Synthesis, Chromatographic Resolution and Chiroptical Properties of Carboxyibuprofen Stereoisomers: Major Metabolites of Ibuprofen in Man

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ABSTRACT The chromatographic resolution of the four stereoisomers of carboxyibuprofen, a major metabolite of ibuprofen in man, was achieved using a Chiralpak AD chiral stationary phase (CSP) (J.T. Baker, Milton, Keynes, UK). The elution order of the stereoisomers was determined to be 2′*S*,2*R*; 2′*R*,2*R*; 2′*R*,2*S*; 2′*S*,2*S* by a combination of stereoselective synthesis of diastereoisomeric mixtures and analysis of the two diastereoisomers isolated from human urine following the administration of (*S*)-ibuprofen. The individual stereoisomers were isolated by semipreparative chiral phase chromatography and characterized by circular dichroism spectroscopy. *Chirality 9:75–87, 1997.*

KEY WORDS: stereoselective synthesis; carboxyibuprofen diastereoisomers; circular dichroism; chiral LC separation; ibuprofen

(*R,S*)-Ibuprofen [(±)-(*R,S*)-2-(4-isobutylphenyl)propionic acid, **1**] is an important non-steroidal anti-inflammatory drug (NSAID) of the 2-arylpropionic acid group used for the treatment of a variety of rheumatic and musculoskeletal disease states. Ibuprofen shows stereoselectivity in both action and disposition[1–5] and was the first compound of this group reported to undergo metabolic chiral inversion from the relatively inactive *R*-enantiomer to its active *S*-antipode.[6,7] Ibuprofen undergoes metabolic oxidation to yield two major products, hydroxyibuprofen (2-[4-(2-hydroxy-2-methylpropyl)phenyl]propionic acid, **3**) and carboxyibuprofen (2-[4-(2-carboxypropyl)phenyl]propionic acid, **2**)[8,9] (Fig. 1). The urinary excretion of these two metabolites, both free and conjugated with glucuronic acid, together with ibuprofen accounts for approximately 80% of an oral dose following administration of the racemate to man.[10,11] The observation that both metabolites were dextrorotatory irrespective of the form, i.e., either individual enantiomer or racemate, in which the drug was administered ultimately resulted in the discovery of the chiral inversion reaction for this series of NSAIDs.[2,3]

As a result of the interest in the stereochemical aspects of its metabolism, together with the possible implications of the chiral inversion reaction, the metabolism of ibuprofen has been investigated extensively both in vivo and in vitro.[2–5] However, compared to the unchanged drug, the stereochemical composition of the two major oxidation products has received relatively little attention. The formation of carboxyibuprofen results in the introduction of a second chiral center in the molecule and therefore, following the administration of racemic ibuprofen, four stereoisomers are possible.[7] The lack of attention to the stereochemical composition of the metabolites arises in part as a result of their limited availability and problems associated with the chromatographic resolution of the stereoisomers of carboxyibuprofen. Neither the original packed column gas-liquid chromatography (GLC) assay[7] nor the more recent capillary gas chromatography-mass spectrometric (GC-MS) assays,[12,13] based on the indirect approach to enantiomeric analysis, resulted in the separation of all four stereoisomers. The application of a Pirkle type chiral stationary phase (CSP), (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine, to the resolution resulted in the separation of the two pairs of diastereoisomers but not an enantiomeric resolution.[14] The only successful chromatographic resolution reported to date is that of Rudy et al.,[15] which is based on the indirect approach follow-ing derivatization of carboxyibuprofen with (*S*)-1-phenylethylamine followed by high-performance liquid chromatography (HPLC) using a C_8 stationary phase. The lack of authentic standards has also

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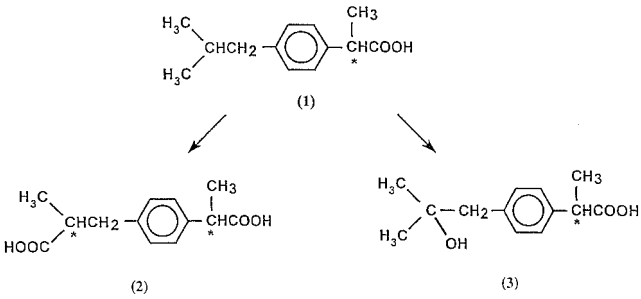

Fig. 1. Metabolism of ibuprofen **(1)** to yield hydroxy- **(3)** and carboxy-ibuprofen **(2).**

resulted in problems associated with the determination of the chromatographic elution order of the stereoisomers. The stereochemical assignment of the chromatographic peaks was based[13,15] by reference to that published by Kaiser et al.,[7] which was itself empirical. The (*S*)-1-phenylethylamine derivatives of (*S*)-ibuprofen and hydroxyibuprofen eluted before those of the *R*-configuration so that it was deduced that the corresponding diamide derivatives of the diacid metabolite would elute in the order *S,S* before *R,S/S,R* before *R,R*.[7]

In order to examine the stereochemical aspects of ibuprofen metabolism in man we have investigated methods for the synthesis, chromatographic resolution, and chiroptical characterization of the stereoisomers of carboxyibuprofen. As pointed out above, carboxyibuprofen is chemically designated as 2-[4-(2-carboxypropyl)phenyl]propionic acid. Thus, both chiral centers are indicated as being at the 2-position of the two side chains. In this report, the chiral center introduced by metabolic oxidation of the isobutyl group of ibuprofen is indicated as the 2′-position, whereas the original chiral center in the propionic acid moiety is indicated as the 2-position.

MATERIALS AND METHODS

Chemicals

(*R*)- and (*S*)-ibuprofen were the generous gifts of Boots Co. Ltd. (Nottingham, UK). The following compounds were purchased from the companies indicated: diethyl 2-p-tolylmalonate, diethyl 2-methylmalonate, bis(triphenylphosphine)pallidium (II) chloride ($Pd(PPh_3)_2Cl_2$), (4*S*)-4-isopropyl-2-oxazolidinone, butyllithium, N-bromosuccinimide, benzoyl peroxide, lithium diisopropylamide (LDA), tributyltin hydride, and methyl propiolate from the Aldrich Chemical Co. Ltd. (Gillingham, Dorset, UK); (4*R*,5*S*)-4-methyl-5-phenyl-2-oxazolidinone from Fluka Chemicals (Gillingham, Dorset, UK); tetrakis(triphenylphosphine)palladium (O) ($Pd(PPh_3)_4$) from Lancaster Synthesis (Morecambe, Lancs, UK); trifluoroacetic acid (TFA), hydrogen chloride gas, and all other reagents from BDH (Poole, Dorset, UK). Solvents for HPLC were of HPLC grade and were purchased from Rathburn (Walkerburn, UK). Unless otherwise stated, petroleum ether refers to the fraction of b.p. range 60–80°.

Instrumentation

Nuclear magnetic resonance (NMR) spectra NMR spectra were recorded using either Perkin Elmer R32 (90 MHz), GE NMR QE 300 (300 MHz), or Bruker AM 360 (360 MHz) spectrometers. ^{13}C-NMR spectra were recorded using the Bruker instrument at 90 MHz. Samples for NMR were prepared in $CDCl_3$ or d_6-DMSO solution using tetramethylsilane as internal standard.

Mass spectrometry (MS) Electron impact mass spectra were recorded using a VG-Micromass 16F mass spectrometer or a JEOL AX505W instrument at an ionization potential of 70 eV. High resolution mass spectra (HRMS) were recorded using the JEOL AX505W instrument by the peak matching technique.

Elemental analyses These were carried out by Butterworth Laboratories (Teddington, UK).

Circular dichroism (CD) CD spectra were recorded using a JASCO J-600 spectropolarimeter. All spectra were recorded using a scan speed of 10 nm/min and time constant of 4 sec. A band width of 2.0 nm was used for recording spectra in the far-ultraviolet (UV) region, from 260 to 180 nm, while a band width of 1.0 nm was used for the spectra in the near-UV region, from 320 to 240 nm. The analytes were dissolved in acetonitrile and the solutions held in cylindrical cells maintained at 25°C in the spectropolarimeter. The far-UV spectra were recorded using a 0.02 cm pathlength cell and analyte concentration of 0.3 mg/ml, while for the near-UV the pathlength was 1.0 cm and the concentration was 1 mg/ml. In each case a solvent baseline was recorded and subtracted from the analyte spectrum before converting to $\Delta\epsilon$. All data acquisition and processing were carried out using JASCO JUP342 software.

Chromatography

HPLC HPLC was carried out using either a LDC Constametric 3000 pump linked to an LDC Spectromonitor 3100 detector and a CI 4000 computing integrator or a Perkin Elmer Integral 4000 system. Samples were introduced on column using a Rheodyne 7125 injection valve fitted with a 20 µl sample loop.

Achiral chromatography Achiral chromatography was carried out using a Partisil silica column (5 µm, 250 × 4.6 mm) obtained from Whatman (Maidstone, Kent, UK) and a Resolve C_{18} column obtained from Anachem (Luton, Beds, UK). Refillable guard columns (10 × 2.1 mm) were packed with pellicular silica (40–63 µm) obtained from Alltech (Lancs, UK).

Chiral phase chromatography Chiral phase chromatography was carried out using a Chiralpak AD CSP [amylose tris (3,5-dimethylphenylcarbamate)] stationary phase (10 µm, 250 × 4.6 mm) (J.T. Baker, Milton Keynes, UK). The mobile phase consisted of hexane:ethanol mixtures with the proportions varying with the analyte (see Results and Discussion) containing TFA (0.05%, v/v) for examination of the free acids, at a flow rate of 1 ml/min at ambient temperature. Detection was by UV at 220 nm.

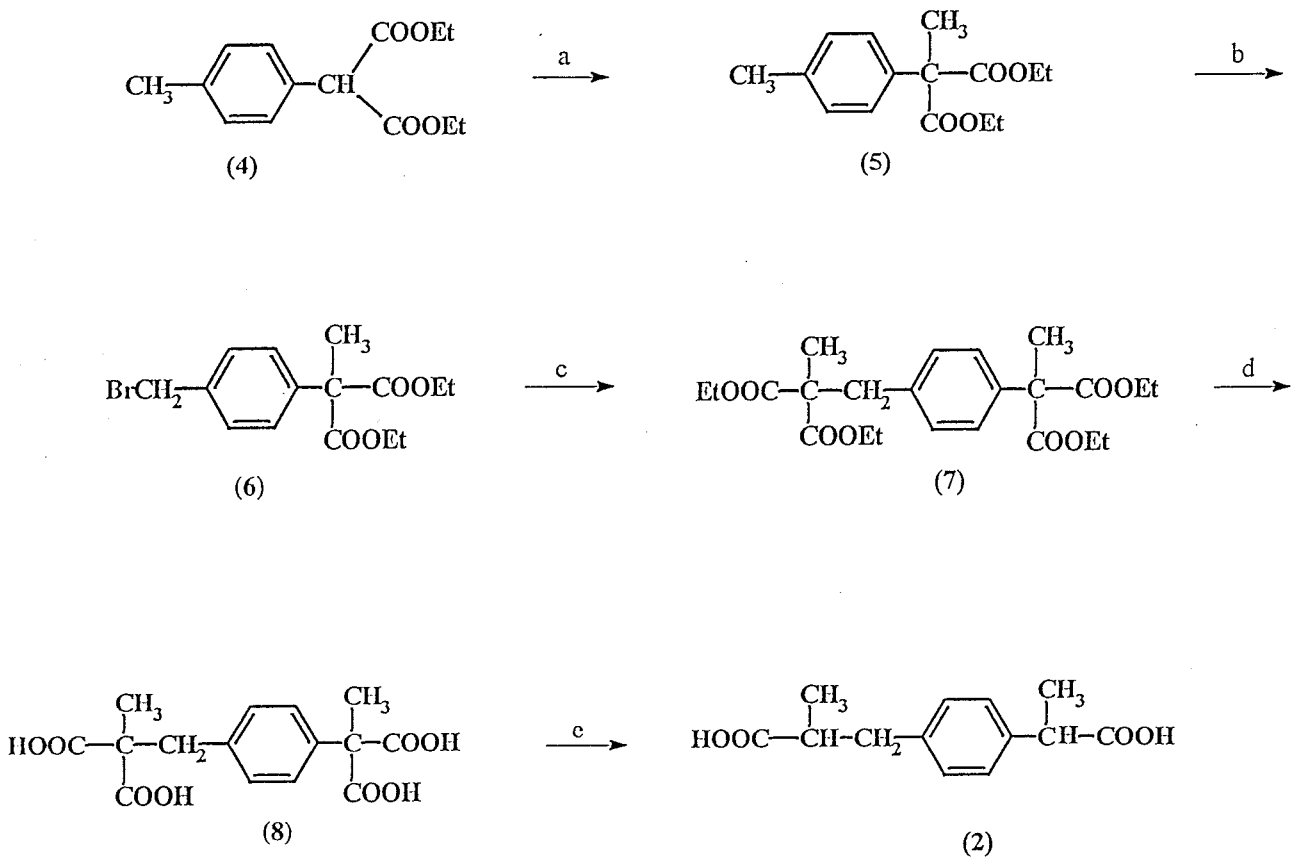

Fig. 2. Synthetic route adopted for the preparation of (2′*R,S,* 2*R,S*)-carboxyibuprofen **(2). (a)** CH_3I, EtONa; **(b)** N-bromosuccinimide, benzoyl peroxide, CCl_4; **(c)** diethyl 2-methylmalonate, NaH, THF; **(d)** NaOH, EtOH 50%; H_2SO_4; **(e)** heat.

Preparative separation of carboxyibuprofen stereoisomers This was carried out using the Chiralpak AD CSP (see above). The mobile phase consisted of hexane:methanol:ethanol (95:3.5:1.5, v/v) containing TFA (0.05%, v/v). The flow rate used was 1.0 ml/min at ambient temperature. Detection was by UV at 220 nm.

Flash chromatography Flash chromatography was carried out on silica gel (60 mesh; Merck 9385) using gradient elution with increasing proportions of ethyl acetate in petroleum ether as the mobile phase unless otherwise stated.

Thin-layer chromatography (TLC) TLC was carried out using silica gel G/UV_{254}, 0.25 mm thick, coated microplates (Camlab, Cambridge, UK). The chromatograms were developed using varying mixtures of ethyl acetate in petroleum ether depending on the analyte. The spots on the TLC plate were visualized by examination under UV light (254 nm).

Administration of (S)-Ibuprofen and Isolation of Carboxyibuprofen

A healthy male volunteer (36 yr old, weight 59 kg) received (*S*)-ibuprofen (50 mg; filled into a size 5 gelatin capsule) in the morning on an empty stomach. All urine voided up to 6 h post-drug administration was collected into plastic bottles and stored at −20°C prior to analysis.

An aliquot of the pooled urine sample (0.5 ml) was acidified by adding hydrochloric acid (1.0 M; 100µl), buffered to pH 3.8 with 1.5 ml phosphate buffer (pH 3.8, 1.0 M), and extracted with dichloromethane:ethyl acetate (14:1, v/v; 5 ml). After phase separation by centrifugation (100*g,* 5 min), the lower organic layer was separated and evaporated under a gentle stream of nitrogen at 40°C on a dry heating block. The residue was then reconstituted in 150 µl HPLC mobile phase and injected onto the Partisil silica column (mobile phase, hexane:ethanol, 98.2:1.8, v/v with 0.05% v/v TFA, at a flow rate of 2 ml/min and detection at 220 nm). Under these conditions, carboxyibuprofen eluted with a retention time of 12.6 min. The fraction eluting between 12.2 and 13.0 min was collected and the solvent evaporated under nitrogen at 40°C. The residue was reconstituted in mobile phase (100 µl; hexane:ethanol, 92:8, v/v containing 0.05% v/v TFA) and examined by chiral phase HPLC.

SYNTHESIS

*Synthesis of (2′*R,S, *2*R,S)-Carboxyibuprofen (Fig. 2)*

Diethyl 2-methyl-2-(4-methylphenyl)malonate (5) To a solution of sodium ethoxide, prepared from sodium (12.74 g, 0.554 mol) and superdry ethanol (235 ml), was

added ethyl acetate (dried over calcium sulfate; 2.5 ml) and the mixture held at 60°C for 30 min. Diethyl 2-p-tolylmalonate **(4)** (125.4 g, 0.501 mol) and superdry ethanol (20 ml) was placed in a 1-liter three necked flask fitted with a sealed mechanical stirrer, a tap funnel, a calcium chloride guard tube, a nitrogen inlet, and a condenser fitted with a calcium chloride guard tube leading to a water seal. The apparatus was purged with dry nitrogen (250 ml/min for 15 min) and the flow then stopped. Methyl iodide (39 ml, 0.625 mol) was added to the mixture, which was then stirred, heated to 80°C, and the sodium ethoxide solution dropped in over 4 h. After the addition of further methyl iodide (2 ml), heating was continued for a further 1 h. The mixture was cooled under a nitrogen purge and then left overnight, the condenser stoppered. After removal of the ethanol by distillation, water (150 ml) and glacial acetic acid (5 ml) were added and the product extracted with ether (200 ml). The ethereal solution was washed successively with water (50 ml), 0.1 M sodium thiosulfate (50 ml), and finally water (3 × 50 ml). The solution was then dried ($MgSO_4$) overnight. Removal of the desiccant and solvent was followed by distillation of the product in vacuo to give 124.7 g (94%) of a colorless oil b.p. 130–132° (mainly 131–131.5°) at 1.5 mm; NMR: (90 MHz, $CDCl_3$), δ ppm, 1.22 (t, 6H, CH_2CH_3), 1.82 (s, 3H, C-CH_3), 2.31 (s, 3H, ArCH_3), 4.21 (q, 4H, CH_2CH_3), 7.20 (m, 4H, ArH). Elemental analysis: found (%), C, 67.8; H, 7.8; required (%) for $C_{15}H_{20}O_4$: C, 68.2; H, 7.6.

Diethyl 2-(4-bromomethylphenyl)-2-methylmalonate (6) A mixture of the malonate ester **(5)** (15.86 g, 60 mmol), N-bromosuccinimide (10.68 g, 60 mmol), and dry benzoyl peroxide (60 mg) in CCl_4 (55 ml) was stirred and gently refluxed for 3.5 h. After this time the mixture was allowed to cool and water (50 ml) was added. The organic layer was washed with sodium bicarbonate solution (250 mg in 50 ml water) followed by water (2 × 50 ml) and was then dried with anhydrous $MgSO_4$. Removal of the desiccant and solvent yielded 21.3 g (103%; theoretical 20.6 g) of the required bromo compound **(6)** as a pale straw-colored liquid, which was used without further purification.

Tetraethyl 2-[4-(2,2-dicarboxypropyl)phenyl]-2-methylmalonate (7) The apparatus (250 ml conical flask fitted with a three way adapter carrying a condenser, a filter stick or tap funnel, a nitrogen inlet, and the requisite calcium chloride guard tubes) was purged with dry nitrogen. Sodium hydride in oil (60%; 2 g, 50 mmol) was placed in the flask and washed with petroleum ether (dried over calcium hydride; 3 × 15 ml). Anhydrous tetrahydrofuran (THF) (30 ml) was added to the washed hydride followed by dropwise addition of diethyl 2-methylmalonate (8.6 ml, 50 mmol) to the magnetically stirred mixture. The mixture was refluxed and the bromoester **(6)** (17.58 g, 51.2 mmol) dissolved in dry THF (27 ml) added dropwise over 30 min. After continuing reflux for a further 1.5 h, the mixture was cooled, glacial acetic acid (2 ml) added, and the solvent removed. The cooled residue was treated with water (30 ml) and ether (50 ml). The ethereal layer was washed thrice with water (20 ml) containing sodium bicarbonate (1 g) followed by water (4 × 20 ml) and then dried (magnesium sulfate). Removal of the desiccant and solvent left an orange oil (21.49 g) which was distilled in vacuo to yield a faintly straw-colored oil (16.27 g) b.p. 200–216° (mainly 208–211°) at 1.2 mm. The oil was dissolved in warm hexane (40 ml), the solution allowed to cool till slightly hazy and then cooled in ice with vigorous shaking until crystallization was achieved. After 1 h the crystals were collected, washed with ice-cold hexane (3 × 10 ml), and dried in vacuo to give white microcrystals (11.45 g; 52%), m.p. 42–45.5°C; NMR: (90 MHz, $CDCl_3$), δ ppm, 1.22 (t, 12H, CH_2CH_3), 1.33 (s, 3H, CH_2CCH_3), 1.81 (s, 3H, CCH_3), 3.21 (s, 2H, CH_2CCH_3), 4.12 (dq, 8H, CH_2CH_3), 7.2 (m, 4H, ArH). Elemental analysis: found (%), C, 62.97; H, 7.33; required (%) for $C_{23}H_{32}O_8$; C, 63.28, H, 7.40.

2-[4-(2,2-Dicarboxypropyl)phenyl]-2-methylmalonic acid (8) A mixture of the tetraethyl ester **(7)** (8.6 g) and sodium hydroxide (6.3 g) in 50% ethanol (100 ml) was stirred magnetically and refluxed for 10 h. The ethanol was removed by distillation and the aqueous residue filtered. Acidification of the ice-cold filtrate with concentrated sulfuric acid (6 ml) gave a very fine precipitate, which was collected, washed with ice-cold water until free of sulfate and then dried (100°C). Yield: 4.81 g (75.3%); m.p. 147.5°C decomp. Crystallization of 2.5 g from 2 M hydrochloric acid gave 2.15 g (86%) of white, very fine needles; m.p. 147–150°C decomp; NMR: (90 MHz, d_6-DMSO), δ ppm, 1.19 (s, 3H, CH_2CCH_3), 1.70 (s, 3H, CCH_3), 3.09 (s, 2H, CH_2CCH_3), 7.25 (m, 4H, ArH), 9.1 (br. s, 4H, COOH). Elemental analysis: found (%), C, 52.45; H, 5.14; required (%) for $C_{15}H_{16}O_8.H_2O$: C, 52.62; H, 5.31.

(2′*R,S*,2*R,S*)-2-[-4-(2-carboxypropyl)phenyl]propionic acid (2) [(2′*RS*,2*RS*)-carboxyibuprofen] Crude tetracarboxylic acid **(8)** (4.83 g) was heated to 160°C (oil bath) during 1 h and held at 160–164°C for 80 min to give 3.35 g (95.2%) of a yellowish solid when cold. Three crystallizations of 2.81 g from 15% v/v acetic acid (about 20 ml) gave 1.91 g (68%) of white, minute crystals, m.p. 114–119°C; NMR: (300 MHz, $CDCl_3$), δ ppm, 1.22 (dd, 3H, CH_2CHCH_3), 1.48 (dd, 3H, $CHCH_3$), 2.76 (complex m, 2H, CH_3CHCH_2), 2.96 (complex m, 1H, CH_3CHCH_2), 3.69 (dq, 1H, $CHCH_3$), 7.23 (complex m, 4H, ArH), 11.6 (br. s, ~2H, COOH); MS (EI, 70 eV), *m/z* (relative intensity, %): 236 (M^+; 22), 192 (5), 191 (M^+ –COOH; 72), 164 (5), 163 (100), 145 (21), 119 (10), 118 (34), 117 (59), 107 (29), 91 (29), 77 (5), 45 (24). Elemental analysis: found (%), C, 66.04; H, 6.88; required (%) for $C_{13}H_{16}O_4$: C, 66.08; H, 6.84.

Synthesis of the Dimethyl, Diethyl, and Diisopropyl Esters of Carboxyibuprofen

To (2′*R,S,* 2*R,S*)-carboxyibuprofen **(2)** (250 mg) was added acidified methanol (10 ml, prepared by passing HCl gas through methanol (100 ml) until an increase

in weight of about 3.6 g was noted) and the whole heated under reflux for 2 h. After this time the mixture was diluted with water (10 ml) and extracted with diethyl ether (3 × 10 ml). The combined ether extracts were dried (Na_2SO_4:$NaHCO_3$, 4:1, w/w), and filtered and evaporated to dryness to yield a colorless oily residue. NMR: (90 MHz, $CDCl_3$), δ ppm, 1.13 (d, 3H, CH_2CHCH_3), 1.45 (d, 3H, $CHCH_3$), 2.67 (m, 2H, CH_2CHCH_3), 2.90 (m, 1H, CH_2CHCH_3), 3.61 (s, 6H, $COOCH_3$), 3.68 (q, 1H $CHCH_3$), 7.17 (dd, 4H, ArH).

The same procedure was repeated using ethanol and isopropanol for the synthesis of the corresponding diesters. Diethyl carboxyibuprofen, NMR: (90 MHz, $CDCl_3$), δ ppm, 1.17 (d, dt, 9H, CH_3CHCH_2; CH_3CH_2O), 1.41 (d, 3H, CH_3CHCO), 2.75 (m, 3H, CH_3CHCH_2), 3.65 (q, 1H, CH_3CHCO), 4.18 (dq, 4H, CH_2CH_3), 7.17 (dd, 4H, ArH). Diisopropyl carboxyibuprofen, NMR: (90 MHz, $CDCl_3$), δ ppm, 1.13 (d, 15H, CH_3CHCH_2; 4d, $(CH_3)_2CHO$), 1.44 (d, 3H, CH_3CH), 2.6–3.0 (complex m, 3H, CH_3CHCH_2), 3.62 (q, 1H, CH_3CHCO), 4.98 (m, 2H, $(CH_3)_2CHO$), 7.17 (dd, 4H, ArH).

Synthesis of (2′S, 2R,S)-Carboxyibuprofen Diastereoisomers (see Fig. 5)

(4*R*,5*S*)-4-methyl-5-phenyl-3-propanoyl-2-oxazolidinone (9) A 100 ml one necked round bottom flask containing a stirred solution of (4*R,* 5*S*)-4-methyl-5-phenyl-2-oxazolidinone (1.0 g, 5.64 mmol) in dry THF (20 ml) under an atmosphere of nitrogen was immersed in a dry ice/industrial methylated spirit bath (–72°C). After 15 min, butyllithium (4.0 ml of a 1.6 M solution in hexane, 1 eq) was added via syringe. Propionyl chloride was added 15 min later and the mixture was maintained in the cold bath for 30 min, after which time the mixture was allowed to warm to room temperature. Evaporation of the solvent and flash chromatography of the residue afforded 1 g (76%) of the required product as an oil. NMR: (360 MHz, $CDCl_3$), δ ppm, 0.9 (3H, d, J 6.6 Hz, CH_3), 1.18 (3H, t, J 7.3 Hz, CH_3), 2.97 (2H, m, CH_2), 4.76 (1H, quintet, J 6.6 Hz, CH), 5.67 (1H, d, J 7.3 Hz, CH), 7.35 (5H, m, ArH).

4-Iodobenzyl bromide (10) A mixture of 4-iodotoluene (4.5 g, 20.73 mmol), N-bromosuccinimide (4 g), and benzoyl peroxide (50 mg) in CCl_4 was heated under reflux for 4 h. The reaction mixture was then cooled and the solvent removed by evaporation. Diethyl ether was added to the residue and the precipitated succinimide filtered off. The filtrate was stripped of solvent to afford a white solid which is a mixture of 4-iodotoluene and the required 4-iodobenzyl bromide **(10).** A pure sample of **10** was obtained after several crystallizations from petroleum ether, until TLC showed a single spot. The reaction afforded 2.64 g (42%) of a white solid, m.p. 58–62°C; NMR: (360 MHz, $CDCl_3$), δ ppm, 4.42 (2H, s, CH_2), 7.13 (2H, d, J 8.3 Hz, ArH), 7.67 (2H, d, J 8.3 Hz, ArH); ^{13}C-NMR: (90 MHz, $CDCl_3$), δ ppm, 32.59, 128.26, 130.91, 137.45, 138.00; MS (EI) *m/z* (relative intensity, %): 298 (M^+, 25), 296 (25), 217 (100), 90 (16); HRMS calculation for C_7H_6IBr (M^+) 295.8700, found 295.8701.

(2′*S*,4*R*,5*S*)-4-methyl-5-phenyl-3-[2′-(4-iodophenylmethyl)]propanoyl-2-oxazolidinone (11) LDA (2M) (1.3 ml, 1.1 eq) was added to a stirred solution of **9** (0.54 g, 2.32 mmol) in dry THF (10 ml) maintained at –78°C under dry nitrogen. The solution gained a yellow color. After 10 min, 4-iodobenzyl bromide **(10)** (4 g, 6 eq) was added as a solution in THF (5 ml). The mixture was kept at –78°C for 30 min and then brought to 0°C and maintained at this temperature for 1 h. After this time, the reaction mixture was poured into water (50 ml) and extracted with ethyl acetate (2 × 75 ml). Evaporation of the solvent led to a residue which was purified by flash chromatography to afford the expected product **(11)** as a yellow oil (0.97 g; 93%). NMR: (360 MHz, $CDCl_3$), δ ppm, 0.75 (3H, d, J 6.61 Hz, CH_3), 1.17 (3H, d, J 6.7 Hz, CH_3), 2.57 (1H, dd, J 8.01 Hz, 13.3 Hz, CH), 3.06 (1H, dd, J 6.7 Hz, 13.3 Hz, CH), 4.08 (1H, m, CH), 4.77 (1H, m, CH), 5.65 (1H, d, J 7.43 Hz, CH), 7.02 (2H, d, J 8.3 Hz, ArH), 7.4 (5H, m, ArH), 7.59 (2H, d, J 8.3 Hz, ArH); MS (EI) *m/z* (relative intensity, %): 449 (M^+, 100), 369 (12), 306 (16), 244 (56), 217 (29), 150 (33), 118 (25); HRMS calculation for $C_{20}H_{20}O_3NI$ (M^+) 449.0490, found 449.0514.

(2*S*)-methyl 2-methyl-3-(4-iodophenyl)propanoate (12) Butyllithium (2 ml of a 1.6 M solution in hexane, 1 eq) was added dropwise to anhydrous methanol (15 ml) cooled to 0°C under nitrogen. The clear solution thus obtained was transferred to a solution of **11** (0.91 g, 2.02 mmol) in anhydrous methanol. TLC showed no starting material left after 30 min at 0°C. The reaction mixture was poured into water, neutralized by the addition of HCl (1 M), and extracted with dichloromethane (3 × 70 ml). After drying (Na_2SO_4) and evaporation of the solvent, the colorless oil was chromatographed to afford 0.41 g (70%) of the expected product **(12);** NMR: (360 MHz, $CDCl_3$), δ ppm, 1.40 (3H, d, J 6.90 Hz, CH_3), 2.86 (1H, dd, J 7.44 Hz, 14.0 Hz, CH), 2.95 (1H, m, $CHCH_3$), 3.2 (1H, dd, J 7.08 Hz, 14.19 Hz, CH), 3.88 (3H, s, CH_3), 7.14 (2H, d, J 8.3 Hz, ArH), 7.84 (2H, d, J 8.3 Hz, ArH); MS (EI) *m/z* (relative intensity, %): 305 (M^+ 45), 304 (45), 244 (38), 217 (100), 117 (20), 90 (36); HRMS calculation for $C_{11}H_{13}O_2I$ (M^+) 303.9962, found 303.9980.

Methyl 2-tributyltinacrylate (13) Tributyltin hydride (1 ml, 3.72 mmol) was added dropwise to a suspension of methyl propiolate (0.32 g, 3.8 mmol) and catalyst Pd $(PPh_3)_2Cl_2$ (20 mg) in dry THF (10 ml). After 1 h the reaction went to completion. The reaction mixture was impregnated on silica and chromatographed (diethyl ether 5% v/v in petroleum ether 60–80°C) to afford 1.15 g (79%) of the expected product **(13)** as an oil. NMR: (360 MHz, $CDCl_3$), δ ppm, 0.88 (9H, t, J 7.26 Hz, 3 × CH_3), 0.97 (6H, m, 3 × CH_2), 1.30 (6H, sextet, J 7.2 Hz, 3 × CH_2), 1.48 (6H, m, 3 × CH_2), 3.73 (3H, s, OCH_3), 5.92 (1H, d, J 2.7 Hz, =CH), 6.89 (1H, d, J 2.7 Hz, =CH); ^{13}C-NMR: (90 MHz, $CDCl_3$), δ ppm, 9.87 (CH_2), 13.54 (CH_3), 27.09 (CH_2), 28.76 (CH_2), 51.63 (OCH_2), 139.78 (CH), 145.72 (C), 170.86 (CO); MS (EI) *m/z* (relative intensity, %): 375 (M^+, 0.16), 319 ([M-Bu]$^+$, 100), 317 (82), 289 (10), 265 (18), 263 (20), 233 (8), 231 (5), 205 (9), 177 (15).

(2S)-dimethyl 2-methyl-3-[4-(2-propenoyl)phenyl]propanoate (14) Methyl 2-tributyltinacrylate **(13)** (1.77 g, 2.5 eq) was dissolved in dimethylformamide (DMF) (10 ml) and added via syringe to a solution of **12** (0.54 g, 1.86 mmol) in dry DMF (10 ml) containing copper(I) iodide (0.265 g, 0.75 eq) and $Pd(PPh_3)_4$ (0.214 g) under dry nitrogen. The mixture turned black and stirring was continued under dry nitrogen for 2 days. The black suspension was diluted with diethyl ether and filtered through celite. The filtrate was washed with water (2 × 50 ml), brine (2 × 50 ml), and dried over Na_2SO_4. Evaporation of the solvent and chromatography on the residue afforded 0.49 g of a caramel oil. NMR analysis of the product showed the presence of a 2:1 mixture of the required product **14** and the diene **20** (Fig. 6). Therefore, the yield was estimated as 0.31 g (71%). NMR: (360 MHz, $CDCl_3$), δ ppm, 1.16 (3H, d, J 6.80 Hz, CH_3), 2.66 (1H, m, CH), 2.74 (1H, m, CH), 3.04 (1H, m, CH), 3.65 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 5.88 (1H, d, J 1.22 Hz, =CH), 6.32 (1H, d, J 1.22 Hz, =CH), 7.15 (2H, d, J 8.28 Hz, ArH), 7.33 (2H, d, J 8.28 Hz, ArH). In addition, the following signals due to the diene **(20)** impurity were also discernible: 3.76 (6H, s, OCH_3), 5.81 (2H, d, J 1.3 Hz, CH), 6.28 (2H, d, J 1.3 Hz, CH).

(2′S, 2R,S)-dimethyl 2-[4-(2-carboxypropyl)phenyl] propionate (15) Pd/C (0.4 g; 10% w/w) was added to a solution of **14** (0.49 g) containing a mixture of **14** and the diene **20** in ethanol. The reaction was kept under hydrogen for 24 h until 45 ml was consumed. Removal of the catalyst and evaporation of the solvent afforded 0.45 g of an oil that appeared to be a mixture of the required product **(15)** and the reduced diene, dimethyl succinate. NMR: (360 Mz, $CDCl_3$), δ ppm, 1.15 (3H, t, CH_3), 1.47 (3H, t, CH_3), 2.67 (2H, m, CH_2), 3.00 (1H, q, CH), 3.64 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 3.70 (1H, m, CH), 7.12 (2H, d, ArH), 7.22 (2H, d, ArH). In addition, the following signals due to the dimethyl succinate impurity were also discernible: 1.27 (6H, d, 2 × CH_3), 4.12 (2H, q, 2 × CH), 3.65 (6H, s, 2 × OCH_3). MS (EI) *m/z* (relative intensity, %): 264 ($M^{\ddagger}$, 48), 233 (14), 205 (100), 177 (69), 145 (55), 177 (31), 91 (12); HRMS calculation for $C_{15}H_{20}O_4$ ($M^{\ddagger}$) 264.1362, found 264. 1360.

(2′S,2R,S)-2-[4-(2-carboxypropyl)phenyl]propionic acid (2) [(2′S,2R,S)-carboxyibuprofen] Sodium hydroxide 2 M (2 ml) was added to a stirred solution of **15** (mixture of the 2 products obtained in reaction from the synthesis of **15)** (0.45 g) in methanol (5 ml). The mixture was cooled to 0°C and allowed to react for 4 h. After this time further NaOH (2 M, 2 ml) was added. The aqueous layer was extracted with dichloromethane to remove the unreacted starting material, acidified (HCl, 1 M), and extracted with dichloromethane. Drying (Na_2SO_4) and evaporation of solvent afforded 0.16 g (50%) of the required product **15.** NMR: (360 MHz, $CDCl_3$), δ ppm, 1.17 (3H, d, J 6.75 Hz, CH_2CHCH_3), 1.50 (3H, d, J 7.1 Hz, $CHCH_3$), 2.65 (1H, m, ArCH(H)), 2.76 (1H, m, ArCH(H)), 3.03 (1H, m, CH_3CHCH_2), 3.7 (1H, m, $CHCH_3$), 7.15 (2H, d, J 8.0 Hz, ArH), 7.24 (2H, d, J 8.0 Hz, ArH) 11.09 (2H, br s, CO_2H); ^{13}C-NMR: (90 MHz), δ ppm, 16.85 (CH_3), 18.0 (CH_3), 38.8 (CH_2), 41.2 (CH), 45.11 (CH), 127.66 (aromatic CH), 129.3 (aromatic CH), 137.8 (aromatic C), 138.3 (aromatic C), 181.0 (C=O), 182.6 (C=O); MS (EI) *m/z* (relative intensity, %): 236 ($M^{\ddagger}$, 54), 219 (6), 191 (91), 163 (100), 145 (19), 117 (34), 107 (21), 91 (14); HRMS calculation for $C_{13}H_{16}O_4$ ($M^{\ddagger}$) 236.1048, found 236.1022.

Synthesis of (2′R,2R,S)-Carboxyibuprofen Diastereoisomers (see Fig. 6)

(2′R,4S)-4-isopropyl-3-[2′-(4-iodophenylmethyl)]propanoyl-2-oxazolidinone (17) LDA (2 M) (3.0 ml, 1.2 eq) was added to a stirred solution of (4*S*)-4-isopropyl-2-oxazolidinone **(16)** (0.90 g, 4.87 mmol) in dry THF (20 ml) maintained at −78°C under dry nitrogen. The solution gained a cherry red color. After 10 min, 4-iodobenzyl bromide **(10)** (1.80 g, 1.2 eq) was added as a solution in THF (5 ml). The solution was kept at −78°C for 30 min and then brought to 0°C and maintained at this temperature for 1 h. The reaction mixture was poured into water (50 ml) and was extracted with ethyl acetate (2 × 75 ml). Evaporation of the solvent led to a residue which was chromatographed to afford the required product **(17)** as a colorless oil (1.36 g; 70%), NMR: (360 MHz, $CDCl_3$), δ ppm, 0.61 (3H, d, J 7.0 Hz, isopropyl CH_3), 0.85 (3H, d, J 7.0 Hz, isopropyl CH_3), 1.15 (3H, d, J 7.0 Hz, CH_3), 2.14 (1H, hept, J 7.0 Hz, $CHMe_2$), 2.58 (1H, dd, J 7.5 Hz, 13.2 Hz, ArCH), 3.06 (1H, dd, J 7.5 Hz, 13.2 Hz, ArCH), 4.10–4.27 (3H, m, 4-CH and 5-CH_2), 4.42 (1H, m, CHCO), 7.42 (2H, d, J 8.3 Hz, ArH), 7.59 (2H, d, J 8.3 Hz, ArH).

(2R)-benzyl 2-methyl-3-(4-iodophenyl)propanoate (18) Butyllithium (1.6 M) (3.5 ml, 5.6 mmol) was added dropwise to a solution of anhydrous benzyl alcohol (0.75 g, 6.95 mmol) in dry THF (10 ml) cooled to 0°C under nitrogen. The clear solution obtained was transferred to a solution of **17** (1.35 g, 3.36 mmol) in anhydrous THF (5 ml). TLC analysis showed no starting material remaining after 30 min at 0°C. The reaction mixture was poured into hydrochloric acid (1 M, 15 ml) and extracted with dichloromethane (3 × 70 ml). After drying (Na_2SO_4) and evaporation of the solvent, the colorless oil was chromatographed to afford the starting oxazolidinone **(16)** (0.45 g; 100%) as well as the required product **18** (1.29 g; 98%), as a yellow oil. NMR: (360 MHz, $CDCl_3$), δ ppm, 1.22 (3H, d, J 7.0 Hz, CH_3), 2.67 (1H, dd, J 7.0 Hz, 13.2 Hz, ArCH(H)CH), 2.80 (1H, m, CHCO), 2.94 (1H, dd, J 7.0 Hz, 13.2 Hz, ArCH(H)CH), 5.15 (2H, s, $PhCH_2$), 6.88 (2H, d, J 8.3 Hz, ArH), 7.20–7.40 (5H, m, ArH), 7.56 (2H, d, J 8.3 Hz, ArH).

(2R)-benzyl methyl 2-methyl-3-[4-(2′-propenoyl) phenyl]propanoate (19) Methyl 2-tributyltinacrylate **(13)** (2.8 g, 7.33 mmol, 2.3 eq) was added via syringe to a solution of **18** (1.20 g, 3.16 mmol) in dry DMF (10 ml) containing copper(I) iodide (0.45 g, 2.34 mmol, 0.75 eq) and $Pd(PPh_3)_4$ (0.36 g, 0.32 mmol) under dry nitrogen. The mixture turned black and stirring was continued under nitrogen for 2 days. The black suspension was diluted with diethyl ether and filtered through celite. The filtrate was washed with water (2 × 50 ml), brine

(2 × 50 ml), and dried over Na_2SO_4. Evaporation of the solvent and flash chromatography of the residue afforded the required product **19** (0.45 g; 42%) as an oil as well as unreacted starting material **(18)** (0.6 g; 50% recovery). NMR: (360 MHz, $CDCl_3$), δ ppm, 1.19 (3H, d, J 7.0 Hz, CH_3), 2.70 (1H, dd, J 7.0 Hz, 13.2 Hz, ArCH(H)CH), 2.81 (1H, m, CHCO), 3.04 (1H, dd, J 7.0 Hz, 13.2 Hz, ArCH-(H)CH), 3.82 (3H, s, OCH_3), 5.08 (2H, s, $PhCH_2$), 5.87 (1H, d, J 1.1 Hz, =CH), 6.33 (1H, d, J 1.1 Hz, =CH), 7.12–7.36 (9H, m, ArH).

(2′*R*,2*R*,*S*)-2-[4-(2-carboxypropyl)phenyl]propionic acid (2) [(2′*R*,2*R*,*S*)-carboxyibuprofen] Pd/C (0.4 g, 10% w/w) was added to a solution **19** (0.42 g) in ethanol (25 ml) and ethyl acetate (10 ml). The reaction mixture was kept under hydrogen until no more gas was consumed. Removal of the catalyst and evaporation of the solvent afforded a gum that was suspended in sodium hydroxide (2 M, 2 ml) at 0°C for 10 min. The aqueous solution was extracted with dichloromethane to remove the unreacted starting material, and then acidified (HCl, 1 M) and extracted with dichloromethane. Drying (Na_2SO_4) and evaporation of solvent afforded 0.15 g (48%) of the required product **2,** m.p. 116–122°C; NMR: (360 MHz, d_6-DMSO), δ ppm, 1.02 (3H, d, J 6.5 Hz, CH_2CHCH_3), 1.32 (3H, d, J 7.1 Hz, $CHCH_3$), 2.49–2.63 (2H, m, $ArCH_2$), 2.86 (1H, m, CH_2CHCH_3), 3.61 (1H, q, J 7.0 Hz, ArCH), 7.12 (2H, d, J 8Hz, ArH), 7.18 (2H, d, J 8Hz, ArH), 11.5 (2H, br.s, CO_2H); ^{13}C-NMR: (90 MHz, d_6-DMSO), δ ppm, 16.68 (CH_3), 18.63 (CH_3), 38.67 (CH_2), 40.7 (CH), 44.4 (CH), 127.35 (aromatic CH), 129.05 (aromatic CH), 138.17 (aromatic C), 139.08 (aromatic C), 175.6 (CO), 176.99 (CO); MS (EI) *m/z* (relative intensity, %): 236 (M^+, 39), 191 (92), 163 (100), 147 (36), 145 (34), 119 (85), 117 (87), 107 (41), 91 (86); HRMS calculation for $C_{13}H_{16}O_4$ (M^+) 236.1048, found 236.1053.

(2*R*)-2-methyl-3-phenylpropionic acid (21, Fig. 6) The title compound prepared by the method of Evans et al.,[21] from the oxazolidinone **16,** yielded an NMR spectrum consistent with the structure. The stereochemical purity of **21** was determined to be *R*:*S* 91:9 by derivatization with (*R*)-1-(naphthen-1-yl)ethylamine[17] followed by reversed phase HPLC (column, C_{18}, 150 × 3.9 mm, 5 μm; mobile phase, acetonitrile:phosphate buffer (0.01 M, pH 3.5), 50:50, v/v; flow rate 1.5 ml/min; detection, UV, λ = 220 nm).

RESULTS AND DISCUSSION

Synthesis of "Racemic" Carboxyibuprofen

The synthesis of "racemic" carboxyibuprofen was carried out according to the scheme outlined in Figure 2. The synthesis of diethyl 2-methyl-2-(4-methylphenyl)malonate **(5)** was adapted from that presented in Furniss et al.[16] for the preparation of the corresponding 2-ethyl derivative. During the initial studies the synthesis was attempted without the use of a nitrogen atmosphere which resulted in the repeated formation of iodine during the purification of the product. Treatment of **5** with N-bromosuccinimide in the presence of benzoyl peroxide resulted in the formation of crude **6,** which was subsequently used for the synthesis of **7** without further purification. Attempts to purify **6** by vacuum distillation were unsuccessful, yielding a product which gave unsatisfactory elemental analyses. Treatment of crude **6** with diethyl 2-methylmalonate in the presence of sodium hydride yielded the tetraethyl ester **7.** An alternative reaction pathway involving treatment of **6** with diethyl methylmalonate and sodium ethoxide resulted in a lower yield (44%). Hydrolysis of **7** resulted in the formation of the tetra acid **8,** which on heating at about 160°C for 80 min yielded the required diacid **2,** carboxyibuprofen, in approximately 21% overall yield with respect to the starting material **4.**

Chromatographic Resolution

Following the reported success of Rudy et al.,[15] initial attempts to resolve the stereoisomers of carboxyibuprofen **(2)** were based on the indirect approach. The racemic mixture of the diacid **2** was derivatized with (*R*)-1-(naphthen-1-yl)ethylamine, using 1-hydroxybenzotriazole and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide as coupling agents,[17] and chromatography was carried out using a reversed phase (C_{18}) stationary phase and a mobile phase consisting of a 50:50 (v/v) mixture of acetonitrile:phosphate buffer (0.1 M, pH 3.5) run at a flow rate of 1.5 ml/min. These attempts, however, proved to be unsuccessful, two of the isomeric diamide derivatives coeluting. Variation of the stationary phase and/or mobile phase composition did not result in any marked improvement (data not shown). As the resolution of stereoisomers with a carboxyl group bonded to the chiral center has been reported, following esterification, using the derivatized polysaccharide CSPs[18,19] a range of ester derivatives (dimethyl, diethyl, diisopropyl) was prepared and examined using the derivatized amylose CSP, Chiralpak AD. The dimethyl ester derivative gave near baseline resolution of all four stereoisomers using a mobile phase of hexane:ethanol (99.5:0.5 v/v). However, increasing the size of the ester alkyl group resulted in a reduction in analyte retention and resolution (Fig. 3) with, in the case of the diisopropyl derivative, only three peaks being observed in the chromatogram. It is therefore apparent that increasing both the lipophilicity and steric bulk of the ester functionality reduced both analyte retention and resolution.

Chromatographic resolution of the free diacids was achieved by modification of the mobile phase composition (hexane:ethanol 92:8 v/v) and the addition of TFA (0.05% v/v) to improve peak symmetry. Baseline resolution of the four stereoisomers was achieved in an overall run time of 21.5 min (Fig. 4). For semipreparative isolation of the diacids the mobile phase composition was further modified by the addition of methanol (final composition, hexane:methanol:ethanol, 95:3.5:1.5 by volume containing TFA 0.05% v/v), in order to improve the resolution. Multiple injections (30) of 0.5 mg material on-column per injection resulted in the isolation of approximately 3 mg of each stereoisomer. The stereochemical purity of each sample was determined by reinjection onto the CSP, using the

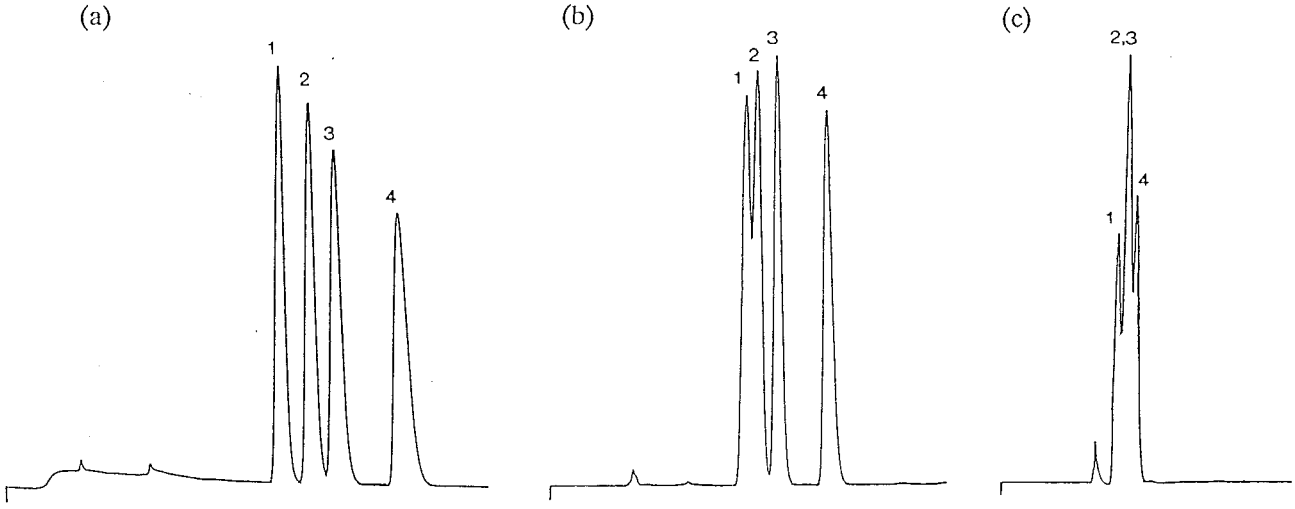

Fig. 3. Chromatographic resolution of carboxyibuprofen diesters using a Chiralpak AD CSP. **a:** Dimethyl ester; retention times, peak 1: 12.2 min; peak 2: 13.5 min (α = 1.16, R_s = 1.1); peak 3: 14.6 min (α = 1.12, R_s = 1.1); peak 4: 17.6 min (α = 1.27, R_s = 1.72). **b:** Diethyl ester; retention times, peak 1: 7.6 min; peak 2: 7.9 min (α = 1.13, R_s = 0.6); peak 3: 9.7 min (α = 1.13 R_s = 0.8); peak 4: 12.1 min (α = 1.32, R_s = 1.9). **c:** Diisopropyl ester; retention times, peak 1: 5.0 min; peak 2: 5.3 min (α = 1.45, R_s = 0.52); peak 3: 5.3 min (α = 1.0, R_s = 0); peak 4: 5.6 min (α = 1.20, R_s = 0.46).

original mobile phase composition, which was found to be greater than 99% based on peak area measurements (for a typical chromatogram see Fig. 4).

In order to determine the elution order of the carboxyibuprofen stereoisomers, the two diastereoisomeric metabolites isolated from the urine of a volunteer following the oral administration of (*S*)-ibuprofen were examined. Chromatographic analysis of the sample indicated the presence of the two later eluting peaks with retention times of 16.9 and 20.1 min. It was therefore concluded that the first two eluting diastereoisomers had the *R*-configuration of the propionic acid moiety and the later eluting pair had the *S*-configuration at the same chiral center. Examination of the four stereoisomers, isolated by preparative HPLC, was carried out by CD spectroscopy. However, the differences in the spectra obtained, even with a knowledge of the stereochemistry of the propionic acid moiety, were not sufficiently obvious to allow unequivocal assignment of the second chiral center (see below) and hence the chromatographic elution order. Additional synthetic approaches were therefore examined in which the stereochemistry of the metabolically introduced chiral center was defined.

Synthesis of Diastereoisomeric Mixtures of Carboxyibuprofen

The use of chiral oxazolines and oxazolidones for the enantioselective synthesis of α-substituted carboxylic acids has been reported previously,[16,20–24] and this approach was investigated for the preparation of diastereoisomeric mixtures of carboxyibuprofen. The synthesis of diastereoisomeric mixtures of carboxyibuprofen with the *S*- and *R*-configurations at the 2′-chiral center was achieved via the routes presented in Figures 5 and 6, respectively.

The (Z)-chiral enolate **(9),** prepared according to the method of Evans *et al.,*[20,21] was reacted with 4-iodobenzyl bromide **(10),** prepared by treatment of 4-iodotoluene with N-bromosuccinimide in the presence of benzoyl peroxide. The diastereoisomeric purity of the product **(11)** was high and following column chromatography the alternative diastereomer could not be detected on NMR analysis. Following alcoholysis, and recovery of the chiral auxiliary, the iodoester **(12)** was subjected to palladium-catalyzed coupling with 2-tributyltinacrylate **(13).**[25,26] Under normal conditions, such palladium-catalyzed coupling affords the corresponding cinnamates. However, addition of copper(I) iodide and using the method of Levin[27] the required product was obtained in good yield. NMR examination of the product, however, indicated contamination with the homo coupled diene (**20,** Fig. 6) which arises in the presence of copper(II) salts.[26] Hydrogenation of **14** followed by hydrolysis resulted in the required mixture of 2′*S*-carboxyibuprofen diastereomers in reasonable yield (Fig. 5).

The synthesis of the 2′*R*-diastereoisomers (Fig. 6) was carried out in a similar manner using (4*S*)-4-isopropyl-2-oxalidinone **(16)** as a chiral selector and involved the preparation of the mixed benzyl and methyl esters to chemically distinguish between the two carboxy groups in the product diester.

Chiral phase chromatographic analysis of the synthetic diastereoisomeric mixture containing (2′*S,* 2*R*)- and (2′*S,* 2*S*)-carboxyibuprofen gave two peaks with retention times of 11.0 and 20.1 min, respectively, corresponding to peaks 1 and 4 in the chromatogram of the racemic product (Fig. 4). The mixture of the 2′*R,*2*R*- and 2′*R,*2*S*-diastereoisomers yielded the corresponding peaks 2 and 3. Thus, using the synthetic compounds, together with the metabolically generated samples following oral administration of (+)-(*S*)-ibuprofen, the chromatographic elution order of the carboxyibuprofen stereoisomers was determined to be 2′*S,* 2*R* (11.0 min), 2′*R,* 2*R* (12.1 min), 2′*R,* 2*S* (16.9 min), and 2′*S,* 2*S* (20.1 min).

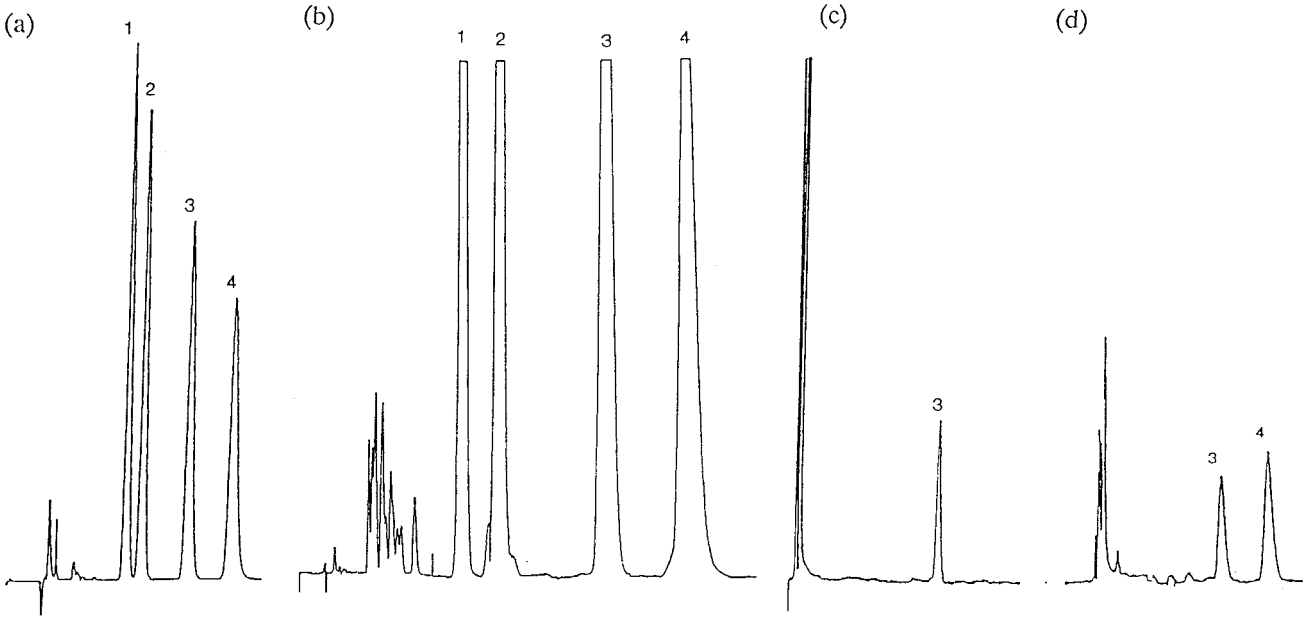

Fig. 4. a: Chromatographic resolution of carboxyibuprofen, mobile phase, hexane:ethanol (92:8 v/v) with TFA (0.05%, v/v); retention times peak 1: 11.0 min; peak 2: 12.1 min (α = 1.20, R_s = 1.3); peak 3: 16.9 min (α = 1.5, R_s = 3.3); peak 4: 20.1 min (α = 1.3, R_s = 2.4). **b:** Semipreparative resolution of carboxyibuprofen diastereoisomers, mobile phase, hexane:methanol:ethanol (95:3.5:1.5, v/v) with TFA (0.05%, v/v); retention times, peak 1: 21.2 min; peak 2: 26.2 min (α = 1.23, R_s = 2.9); peak 3: 39.4 min (α = 1.57, R_s = 7.6); peak 4: 50.2 min (α = 1.3, R_s = 4.7). **c:** Chromatographic analysis of peak 3 after semipreparative isolation; retention time, 16.9 min. **d:** Carboxyibuprofen diastereoisomers isolated from human urine following the administration of (+)-(*S*)-ibuprofen (using the system presented in a).

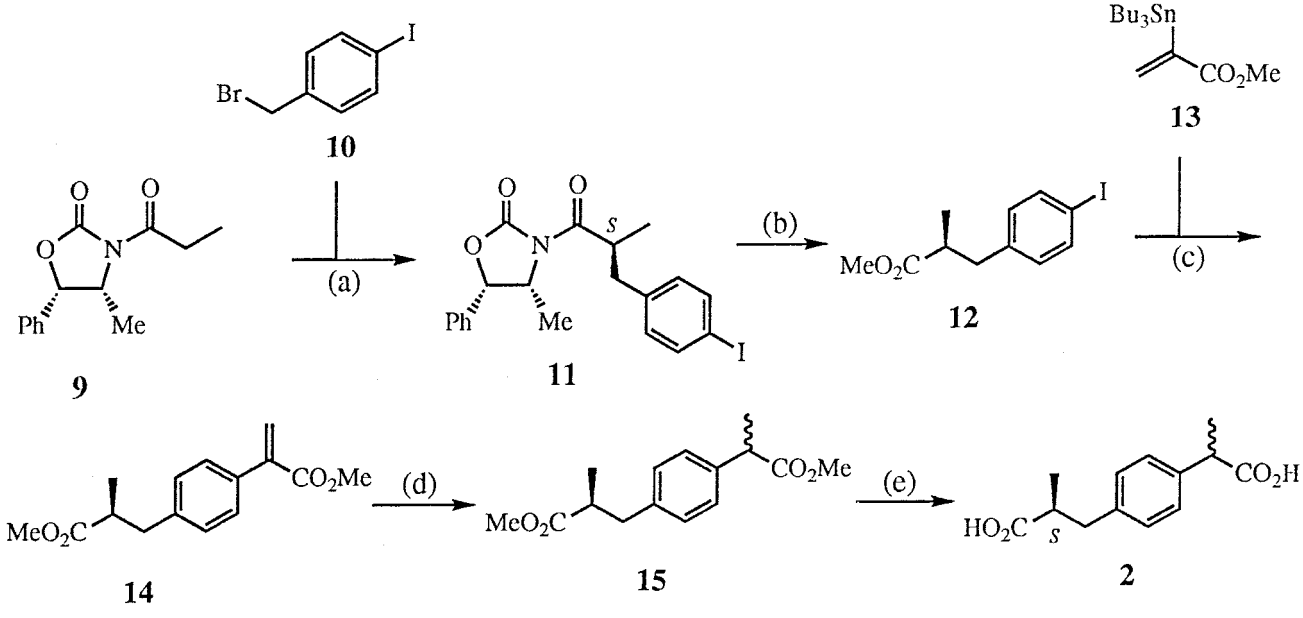

Fig. 5. Synthetic route adopted for the preparation of (2′*S*,2*R*,*S*)-carboxyibuprofen diastereoisomers **(2). (a)** LDA, THF, –78°C; **(b)** butyllithium, anhydrous MeOH, 0°C; **(c)** CuI, $Pd(PPh_3)_4$, THF; **(d)** Pd/C, EtOH; **(e)** NaOH (2 M), 0°C.

CD

The far-UV CD spectra of the enantiomers of ibuprofen exhibit bands at approximately 194 and 226 nm (Fig. 7). The near-UV spectra show a series of much weaker bands, with maxima at 259, 265, and 272 nm (data not shown). The spectra obtained for (*S*)-ibuprofen are consistent with those of related *S*-α-substituted phenylacetic acid derivatives reported in the literature.[28,29] The band at 226 nm was assigned to the n → π* transitions of the carbonyl group and those in the 250–280 nm region to ^1L_b transitions of the phenyl moiety.[28] In the case of the carboxyibuprofen stereoisomers, the main differences in the spectra were ob-

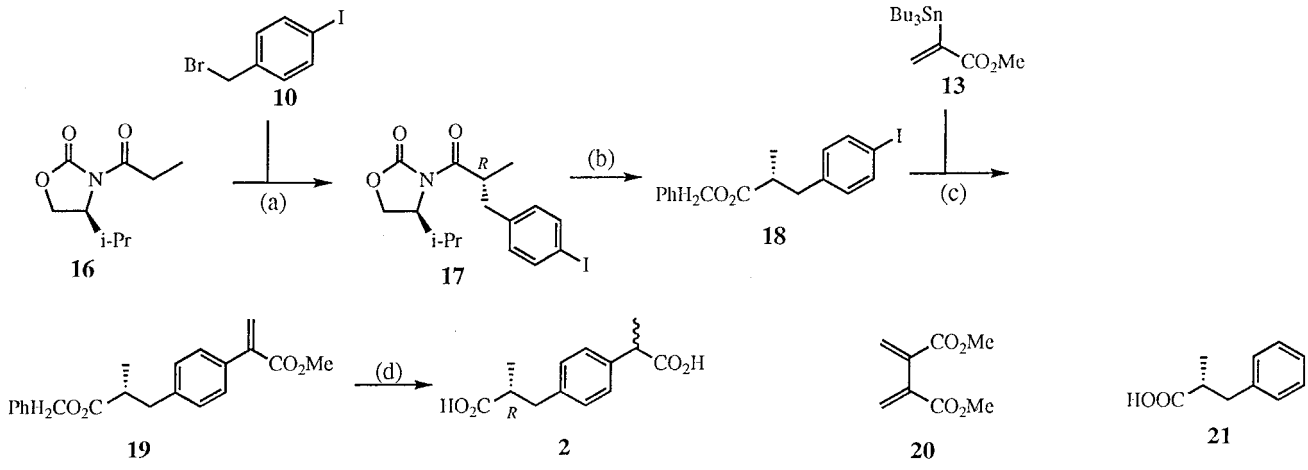

Fig. 6. Synthetic route adopted for the preparation of (2′*R*,2*R*,*S*)-carboxyibuprofen diastereoisomers **(2). (a)** LDA, THF, −78°C; **(b)** butyllithium, $PhCH_2OH$, THF, 0°C; **(c)** dry DMF, CuI, $Pd(PPh_3)_4$; **(d)** Pd/C, EtOH, NaOH, 0°C.

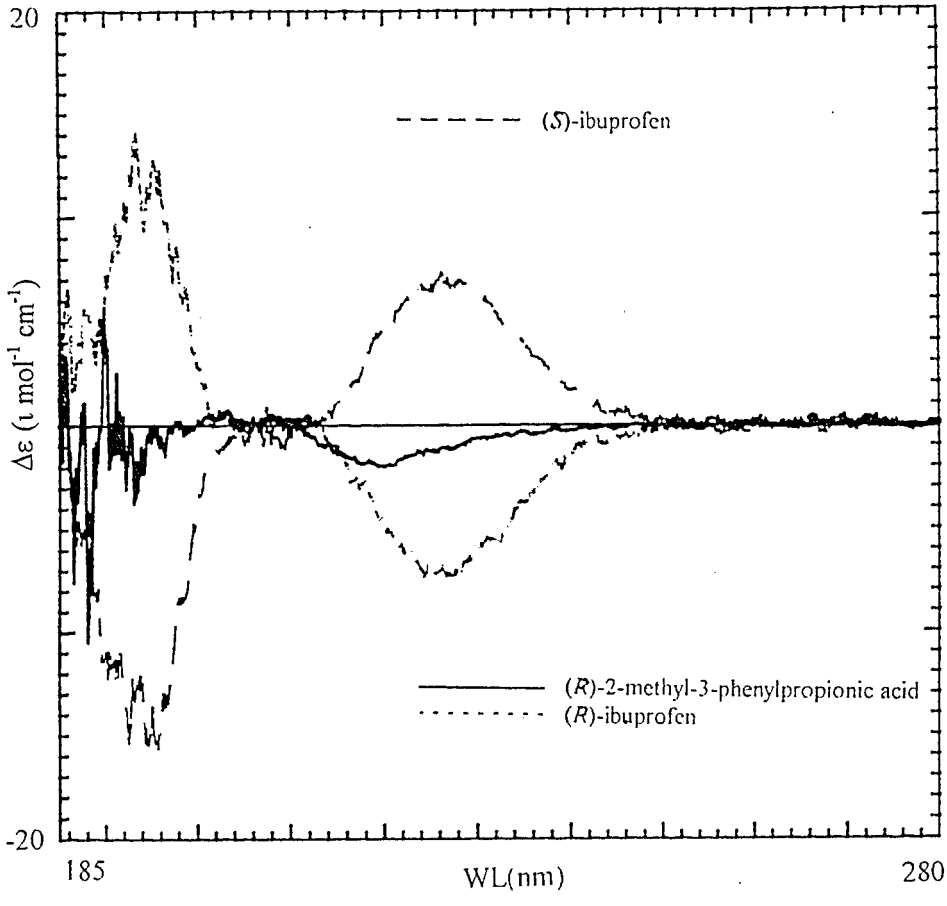

Fig. 7. CD spectra presented from 185 to 280 nm for (*R*)-2-methyl-3-phenylpropionic acid, (*R*)- and (*S*)-ibuprofen. Samples dissolved (0.3 mg/ml) in acetonitrile; pathlength 0.02 cm; spectra recorded at 25°C.

served in the region of the 226 nm band (Fig. 8). The spectrum of the 2′*R,* 2*R*-stereoisomer was similar to that observed for (*R*)-ibuprofen (Fig. 8), although the intensity of the negative band for (2′*R,* 2*R*)-carboxyibuprofen was greater. It is therefore evident that the *R*-configuration of the carboxypropyl moiety exhibits a similar CD to the original chiral center at this wavelength although the intensity is weaker. Thus, the contribution of the two chiral centers of the same configuration is additive, resulting in a greater negative CD than that observed for (*R*)-ibuprofen. With the 2′*S,* 2*R*-diastereoisomer, the contributions of the two centers are opposite and, since that of the 2′-center is

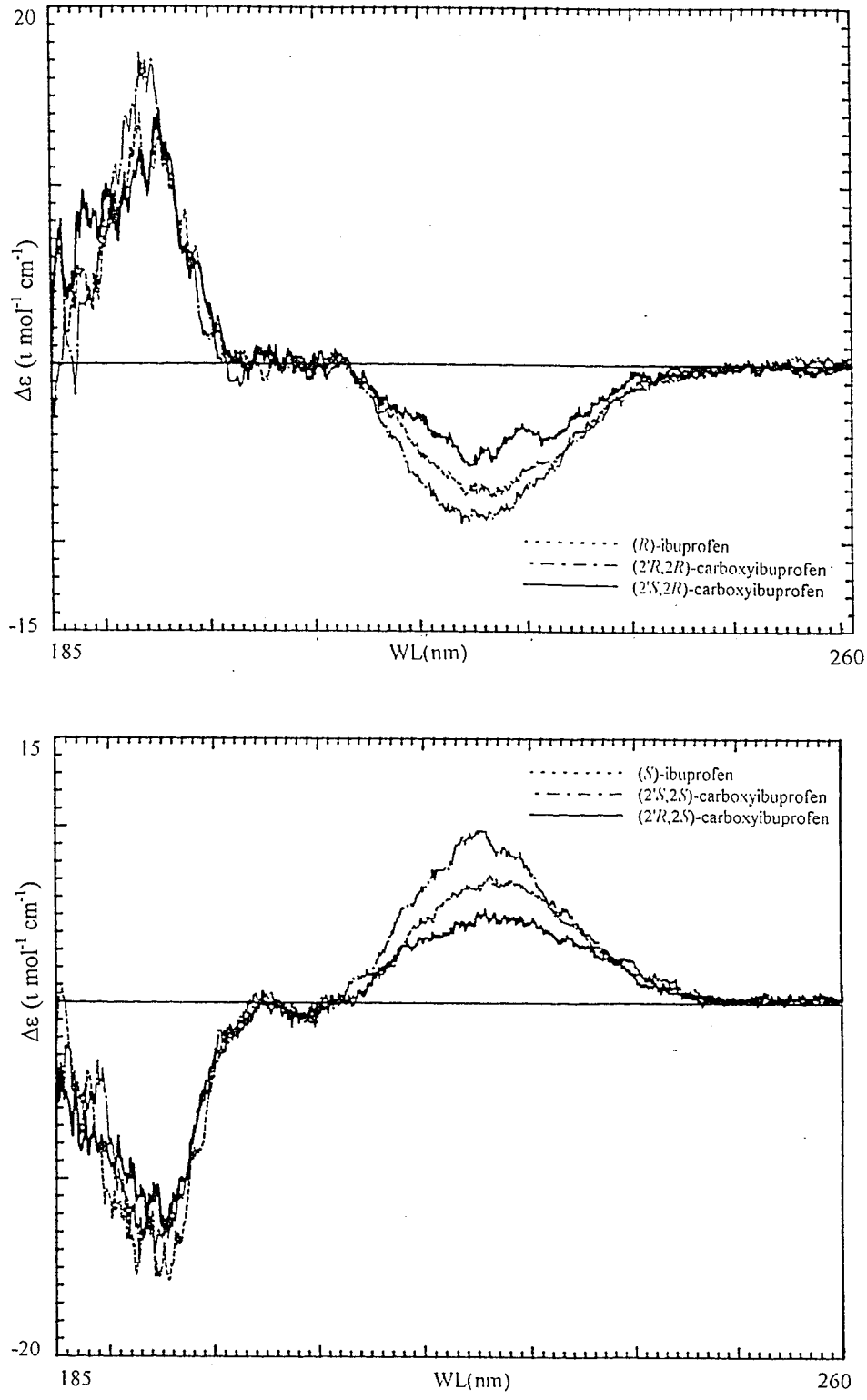

Fig. 8. CD spectra presented from 185 to 280 nm for **(top)** (2′*R,2R*)-carboxyibuprofen, (2′*S,2R*)-carboxyibuprofen, and (*R*)-ibuprofen, and **(bottom)** (2′*S,2S*)-carboxyibuprofen, (2′*R,2S*)-carboxyibuprofen, and (*S*)-ibuprofen. Samples dissolved (0.3 mg/ml) in acetonitrile; pathlength 0.02 cm; spectra recorded at 25°C.

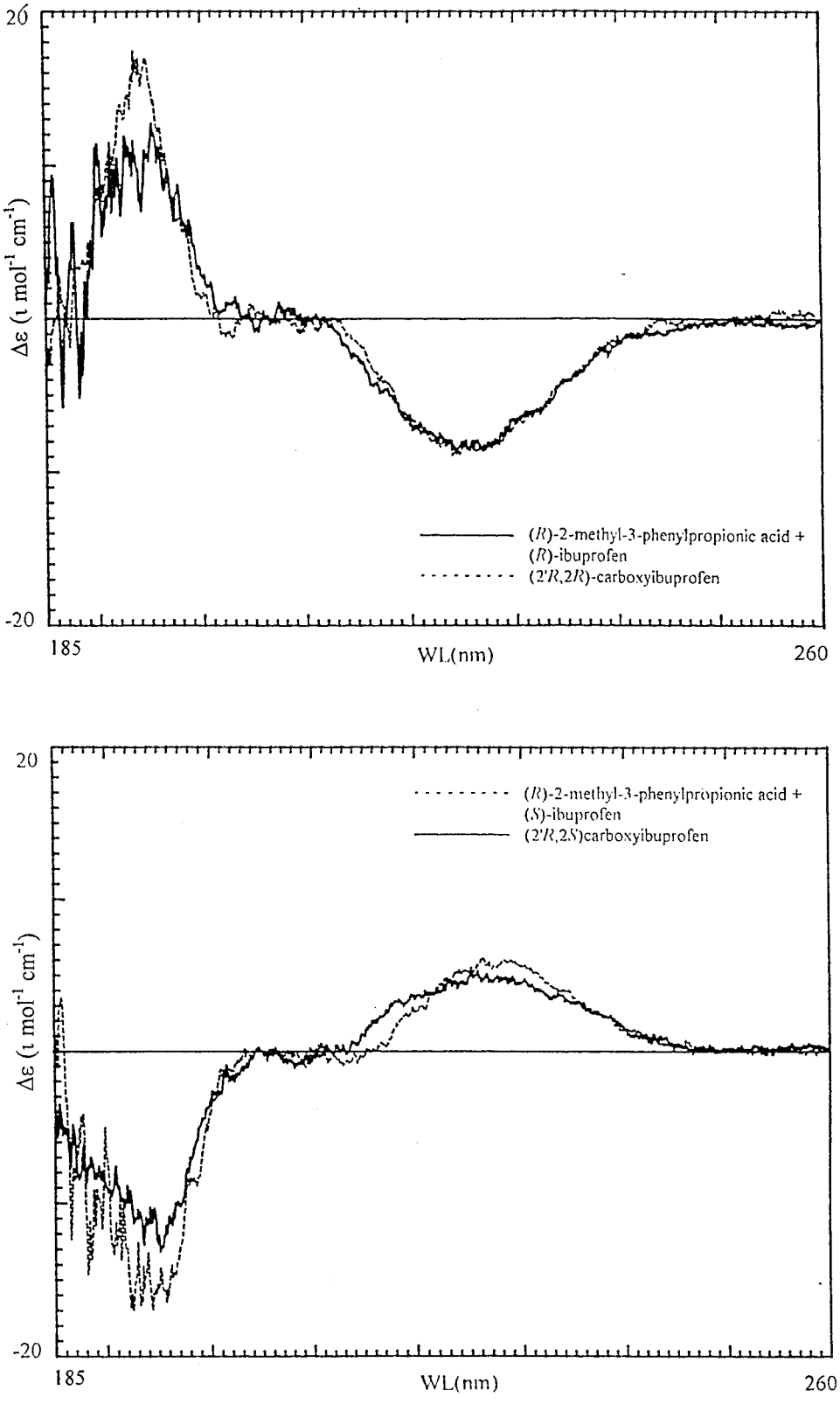

Fig. 9. Top: Composite CD spectrum presented from 185 to 260 nm after the addition of the CD spectrum of (*R*)-2-methyl-3-phenylpropionic acid to that of (*R*)-ibuprofen, overlaid with the CD spectrum for (2′*R*,2*R*)-carboxyibuprofen. **Bottom:** Composite spectrum of (*R*)-2-methyl-3-phenylpropionic acid and (*S*)-ibuprofen, overlaid with the spectrum of (2′*R*,2*S*)-carboxyibuprofen. Samples dissolved (0.3 mg/ml) in acetonitrile; pathlength 0.02 cm; spectra recorded at 25°C.

weaker, a negative maximum is still observed but is attenuated compared to (*R*)-ibuprofen. An analogous situation applies to (2′*S,* 2*S*)- and (2′*R,* 2*S*)-carboxyibuprofen, but in these cases the CD bands are positive (Fig. 8).

As (*R*)-2-methyl-3-phenylpropionic acid is structurally similar to the carboxypropyl moiety of carboxyibuprofen, examination of its CD spectrum should provide information on the relative contributions of the two chiral centers in carboxyibuprofen. The far-UV CD spectrum of (*R*)-2-methyl-3-phenylpropionic acid shows a negative maximum at 220 nm which is quantitatively weaker than that of (*R*)-ibuprofen (Fig. 7). Addition of the two spectra yields a composite spectrum similar to that of (2′*R,*2*R*)-carboxyibuprofen, supporting the observation that the spectral contributions are additive (Fig. 9). Similar addition of the spectrum of (*R*)-2-methyl-3-phenylpropionic acid to that of (*S*)-ibuprofen yields a composite spectrum similar to that of (2′*R,* 2*S*)-carboxyibuprofen.

Examination of the 255–280 nm region of the spectra of the four stereoisomers of carboxyibuprofen and the enantiomers of ibuprofen shows that the carboxypropyl group causes a small reduction in the CD intensity in this region, independent of the absolute configuration of the carboxypropyl center (data not shown).

CONCLUSIONS

In conclusion, the four stereoisomers of carboxyibuprofen have been chromatographically resolved for the first time, and the elution order unequivocally assigned by a combination of synthetic and metabolic approaches. The chromatographic resolution has enabled the characterization of the stereoisomers by CD spectroscopy and the influence of the metabolically introduced chiral center on the spectra has been defined. The adaptation of the chromatographic system described above for the routine determination of the stereochemical composition of carboxyibuprofen resulting from metabolic studies is currently in progress.

LITERATURE CITED

1. Adams, S.S., Bresloff, P., Mason, C.G. Pharmacological differences between the optical isomers of ibuprofen: evidence for the metabolic inversion of the (–)-isomer. J. Pharm. Pharmacol. 28:256–257, 1976.
2. Hutt, A.J., Caldwell, J. The metabolic chiral inversion of 2-arylpropionic acids—a novel route with pharmacological consequences. J. Pharm. Pharmacol. 35:693–704, 1983.
3. Caldwell, J., Hutt, A.J., Fournel-Gigleux, S. The metabolic chiral inversion and dispositional enantioselectivity of the 2-arylpropionic acids and their biological consequences. Biochem. Pharmacol. 37:105–114, 1988.
4. Williams, K.M. Enantiomers in arthritic disorders. Pharmacol. Ther. 46:273–295, 1990.
5. Evans, A.M. Enantioselective pharmacodynamics and pharmacokinetics of chiral non-steroidal anti-inflammatory drugs. Eur. J. Clin. Pharmacol. 42:237–256, 1992.
6. Wechter, W.J., Loughhead, D.G., Reischer, R.J., Van Giessen, G.J., Kaiser, D.G. Enzymatic inversion at saturated carbon: nature and mechanism of the inversion of (*R*)-(–)-p-isobutylhydratropic acid. Biochem. Biophys. Res. Commun. 61:833–837, 1974.
7. Kaiser, D.G., Van Giessen, G.J., Reischer, R.J., Wechter, W.J. Isomeric inversion of ibuprofen *R*-enantiomer in humans. J. Pharm. Sci. 65:269–273, 1976.
8. Adams, S.S., Cliffe, E.E., Lessel, B., Nicholson, J.S. Some biological properties of 2-(4-isobutylphenyl)propionic acid. J. Pharm. Sci. 56:1686, 1967.
9. Mills, R.F.N., Adams, S.S., Cliffe, E.E., Dickinson, W., Nicholson, J.S. The metabolism of ibuprofen. Xenobiotica 3:589–598, 1973.
10. Lockwood, G.F., Albert, K.S., Gillespie, W.R., Bole, G.G., Harkcom, T.M., Szpunar, G.J., Wagner, J.G. Pharmacokinetics of ibuprofen in man. I. Free and total area/dose relationships. Clin. Pharmacol. Ther. 34:97–103, 1983.
11. Evans, A.M., Nation, R.L., Sansom, L.N., Bochner, F., Somogyi, A.A. The relationship between the pharmacokinetics of ibuprofen enantiomers and the dose of racemic ibuprofen in humans. Biopharm. Drug Dispos. 11:507–518, 1990.
12. Young, M.A., Aarons, L., Davidson, E.M., Toon, S. Stereospecific assay of ibuprofen and its metabolites. J. Pharm. Pharmacol. 38:60P, 1986.
13. Baillie, T.A., Adams, W.J., Kaiser, D.G., Olanoff, L.S., Halstead, G.W., Harpootlian, H., Van Giessen, G.J. Mechanistic studies of the metabolic chiral inversion of (*R*)-ibuprofen in humans. J. Pharmacol. Exp. Ther. 249:517–523, 1989.
14. Nicoll-Griffith, D.A., Inaba, T., Tang, B.K., Kalow, W. Method to determine the enantiomers of ibuprofen from human urine by high performance liquid chromatography. J. Chromatogr. 428:103–112, 1988.
15. Rudy, A.C., Anliker, K.S., Hall, S.D. High-performance liquid chromatographic determination of the stereoisomeric metabolites of ibuprofen. J. Chromatogr. 528:395–405, 1990.
16. Furniss, B.S., Hannaford, A.J., Rogers, V., Smith, P.W.G., Tatchell, A.R. In: Vogel's Textbook of Practical Organic Chemistry, 5th ed. London: Longman, 1989: 687–690, 1178.
17. Hutt, A.J., Fournel, S., Caldwell, J. Application of a radial compression column to the high-performance liquid chromatographic separation of the enantiomers of some 2-arylpropionic acids as their diastereoisomeric *S*-(–)-1-(naphthen-1-yl)ethylamides. J. Chromatogr. 378:409–418, 1986.
18. Wainer, I.W. A Practical Guide to the Selection and Use of HPLC Chiral Stationary Phases. Phillipsburg: J.T. Baker, 1988:11–14.
19. Shibata, T., Mori, K., Okamota, Y. Polysaccharide phases. In: Chiral Separations by HPLC. Applications to Pharmaceutical Compounds. Krstulovic, A.M., ed. Chichester: Ellis Horwood, 1989:336–398.
20. Evans, D.A., Bartroli, J., Shih, T.L. Enantioselective aldol condensations 2. Erythro-selective chiral aldol condensations via boron enolates. J. Am. Chem. Soc. 103:2127–2129, 1981.
21. Evans, D.A., Ennis, M.D., Mathre, D.J. Asymmetric alkylation reactions of chiral imide enolates. A practical approach to the enantioselective synthesis of α-substituted carboxylic acid derivatives. J. Am. Chem. Soc. 104:1737–1739, 1982.
22. Meyers, A.I., Temple, D.L., Nolen, R.L., Mihelich, E.D. Oxazolines IX. Synthesis of homologated acetic acids and esters. J. Org. Chem. 39: 2778–2783, 1974.
23. Meyers, A.I., Knaus, G., Kamata, K., Ford, M.E. Asymmetric synthesis of *R* and *S* α-alkylalkanoic acids from metalation and alkylation of chiral 2-oxazolines. J. Am. Chem. Soc. 98:567–576, 1976.
24. Meyers, A.I. Asymmetric carbon-carbon bond formation from chiral oxazolines. Acc. Chem. Res. 11:375–381, 1978.
25. Miyake, H., Yamamura, K. Palladium (O) catalysed hydrostannylation of alkynes. Stereospecific synthetic addition of tributyltin hydride. Chem. Lett. 980–984, 1989.
26. Zhang, H.X., Guibé, F., Balavoine, G. Palladium and molybdenum-catalysed hydrostannation of alkynes. A novel access to regio and stereodefined vinylstannanes. J. Org. Chem. 55:1857–1867, 1990.
27. Levin, J.I. Palladium-catalysed coupling of an α-stannyl acrylate to aryl iodides and triflates. A one-step synthesis of arylpropenoic esters. Tetrahedron Lett. 34:6211–6214, 1993.
28. Barth, G., Voelter, W., Mosher, H.S., Bunnenberg, E., Djerassi, C. Optical rotatory dispersion studies CXVII. Absolute configurational assignments of some α-substituted phenylacetic acids by circular dichroism measurements. J. Am. Chem. Soc. 92:875–886, 1970.
29. Teulon, J.M., Cognacq, J.C., Hertz, F., Lwoff, J.M., Foulon, M., Baert, F., Brienne, M.J., Lacombe, L., Jacques, J. Antiinflammatory and analgesic diastereoisomeric derivatives of indan-5-acetic acid. J. Med. Chem. 21:901–905, 1978.