–water (1 mL, 1:1, v/v) and Bioflur (10 mL, New England Nuclear), the vials were counted in a Beckman scintillation spectrophotometer. Results (Figure 2) are expressed in terms of the amount (percent) of arachidonic acid present in the sample. In control experiments, the amount of unreacted arachidonic acid represented 12.4 ± 0.3% ($n$ = 5), while $PGE_2$ and $PGF_{2\alpha}$ represented 58.9 ± 1.5% ($n$ = 5) of the radioactivity in the sample. The rest of the radioactivity migrated in the region of monohydroxy fatty acids (probably 11- and 15-HETE, formed via cyclooxygenase). Results with drugs showed inhibition of both PGs, as well as the hydroxy fatty acids (seen also with indomethacin), indicating an inhibition at the level of cyclooxygenase.

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**Registry No.** (±)-I, 87226-38-8; (+)-I, 87249-11-4; (−)-I, 87226-41-3; (1*S*)-etodolac (*S*)-(−)-borneol ester, 87226-39-9; (1*R*)-etodolac (*S*)-(−)-borneol ester, 87226-40-2; prostaglandin synthetase, 9055-65-6.

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# *N*-Arylhydroxamic Acid *N,O*-Acyltransferase. Positional Requirements for the Substrate Hydroxyl Group

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*N*-Arylhydroxamic acid *N,O*-acyltransferase (AHAT) is a cytosolic enzyme system that is capable of converting toxic and carcinogenic *N*-arylhydroxamic acids into electrophilic reactants and of catalyzing the transacetylation of arylamines. The role of the *N*-hydroxyl group in promoting AHAT-catalyzed transacetylation of arylamines was investigated by the synthesis and biochemical evaluation of a series of *o*-hydroxyaryl amides and *N*-arylglycolamides. Several of these compounds are metabolites of carcinogenic aryl amides in vivo. 3-Hydroxy-4-acetamidobiphenyl (**8**) was weakly effective as an acetyl donor when partially purified preparations of hamster or rat hepatic AHAT were used to catalyze the transacetylation of 4-aminoazobenzene. 1-Hydroxy-2-acetamidofluorene (**1**), 3-hydroxy-2-acetamidofluorene (**2**), 2-glycolamidofluorene (**3**), 4-glycolamidobiphenyl (**9**), and *trans*-4-glycolamidostilbene (**5**) were less effective acyl donors than 4-acetamidobiphenyl (**7**) itself. The compounds were also assayed for their abilities to participate in the AHAT-catalyzed conversion of *N*-arylhydroxylamines to electrophilic intermediates that form methylthio adducts upon reaction with *N*-acetylmethionine. None of the compounds exhibited more than 4% of the activity of the prototype compound, *N*-hydroxy-4-acetamidobiphenyl (**10**). These results indicate that the presence of an hydroxyl group on the ring position ortho to the amide group or on the α-position of the acyl group is not sufficient to confer significant acyltransferase activity with AHAT.

Carcinogenic aryl amides are N-hydroxylated by the cytochrome P-450 dependent polysubstrate monooxygenase system to form *N*-arylhydroxamic acids, a class of proximate carcinogens.[1] Arylhydroxamic acid *N,O*-acyltransferase (AHAT) catalyzes the rearrangement of these *N*-arylhydroxamic acids to reactive electrophiles, which form covalent adducts with nucleic acids and other cellular nucleophiles, including those present on AHAT itself (Scheme I).[2,3] Additional, or concurrent, biotransformations of *N*-arylhydroxamic acids by AHAT include deacylation to form mutagenic arylhydroxylamines and transfer of the acyl group to arylamines (Scheme I).[4,5]

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