Enantiomeric Purity of Naproxen by Liquid Chromatographic Analysis of Its Diastereomeric Octyl Esters

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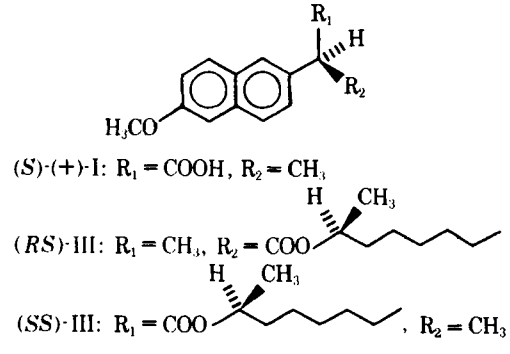
Received May 18, 1978, from the *Pharmaceutical Analysis Department, Syntex Research, Palo Alto, CA 94304.* Accepted for publication June 14, 1978.

Abstract □ A sensitive and specific analytical method was developed to determine the enantiomeric purity of naproxen. A simple derivatization of naproxen with (*S*)-(+)-2-octanol proceeded quantitatively and gave a mixture of diastereomeric esters displaying baseline separation on liquid chromatography. The ratio of these esters was the same as the ratio of enantiomers present in naproxen samples assayed. Reproducibility of the method was excellent.

Keyphrases □ Naproxen—liquid chromatographic analysis of enantiomers □ Liquid chromatography—analysis, enantiomers of naproxen □ Enantiomers—of naproxen, liquid chromatographic analysis □ Anti-inflammatory agents—naproxen, liquid chromatographic analysis of enantiomers

The anti-inflammatory drug naproxen is the (*S*)-(+)-enantiomer of 2-(6′-methoxy-2′-naphthyl)propionic acid (I) (1, 2). During naproxen development, it became necessary to develop a simple, yet sensitive, analytical method for the determination of the enantiomeric purity of this drug.

A chromatographic method was chosen over an NMR or direct rotation measurement because of the small sample size required and to gain the specificity necessary to separate I from any of a variety of potentially optically active compounds (*i.e.*, degradation products, impurities, and metabolites). To provide chromatographic separation of the enantiomers of I, another chiral species must be introduced and a diastereomeric relationship established. The most commonly used technique involves reaction of the enantiomeric mixture with an optically active reagent to form a pair of diastereomers that can be separated using ordinary chromatographic materials[1,2]. For this method to be rigorous (*i.e.*, for the ratio of diastereomers produced to mirror exactly the ratio of enantiomers present in the original sample), the reagent used must be enantiomerically pure and there must be no kinetic resolution or racemization in the reaction that transforms the enantiomer mixture into a diastereomer mixture (6).



This paper describes a simple method for the quantitative reaction of I with (*S*)-(+)-2-octanol [(*S*)-(+)-II] to give the diastereomeric esters (*SS*)-III and (*RS*)-III. The liquid chromatographic separation and quantitation of these esters are also discussed.

EXPERIMENTAL

Materials—The syntheses of naproxen[3] and 2-(6′-hydroxy-2′-naphthyl)propionic acid[3] were reported previously (1, 2). (*S*)-(+)-2-Octanol (>99% optically pure) was obtained commercially[4]. The reagent grade toluene was purified by washing with concentrated sulfuric acid until the acid remained colorless. The toluene was then washed with water until neutral and then with saturated sodium bicarbonate solution. A final wash with water until neutral and drying over anhydrous sodium sulfate afforded toluene of sufficient purity for use as the esterification reaction solvent.

Glass-distilled hexane and ethyl acetate were obtained commercially[5] and were dried prior to use by percolation through neutral aluminum oxide[6] (activity grade I). All other solvents were reagent grade.

Liquid Chromatography Equipment and Operating Conditions—A liquid chromatograph[7] equipped with a variable wavelength UV detector[8] and a 100-μl fixed loop injector[9] were used. The wavelength chosen for detection was 332 nm (0.04 aufs); at lower wavelengths, an impurity found in all batches of octanol gave a detectable peak coincident with that for (*SS*)-III. Integration of peak areas was obtained through the use of a programmed digital integration system[10] interfaced to the detector. A 25-cm × 3-mm (i.d.) commercially available microparticle silica[11] column was operated at ambient temperature. With 0.5% (v/v) ethyl acetate in hexane as the mobile phase and a flow rate of 1.2 ml/min, the column developed a pressure of approximately 49.21 atmospheres.

Esterification Procedure—Approximately 1 mg of I was weighed into a test tube fitted with a polytef-faced screw cap. To this solid was added 100 μl of (*S*)-(+)-II and, on dissolution, 900 μl of purified toluene and 5 μl of concentrated sulfuric acid were also added. The capped tubes were placed in an oil bath for 2 hr at 100°[12]. After cooling to room temperature, 1 ml of 0.02 *M* sodium bicarbonate was added and the mixture was agitated vigorously.

The toluene layer was transferred to a new tube and dried over sodium sulfate. A 100-μl aliquot of this solution was then brought to 10 ml with the mobile phase, and the resulting solution (~10 μg/ml) was analyzed by injecting 100 μl (1 μg) into the chromatograph. The percent (*SS*)-III is then calculated from:

$$\text{percent (SS)-III} = \frac{\text{area peak 1}}{\text{area peak 1} + \text{area peak 2}} \times 100 \quad \text{(Eq. 1)}$$

[1] For an excellent review of the separation of diastereomers by GLC, see Ref. 3.

[2] Direct chromatographic separation of enantiomers by GLC (4) and liquid chromatographic (5) methods, utilizing optically active stationary or mobile phases, has been reported.

[3] Institute of Organic Chemistry, Syntex Corp., Palo Alto, Calif.

[4] Norse Laboratories.

[5] Burdick and Jackson Laboratories.

[6] Woelm, ICN Pharmaceuticals.

[7] Spectra-Physics model 3500B.

[8] Schoeffel model 770.

[9] Valco universal inlet.

[10] Spectra-Physics Systems I.

[11] Spectra-Physics prepacked with 5-μm Spherisorb silica.

[12] The progress of the reaction was followed by TLC. The reaction mixture (50 μl) was spotted directly onto silica gel GF plates and developed with hexane–tetrahydrofuran–acetic acid (50:50:1). In this solvent system, I (R_f 0.71) and III (R_f 0.86) are well separated.

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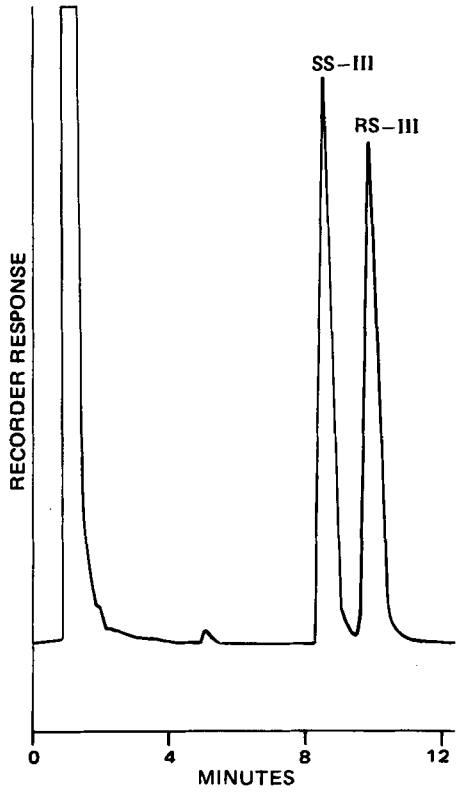


Figure 1—*Chromatogram of the mixture of diastereomeric octyl esters (III) prepared from racemic I and* (S)-(+)-*II.*

where peak 1 is the first eluting peak and corresponds to the (*SS*)-ester and the second peak corresponds to the (*RS*)-ester[13].

Scale-up of this reaction gave a crystalline product (mp 53°) upon workup whose spectral properties were consistent with those of the expected ester III.

Calibration Curve—Approximately 10 mg of (*S*)-(+)-I of high optical purity[14], $[\alpha]_D^{25}$ +67.4° (c 1.0, $CHCl_3$), was accurately weighed into a 10-ml volumetric flask. The solid was dissolved by addition of 1 ml of (*S*)-(+)-II, and the resulting solution was brought to 10 ml with purified toluene. This procedure was repeated using racemic I. Into six culture tubes were added aliquots of the solutions as shown below to give a final volume of 1 ml in each tube:

volume of (*S*)-(+)-I, ml	volume of (±)-I, ml	approximate enantiomeric purity, %
1.0	0	100
0.8	0.2	80
0.6	0.4	60
0.4	0.6	40
0.2	0.8	20
0	1.0	0

To each sample was added 5 μl of concentrated sulfuric acid, and the resulting reaction mixtures were treated as described and analyzed by liquid chromatography. The enantiomeric purity of (*S*)-(+)-I in each calibration sample can be calculated from the following equation[15]:

$$\text{percent enantiomeric purity} = 200\left[\frac{V_+W_+F + \frac{1}{2}V_\pm W_\pm}{V_+W_+ + V_\pm W_\pm}\right] - 100 \quad \text{(Eq. 2)}$$

[13] Actually each peak represents an enantiomeric pair. Peak 1 represents both (*SS*)-III and (*RR*)-III while peak 2 represents both (*RS*)-III and (*SR*)-III. Due to the high degree of optical purity of (*S*)-(+)-II, contributions that (*RR*)-III and (*SR*)-III may make to each peak were ignored.

[14] Optical rotations were measured on a Perkin-Elmer model 141 polarimeter.

[15] If $W_+ = W_\pm$, *i.e.*, if the weights used to prepare the two standard solutions are the same, then Eq. 2 simplifies to:

$$\text{percent enantiomeric purity} = 200\,[V_+F + \tfrac{1}{2}V_\pm/V_+ + V_\pm] - 100$$

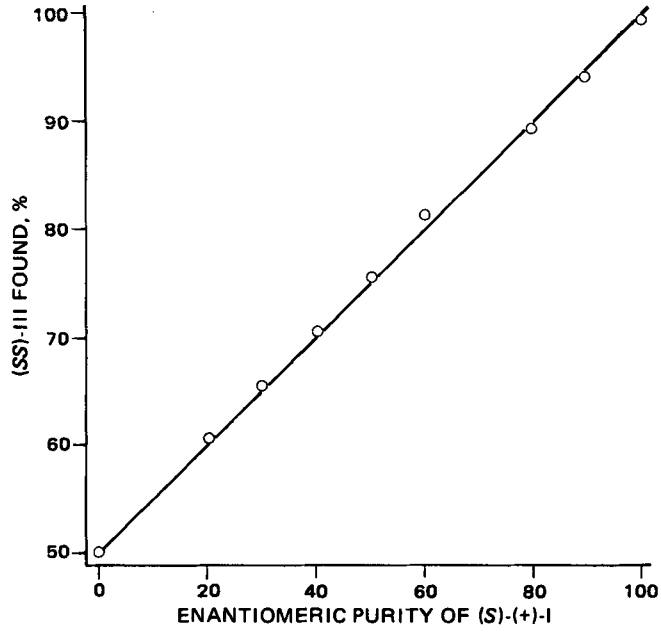


Figure 2—*Calibration plot showing the relationship between the known enantiomeric purity (by weight) of prepared samples of I and the diastereomeric composition of III found after esterification.*

where:

V_+ = volume of (*S*)-(+)-I standard solution in milliliters
$V_\pm$ = volume of racemic I standard solution in milliliters
W_+ = weight of (*S*)-(+)-I used to prepare the standard solution
$W_\pm$ = weight of racemic I used to prepare the standard solution
F = mole fraction of the (*S*)-(+) enantiomer known to be in the batch of I used to prepare the (*S*)-(+)-I standard solution

RESULTS AND DISCUSSION

The esterification reaction rate was followed by TLC. The reaction was complete in approximately 1.5 hr at 100° under the conditions specified. Complete loss of I was observed, and no side products were apparent by TLC. When (*S*)-(+)-I and (*S*)-(+)-II were allowed to react for 23 hr under these same conditions, no evidence of degradation of the ester [(*SS*)-III] was observed by TLC and liquid chromatography. Racemization of I or epimerization of (*SS*)-III under these severe conditions was also ruled out since the percent (*SS*)-diastereomer found (98%) compares well with that found after only 2 hr of reaction (99%). These results indicate that the reaction of I with (*S*)-(+)-II gives only III with no competing reactions and that this ester is completely stable, both chemically and stereochemically, under the reaction conditions.

The liquid chromatographic method used to analyze the reaction mixtures displayed baseline separation of the (*SS*)- and (*RS*)-diastereomers of III (Fig. 1). The specificity of the method for I in the presence of 2-(6′-hydroxy-2′-naphthyl)propionic acid (IV), a major metabolite in humans (7, 8), was also demonstrated by the large separation found between the octyl esters of I and IV on liquid chromatography.

A plot of the percent (*SS*)-III found experimentally *versus* the known enantiomeric purity of (*S*)-(+)-I in each sample is shown in Fig. 2. The excellent fit of the points in this calibration curve to a line of slope 0.5

Table I—Enantiomeric Purity of Several Samples of I [a] by Esterification followed by Liquid Chromatography

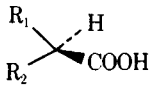
Sample	Enantiomeric Purity of (*S*)-(+)-I, %
1	79.2
2	79.9
3	79.8
4	79.2
5	79.9
Mean ± *SD*	79.6 ± 0.4

[a] Samples are 80.0% enantiomerically pure.

clearly demonstrates that for each sample the ratio of the diastereomeric esters produced is identical to the ratio of enantiomers present in the original sample. If, however, enantiomerically impure (*S*)-(+)-II were used, the slope of the line in Fig. 2 would be less than 0.5 and could be calculated from:

$$\text{slope} = \frac{\text{enantiomeric purity of (S)-(+)-II}}{200} \qquad \text{(Eq. 3)}$$

Table I shows the results of the liquid chromatographic analysis of five samples of (*S*)-(+)-I (80% enantiomerically pure). The data clearly demonstrate the excellent reproducibility of the method. This result is expected since the measurement is not dependent on quantitative recovery of III but rather involves comparison of relative peak areas of two closely eluting diastereomers. The applicability of this method to other



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carboxylic acids of this type (Structure A) can only be speculative but is perhaps not unlikely if R_1 and R_2 are sufficiently dissimilar.

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Stability of E-Type Prostaglandins in Triacetin

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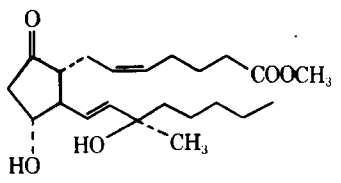
Received January 11, 1978, from *The Upjohn Company, Kalamazoo, MI 49001.* Accepted for publication June 1, 1978.

Abstract □ A drug delivery system for E-type prostaglandins is described. In this system, consisting of drug dissolved in triacetin and filled into soft gelatin capsules, normally unstable prostaglandins show excellent stability at room temperature.

Keyphrases □ Prostaglandins, E type—stability in triacetin drug delivery system □ Stability—E-type prostaglandins in triacetin drug delivery system □ Delivery, drug—E-type prostaglandins in triacetin system, stability

Prostaglandins are a class of biologically active compounds with a wide spectrum of pharmacological responses. Their application in human reproduction (1) and antiulcer therapy (2) demonstrates their clinical usefulness. The general instability of prostaglandins has resulted in the arduous task of developing stable formulations for biological, toxicological, and clinical testing.

The number of asymmetric carbon centers in the molecule can result in the formation of stereoisomers through various degradative pathways. For example, in the F series, dinoprost (prostaglandin $F_{2\alpha}$) undergoes acid-catalyzed epimerization to form the 15-epi derivative (3). Prostaglandins of the E series [*e.g.*, prostaglandin E_1 and prostaglandin E_2 (dinoprostone)] are more labile due to the facile dehydration of the 11-β hydroxyl group in the cyclopentanone ring, forming prostaglandins of the A series; in some cases, subsequent isomerization to B series prostaglandins occurs (4). Recently (5), a more detailed kinetic scheme was elucidated for the decomposition of prostaglandins E_1 and E_2 to include other degradative steps leading to the formation of 8-iso, 15-epi, and 13,15-rearrangement products of the respective prostaglandins.



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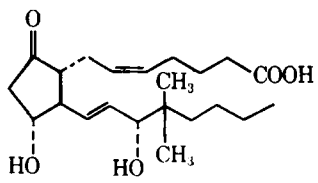

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Table I—Stability of II in Triacetin

Time	Temperature	Triacetin Solution, % label[a]	Soft Elastic Capsule[b], % label
Initial	4°	102	100
1 month	4°	102	108
3 months	4°	103	98.8
6 months	4°	105	91.1
9 months	4°	108	105
12 months	4°	98.7	106[c]
Initial	25°	102	100
1 month	25°	98.8	95.3
3 months	25°	103	93.5
6 months	25°	102	87.9
9 months	25°	103	100
12 months	25°	94.7	94.5
Initial	40°	102[d]	100
1 month	40°	99.1[d]	101
3 months	40°	87.7[d]	91.5
6 months	40°	87.3[d]	70.3

[a] Average values from theoretical concentrations of 0.5 and 2.2 mg/ml unless otherwise indicated. [b] Theoretical concentration was 0.85 mg/ml. [c] Value was for 11 months. [d] Value from a theoretical concentration of 2.2 mg/ml.

This paper reports the use of triacetin for stabilizing stock solutions of (15*R*)-15-methyl prostaglandin E_2 methyl ester (I) and 16,16-dimethyl prostaglandin E_2 (II) for experimental studies and in dosage form design. Nonaqueous solvents usually are employed for solubilizing water-insoluble drugs (6, 7). With prostaglandins, a variety of solvent vehicles also was employed to enhance stability (8) and to provide a milieu for rapid drug dissolution.

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