Isomeric Inversion of Ibuprofen (*R*)-Enantiomer in Humans

D. G. KAISER ˣ, G. J. VANGIESSEN, R. J. REISCHER, and W. J. WECHTER

Abstract □ Enantiomeric compositions of the major urinary metabolites of ibuprofen [(*RS*)-2-(4-isobutylphenyl)propionic acid] were characterized after oral administration of the racemic mixture and oral administration of the individual enantiomers to normal human volunteers. Resolution of the diastereomeric amides, formed by reaction of the urinary metabolites with (*S*)-(−)-α-methylbenzylamine, was achieved by GLC. Only the (*R*)-(−)-enantiomer of the intact drug was inverted to its optical antipode, (*S*)-(+), in humans. However, both (*S*)-(+)- and (*R*)-(−)-enantiomers of the intact drug were transformed independently *in vivo* to the major metabolites, *i.e.*, 2,4′-(2-hydroxy-2-methylpropyl)phenylpropionic acid and 2,4′-(2-carboxypropyl)phenylpropionic acid. *In vivo* metabolism of ibuprofen to its carboxy metabolite was not stereoselective.

Keyphrases □ Ibuprofen—isomeric inversion of enantiomers, determined by characterization of major urinary metabolites □ Isomeric inversion—ibuprofen enantiomers, determined by characterization of major urinary metabolites □ Enantiomers—ibuprofen, isomeric inversion determined by characterization of major urinary metabolites

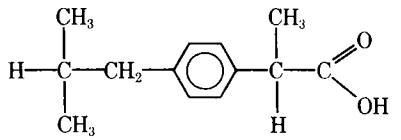
Extensive pharmacological, toxicological, and biochemical studies of ibuprofen[1] [(*RS*)-2-(4-isobutylphenyl)propionic acid] (I) (1, 2), a potent orally active anti-inflammatory agent in animals (3–6) and humans (7–9), have been reported. Interest in the stereochemistry of the metabolism of I was stimulated by the early report (1) that: (*a*) after oral administration of the racemic drug, the major metabolites excreted in human urine (IV and VII) were optically active; and (*b*) the *in vivo* biological activities of the individual enantiomers of the intact drug were equivalent.

Subsequently, it was shown that, after oral administration of the individual enantiomers of I, the major metabolites isolated from human urine were dextrorotatory (5). Utilizing GLC methods for measuring the individual enantiomers (10–12), the facile epimerization of the (*R*)-(−)-isomer of I to its optical antipode, *i.e.*, II, was observed *in vivo* prior to oxidative metabolism (13, 14).

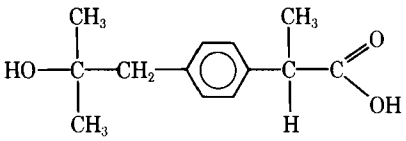
With the availability of suitable analytical methodology for separating the enantiomers of the major metabolites excreted in human urine (10, 12), studies were designed to further characterize the epimerization of the (*R*)-(−)-isomer and metabolism of I in humans.

EXPERIMENTAL

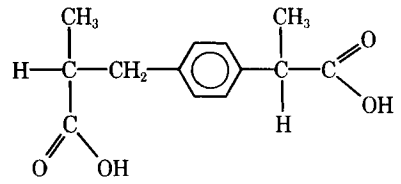
Reagents and Materials—The I used in this study was synthesized[2], and the (*S*)-(+)- and (*R*)-(−)-isomers were resolved (*vide infra*). Compounds IV and VII, used for initial metabolite characterization, were isolated and purified from human urine[3]. The partially resolved (*R*)-(−)-isomer of IV (*i.e.*, VI) was synthesized[4] (15). Tridecanoic acid[5], isophthalic acid[6], (*R*)-(+)-α-methylbenzylamine[7], (*S*)-(−)-α-methylbenzylamine[7], and hydrocarbon-stabilized chloroform[8] were used as supplied. Stock solutions of 1,1′-carbonyldiimidazole (65 mg/ml) in hydrocarbon-stabilized chloroform were prepared fresh daily. All other solvents were analytical grade.



ibuprofen
I: racemate
II: (*S*)-(+)
III: (*R*)-(−)



hydroxy metabolite
IV: isomeric mixture
V: (*S*)-(+)
VI: (*R*)-(−)



carboxy metabolite
VII: isomeric mixture
VIII: (*S*)-(+)/(*S*)-(+)
IX: (*R*)-(−)/(*S*)-(+)
X: (*S*)-(+)/(*R*)-(−)
XI: (*R*)-(−)/(*R*)-(−)

Diethylene glycol succinate on 80–100-mesh Diatoport-S[9], phenyl methyl silicone fluid (OV-17) on 60–80-mesh Gas Chrom Q[5], chromatography plates coated with thin layers (250 μm) of silica gel F-254[10], and silica gel for preparative chromatography[10] (0.05–0.2 mm) were used as supplied. Amberlite XAD-2 resin[11] was washed successively with two bed volumes each of water, acetone, methanol, and water prior to utilization for chromatography.

Resolution of Ibuprofen—(*S*)-(−)-α-Methylbenzylamine (0.291 mole) was added dropwise, with stirring, to an ice-cooled solution of I (0.291 mole) in 600 ml of ether. The resulting solid, *i.e.*,

[1] Motrin, The Upjohn Co., and Brufen, The Boots Co., Ltd.
[2] The Boots Co., Ltd., Nottingham, England.
[3] Supplied by R. Cobb, The Boots Co., Ltd., Nottingham, England.
[4] By The Upjohn Co.
[5] Applied Science Labs., State College, Pa.
[6] Eastman Organic Chemicals, Rochester, N.Y.
[7] Aldrich Chemical Co., Milwaukee, Wis.
[8] Matheson, Coleman and Bell, Milwaukee, Wis.
[9] LAC-728, Hewlett-Packard Co., Avondale, Pa.
[10] Brinkmann Instruments, Westbury, N.Y.
[11] Rohm and Haas, Philadelphia, Pa.

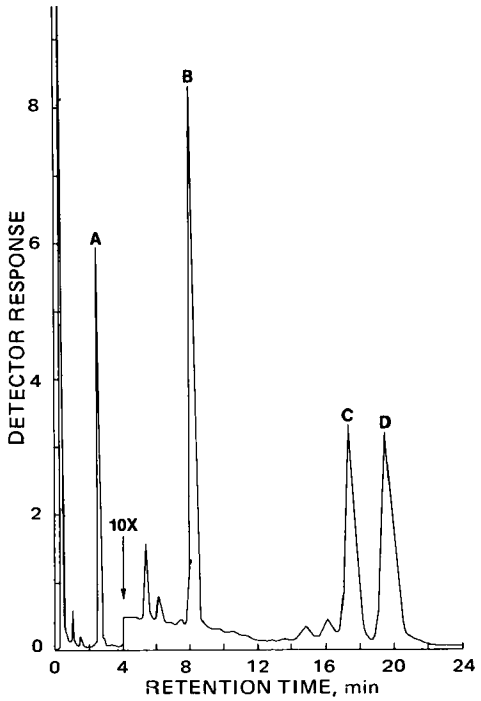


Figure 1—*Gas–liquid chromatogram of methyl esters of ibuprofen, its major urinary metabolites, and isophthalic acid. Key: A, ibuprofen; B, isophthalic acid standard; C, hydroxy metabolite; and D, carboxy metabolite.*

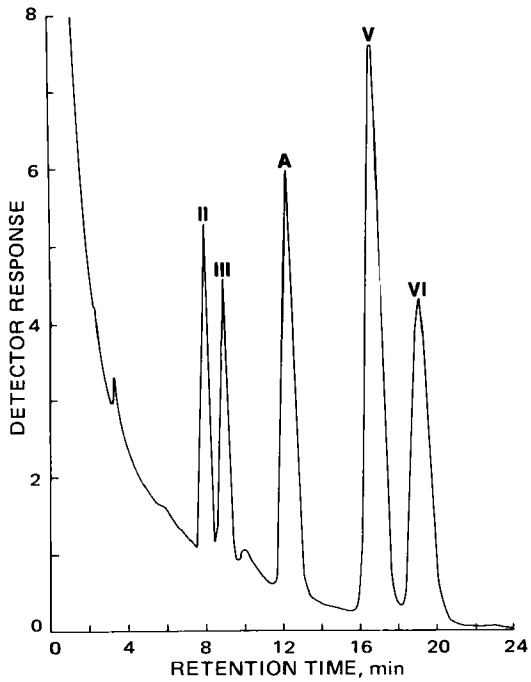


Figure 2—*Gas–liquid chromatogram of* (S)-(−)-*α-methylbenzylamide derivatives of ibuprofen enantiomers and enantiomers of hydroxy metabolite. Key: II,* (S)-(+)-*enantiomer of ibuprofen; III,* (R)-(−)-*enantiomer of ibuprofen; A,* n-*tridecanoic acid standard; V,* (S)-(+)-*enantiomer of hydroxy metabolite; and VI,* (R)-(−)-*enantiomer of hydroxy metabolite.*

the more insoluble (*S*)-(−)-α-methylbenzylamine salt of II (mp 155–170°), was filtered and recrystallized from 300 ml of isopropanol. Two additional recrystallizations, from 200 and 150 ml of absolute ethanol, afforded 12.9 g of white crystals (mp 179–185°). The material was acidified with 3 *N* aqueous sulfuric acid and extracted into ether.

After washing with water and saline, the ether extract was evaporated to dryness. The resulting white solid (mp 49–51°) was recrystallized twice from absolute ethanol to afford 4.5 g of II (mp 50–52°, $[\alpha]_D$ + 57°). GLC analysis, as the (*S*)-(−)-α-methylbenzylamide derivative (*vide infra*), showed an optical purity of 95%.

Anal.—Calc. for $C_{13}H_{18}O_2$: C, 75.69; H, 8.79. Found: C, 75.88; H, 8.92.

The procedure was repeated using (*R*)-(+)-α-methylbenzylamine as the resolving agent. The (*R*)-(+)-α-methylbenzylamine salt of III (mp 153–172°) was filtered and recrystallized from isopropanol. Two additional recrystallizations from absolute ethanol afforded 15.5 g of white crystals (mp 181–185°). The material was acidified with 3 *N* aqueous sulfuric acid and extracted into ether.

After washing with water and saline, the ether extract was evaporated to dryness. The resulting white solid (mp 49–52°) was recrystallized twice from absolute ethanol to afford 6.3 g of III (mp 50–52°, $[\alpha]_D$ −57°). GLC analysis, as the (*S*)-(−)-α-methylbenzylamide derivative (*vide infra*), showed an optical purity of 96%.

Anal.—Calc. for $C_{13}H_{18}O_2$: C, 75.69; H, 8.79. Found: C, 75.86; H, 9.09.

Instrumentation—All GLC measurements were made with a gas chromatograph[12] equipped with dual hydrogen flame-ionization detectors and a −0.2–1.0-mv dual-pen recorder[13]. All gas cylinders used for chromatography (*i.e.*, helium, hydrogen, and oxygen) were fitted with filters containing molecular sieve 4A. All mass spectrometric measurements were made with a combined GLC–mass spectrometer interfaced to a computer (16). Optical rotation measurements, $[\alpha]_D$ in 95% ethanol, were made with a polarimeter[14] at 25° in a 1-dm tube.

TLC—Analytical chromatography was conducted on thin layers of silica gel F-254, ascendingly developed in a solvent system of 10% (v/v) acetic acid in toluene. The separated materials were visualized by irradiation of the plates with a short wavelength (254 nm) UV lamp. Under these chromatographic conditions, I, IV, and VII had R_f values of 0.42, 0.20, and 0.29, respectively.

GLC—The GLC procedure described for the determination of I in plasma (17) as the methyl ester was modified to measure the intact drug and its major metabolites excreted in human urine. Reaction time for the free carboxylic acids with 1,1′-carbonyldiimidazole was increased from 1 to 5 min, and isophthalic acid was selected as an internal standard to replace naphthalene.

During analysis, the column [*i.e.*, 6% (w/w) diethylene glycol succinate on 80–100-mesh Diatoport-S], injection port, and detector block were maintained isothermally at 180, 220, and 240°, respectively. Helium, hydrogen, and oxygen flow rates were 60, 40, and 400 ml/min, respectively. Under these conditions, the methyl esters of I, isophthalic acid, IV, and VII had retention times of 2.6, 8.1, 17.3, and 19.6 min, respectively (Fig. 1).

The individual enantiomers of I, IV, and VII excreted in human urine were measured as the (*S*)-(−)-α-methylbenzylamide derivatives *via* GLC on a column of 3% (w/w) OV-17 (10, 12). With the column temperature maintained isothermally at 220°, the retention times for the derivatives of II, III, V, and VI were 8.0, 9.0, 16.9, and 19.1 min, respectively (Fig. 2). To measure the enantiomers of VII, the column temperature was maintained isothermally at 285°. Three partially resolved peaks, designated VIII, IX–X, and XI, were observed with retention times of 25.2, 28.4, and 30.8 min, respectively (Fig. 3).

For all measurements utilizing combined GLC–mass spectrometry, the temperatures of the ion source and separator were maintained isothermally at 280°. The electron-impact energy was maintained at 70 ev, and helium was used as carrier gas. Columns of 6% (w/w) diethylene glycol succinate and 3% (w/w) OV-17 were maintained isothermally at 180 and 250°, respectively. Mass spectra and tabular presentations of ion intensities were obtained *via* computer data reduction (16).

Column Chromatography—Aliquots (1500 ml) of pooled 24-hr urine speciments were adjusted to pH 3 with acetic acid and applied to columns[11] (65 × 3 cm i.d.). The columns were eluted successively with 600 ml of water, 600 ml of 50% (v/v) ethanol in water, 1100 ml of 95% (v/v) ethanol in water, and 500 ml of acetone. The effluent was monitored by TLC of the free carboxylic

[12] HP model 402, Hewlett-Packard Co., Avondale, Pa.
[13] HP model 7128A, Hewlett-Packard Co., Avondale, Pa.
[14] PE model 141, Perkin-Elmer Corp., Norwalk, Conn.

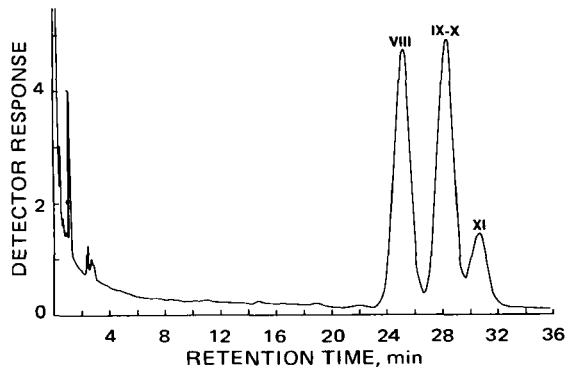


Figure 3—*Gas–liquid chromatogram of* (S)-(−)-α-*methylbenzylamide derivatives of enantiomers of carboxy metabolite. Key: VIII,* (S)-(+)/(S)-(+)-*enantiomer of carboxy metabolite; IX–X, mixture of* (R)-(−)/(S)-(+)- *and* (S)-(+)/(R)-(−)-*enantiomers of carboxy metabolite; and XI,* (R)-(−)/(R)-(−)-*enantiomer of carboxy metabolite.*

acids and GLC of the methyl esters of the carboxylic acids.

Fractions containing I, IV, and VII were pooled and concentrated to 300 ml under reduced pressure (15 mm) at 50°. The concentrate was adjusted to pH 3 with acetic acid and extracted three times with 300 ml of benzene. Then the extracts were pooled and concentrated under reduced pressure (15 mm) at 50°. The resulting residue was reconstituted in 10 ml of 10% (v/v) acetic acid in toluene and applied to a silica gel column (50 × 3 cm i.d.) packed in the same solvent mixture.

The column was developed with 10% (v/v) acetic acid in toluene, and the effluent was monitored by TLC. Fractions containing I, IV, and VII were pooled separately and evaporated to dryness under reduced pressure (15 mm) at 50°. The viscous oil, obtained from I fractions, was too small to crystallize. Residues from IV and VII fractions were recrystallized separately from benzene and hexane. Purification was monitored by TLC and optical rotation measurements of the free carboxylic acids as well as GLC–mass spectrometric analysis of the methyl esters and α-methylbenzylamide derivatives of the acids.

Drug Administration to Humans—Informed written consent was obtained from each of three normal human male volunteers prior to participation. All subjects were between the ages of 27 and 44 years; they ranged in body weight from 70.9 to 93.2 kg and in height from 1.74 to 1.85 m. All subjects were fasted for 12 hr prior to, and 4 hr after, drug administration.

On Days 1, 8, and 15, each subject received a single 800-mg dose of II, III, and I, respectively, in hard-filled gelatin capsules. All doses were taken with 240 ml of water. Total urine specimens were collected for 12 hr prior to drug ingestion and at predetermined time intervals from 0 to 72 hr postadministration. All specimens were stored at −18° until assayed.

RESULTS AND DISCUSSION

Synthesis and Characterization of Methyl Esters—Earlier investigations (17) showed that 1,1′-carbonyldiimidazole was a useful reagent for the facile esterification of I. A series of samples containing known amounts of IV, VII, and isophthalic acid were prepared to determine the optimal reaction conditions for (*a*) imidazolide formation and (*b*) methyl ester formation from the imidazolide and triethylamine in methanol. In studies with IV and VII, known amounts of dimethyl isophthalate were added as the internal standard. The results indicated that imidazolide formation was completed within 1 min for IV and within 5 min for VII and isophthalic acid. Methyl ester formation was completed within 5 min for all compounds.

GLC, in conjunction with mass spectrometry, showed that the methyl ester of IV had a very weak molecular ion of mass 236 (Fig. 4). Major fragmentation peaks were observed at *m/e* 178, 119, 118, 91, and 59. The mass spectrum of VII dimethyl ester showed a molecular ion of mass 264 and major fragmentation peaks at *m/e* 205, 177, 145, 117, and 91 (Fig. 4). The combined results for the methyl esters of IV and VII showed excellent agreement with reported studies (11) using diazomethane in ether for esterification.

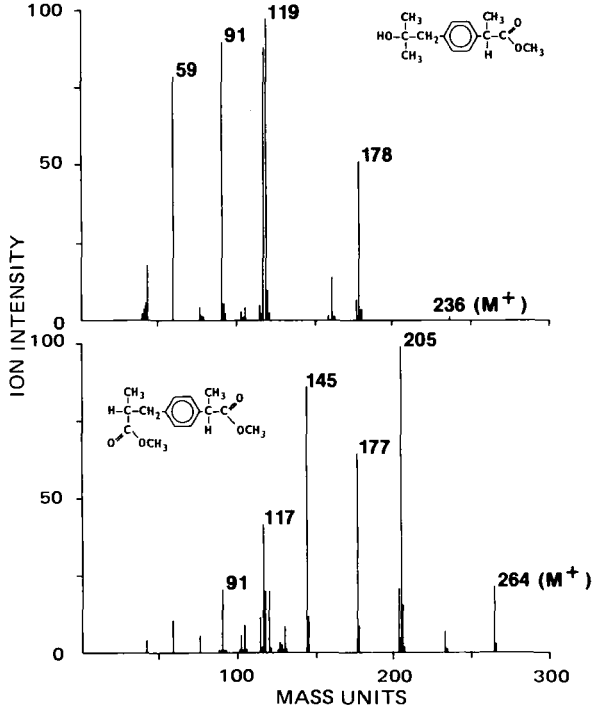


Figure 4—*Mass spectra of methyl esters of major urinary metabolites of ibuprofen. Upper panel: hydroxy metabolite. Lower panel: carboxy metabolite.*

Synthesis and Characterization of (*S*)-(−)-α-Methylbenzylamide Derivatives—Pilot studies using thionyl chloride as an acylating reagent showed that, although I and VII were easily acylated, IV was readily dehydrated and hydrohalogenated. Subsequent reaction of the intermediate(s) of IV with (*S*)-(−)-α-methylbenzylamine produced undesirable side products. Utilization of 1,1′-carbonyldiimidazole, analogous to studies reported for I (10, 12), produced the desired amides of all three compounds.

A series of samples containing known amounts of IV, VII, and *n*-tridecanoic acid was prepared to determine the optimal reaction times for (*a*) imidazolide formation and (*b*) amide formation from the imidazolides and (*S*)-(−)-α-methylbenzylamine. The results

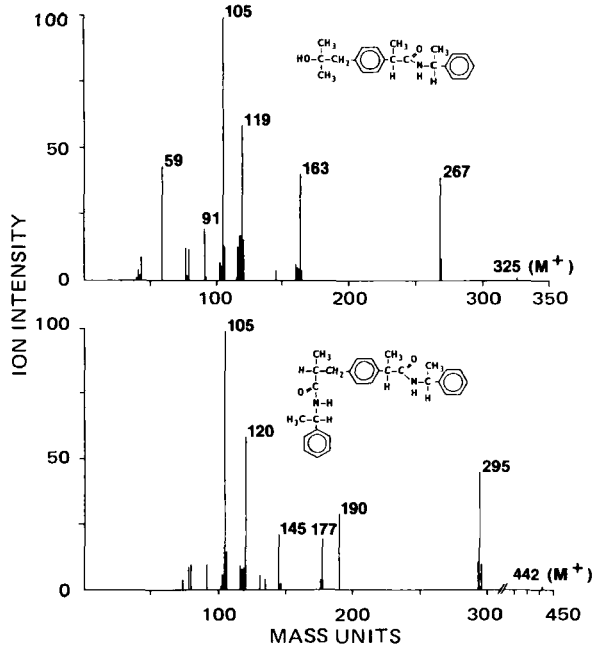


Figure 5—*Mass spectra of* (S)-(−)-α-*methylbenzylamide derivatives of major urinary metabolites of ibuprofen. Upper panel: hydroxy metabolite. Lower panel: carboxy metabolite.*

Table I—Enantiomeric Composition of Drug-Related Materials Excreted in Human Urine after Single-Dose Oral Administration (800 mg) of Ibuprofen or Its Individual Enantiomers

Drug Administered			Drug-Related Materials Excreted in Human Urine					
			Intact Drug		Hydroxy Metabolite		Carboxy Metabolite	
Enantiomer	Optical Rotation	GLC Ratio[a]	Optical Rotation[b]	GLC Ratio[a]	Optical Rotation	GLC Ratio[a]	Optical Rotation	GLC Ratio[c]
II	+57	95:5	+42	95:5	+48 (+35.4)[d]	97:3	+49 (+47.2)[d]	51 (*S*)-(+)/(*S*)-(+) 47 (*R*)-(−)/(*S*)-(+); (*S*)-(+)/(*R*)-(−) 2 (*R*)-(−)/(*R*)-(−)
III	−57	6:94	+24	80:20	+2 (+2.8)[d]	54:46	+6 (+7.2)[d]	33 (*S*)-(+)/(*S*)-(+) 42 (*R*)-(−)/(*S*)-(+); (*S*)-(+)/(*R*)-(−) 25 (*R*)-(−)/(*R*)-(−)
I	0	50:50	+13	71:29	+21 (+29.7)[d]	71:29	+28	43 (*S*)-(+)/(*S*)-(+) 44 (*R*)-(−)/(*S*)-(+); (*S*)-(+)/(*R*)-(−) 13 (*R*)-(−)/(*R*)-(−)

[a] Ratio of (*S*)-(+)- and *R*-( )-enantiomers, as measured by GLC of the *S*-(−)-α-methylbenzylamide derivatives. [b] Chemical purity of isolated materials estimated to be 60–80%. [c] Ratio of (*S*)-(+)/(*S*)-(+)-, (*R*)-( )/(*S*)-(+)-, (*S*)-(+)/(*R*)-(−)-, and (*R*)-(−)/(*R*)-( )-enantiomers, as measured by GLC of the (*S*)-(−)-α-methylbenzylamide derivatives. [d] Optical rotation as reported in Ref. 5.

showed that imidazolide formation from IV, VII, and *n*-tridecanoic acid was completed in 5 min. Amide formation from the imidazolide was completed in 20 min. GLC, in conjunction with mass spectrometry, showed that the amides of V and VI were well resolved on a 1.5-m column of 3% (w/w) OV-17 (Fig. 2) and had extremely weak molecular ions of mass 325 (Fig. 5). Major fragmentation peaks were observed at *m/e* 267, 163, 119, 105, 91, and 59. The mass spectra for the three peaks, designated VIII, IX–X, and XI (Fig. 3), had nearly identical fragmentation patterns. The molecular ions of mass 442 were very weak. Intense fragmentation peaks were observed at *m/e* 295, 190, 120, and 105 (Fig. 5).

Enantiomeric Composition of Drug-Related Materials Excreted in Human Urine—Results from GLC measurement of the major metabolites of I, as the methyl esters, showed that urinary excretion of the unconjugated compounds was greater than 95% complete within 24 hr. Thus, the 0–24-hr urine specimens, from three subjects receiving the individual enantiomers of I, were pooled for determining the enantiomeric compositions of the intact drug and its major metabolites. The results from these studies are summarized in Table I.

The (*S*)-(−)-α-methylbenzylamides of II and III were well resolved on a 1.5-m column of 3% (w/w) OV-17 (Fig. 2). GLC–mass spectrometric analyses established that the materials responding to the assay were identical to known (*S*)-(−)-α-methylbenzylamide derivatives of II and III (10, 12). The enantiomeric composition of the intact drug excreted in urine after oral administration of II was identical to the drug ingested (Table I). However, after oral administration of III and I, 80 and 71% of the intact drugs excreted in urine, respectively, were of the (*S*)-(+)-configuration.

In vitro studies established that known I, II, and III were optically stable between pH 1 and 10 for at least 24 hr. Furthermore, isolation and characterization of known amounts of III added to urine showed that the (*R*)-(−)-configuration was retained. Optical rotation measurements, in combination with GLC, showed a linear relationship between optical rotation, $[\alpha]_D$, and isomeric composition. Optical rotation analyses of the small amounts of intact drug excreted and isolated from human urine indicated that the chemical purities ranged from 60 to 80% (Table I).

As shown in Fig. 2, the (*S*)-(−)-α-methylbenzylamides of V and VI were well resolved on the 3% (w/w) OV-17 column. Measurement of the enantiomeric composition of the major hydroxy metabolite excreted in urine after oral administration of II showed that essentially all of the material was of the (*S*)-(+)-configuration (Fig. 6). Results from the GLC measurement of the enantiomeric composition of VI (Fig. 6), partially synthesized *via* microbial oxygenation, were in excellent agreement with the reported optical rotation (15). After oral administration of III and I, 54 and 71% of the free hydroxy metabolites excreted in urine, respectively, were of the (*S*)-(+)-configuration (Table I). Results from measurement of optical rotations showed good agreement with reported values (5) and were consistent with enantiomeric composition as measured by GLC.

Measurement of the enantiomeric composition of the carboxy metabolite excreted in urine after oral administration of II showed that 51% of the material was associated with the GLC peak designated as VIII [(*S*)-(+)/(*S*)-(+)-configuration] and 47% of the material was associated with the peak designated as IX–X [combination of (*R*)-(−)/(*S*)-(+)- and (*S*)-(+)/(*R*)-(−)-configuration] (Fig. 7). A trace amount (2%) of the material was contained in the peak designated XI [(*R*)-(−)/(*R*)-(−)-configuration], consistent with metabolism of the small amount of III present in the administered drug.

After oral administration of III, 33, 42, and 25% of the carboxy

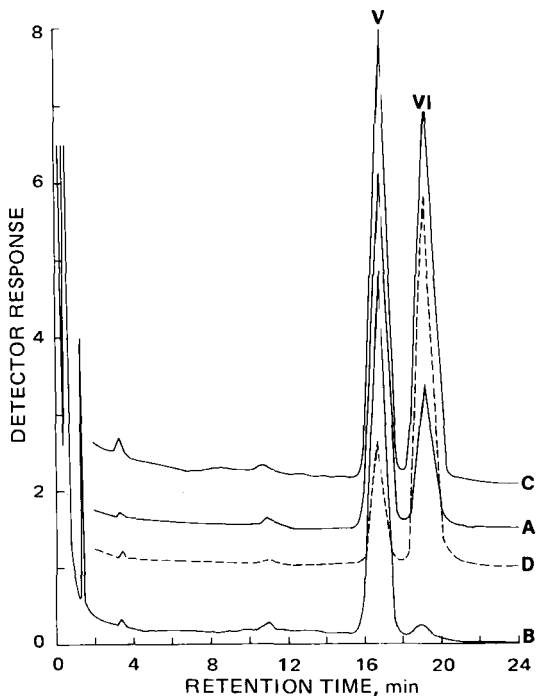


Figure 6—*Gas–liquid chromatograms of* (S)-(−)-α-*methylbenzylamide derivatives of hydroxy metabolites isolated from human urine after single-dose oral administration of 800 mg of ibuprofen (scan A),* (S)-(+)-*enantiomer (scan B), or* (R)-(−)-*enantiomer (scan C). The* (R)-(−)-*enantiomer was partially synthesized via microbial oxygenation (scan D).*

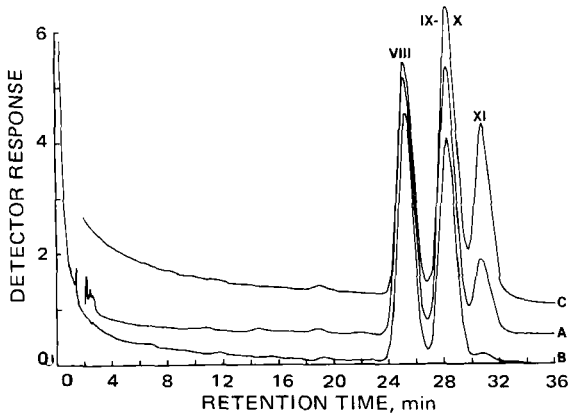


Figure 7—*Gas–liquid chromatograms of* (S)-(−)-α-*methylbenzylamide derivatives of carboxy metabolites isolated from human urine after single-dose oral administration of 800 mg of ibuprofen (scan A),* (S)-(+)-*enantiomer (scan B), or* (R)-(−)-*enantiomer (scan C).*

metabolite were associated with the peaks designated VIII, IX–X, and XI, respectively. Similarly, after oral administration of I, 43, 44, and 13% of the carboxy metabolite were associated with peaks VIII, IX–X, and XI, respectively (Fig. 7).

During oxidative metabolism of II to the carboxy metabolite, a second chiral center is introduced into the molecule. Since the chiral center in the propionic acid side chain retained its (*S*)-(+)-configuration, as observed in the intact drug and hydroxy metabolite, and with the assumption of an equal probability for oxidative metabolism of the isobutyl side chain to result in an (*S*)-(+)- or (*R*)-(−)-configuration in the second carboxyl moiety, two diastereomeric carboxy metabolites could be formed with (*S*)-(+)/(*S*)-(+)- and (*R*)-(−)/(*S*)-(+)-configurations. Two major components were found when the carboxy metabolite, isolated from urine after oral administration of II, was chromatographed as the disubstituted (*S*)-(−)-α-methylbenzylamide derivative. Repetitive mass spectrometric scanning showed that the individual chromatographic peaks were homogeneous and the mass fragmentation patterns were identical. Consistent with GLC behavior of the individual enantiomers of I and IV as well as results from optical rotation measurements, the components with retention times of 25.2 and 28.4 min were designated as VIII and IX, respectively (Fig. 7).

After administration of I or III, potentially four diastereomeric carboxy metabolites could be formed. GLC of the carboxy metabolites as the (*S*)-(−)-α-methylbenzylamide derivatives showed only three components. Results from mass spectral analyses showed that all components were homogeneous and had identical fragmentation patterns. Considering the spatial configuration of the amide derivatives used for GLC separation, it was highly likely that the component with a retention time of 28.4 min was a mixture of IX and X. Thus, the components with retention times of 25.2, 28.4, and 30.8 min were designated VIII, IX–X, and XI, respectively. Metabolic studies are in progress, using tetradeuterated I, to confirm the present assignments and to define the epimerization mechanism.

From these results, it is concluded that only the (*R*)-(−)-enantiomer of I is inverted to its optical antipode, (*S*)-(+), in humans. Both (*S*)-(+)- and (*R*)-(−)-enantiomers are transformed *in vivo* to the hydroxy and carboxy metabolites. *In vivo* oxidation of the isobutyl group of I to the corresponding 2-carboxypropyl moiety is not stereoselective.

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