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REVIEW

The metabolic chiral inversion of 2-arylpropionic acids — a novel route with pharmacological consequences

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1. *Introduction*

It is now well understood that the processes of absorption, distribution, metabolism and excretion of xenobiotics may all exhibit stereo-selectivity or specificity (see Jenner & Testa 1973), and this is especially so with enzymic metabolic transformations. Most such instances involve the stereoselective or specific metabolism of stereoisomers or the selective or specific production of chiral metabolites from prochiral xenobiotics. More recently, an intriguing and biologically fortuitous example of stereospecific metabolism has been discovered in the metabolism of certain carboxylic acids, namely the inversion of the chiral centre of the *R*(−) enantiomers of the 2-arylpropionic acid non-steroidal anti-inflammatory drugs (NSAIDs) to their *S*(+)-isomers. This chiral inversion occurs without any other alteration to the xenobiotic.

Shen (1981) has reviewed the medicinal chemistry of the arylacetic acids and their α-methyl analogues, the 2-arylpropionic acids. The first reports of stereospecificity of action of such agents arose with 3-chloro-4-cyclohexyl-α-methylphenylacetic acid (Shen 1967a) and the indomethacin series of compounds, where it was found that only the dextrorotatory isomers of various α-methylsubstituted analogues had anti-inflammatory activity (Shen 1967b; Shen & Winter 1977) and this was shown to extend to other 2-arylpropionic acid NSAIDs (Shen 1972).

The major metabolic options open to the carboxyl group of the 2-arylpropionic acids and other xenobiotic carboxylic acids are the reactions of amino acid and glucuronic acid conjugation. These were discovered in the 19th century (see Williams 1959). Many subsequent studies have shown the versatility of these pathways in terms both of species distribution and variety of substrates (see Caldwell 1978, 1982; Caldwell et al 1980). Considerable advances in the understanding of the metabolic behaviour of the carboxyl group have been made. Thus, the chemistry of the ester glucuronides has been reinvestigated, notably with respect to intramolecular acyl migration and its consequences (Compernolle et al 1978; Sinclair & Caldwell 1982; Stogniew & Fenselau 1982). Additionally, intimate relationships have been noted between metabolism of carboxylic acids and reactions of lipid biosynthesis (see Caldwell & Marsh 1983). The chiral inversion of the 2-arylpropionates is thus another fascinating new aspect of the metabolic behaviour of an important group of carboxylic acids.

The carboxylic acid group, either as such or readily metabolically generated by the hydrolysis of simple esters, occurs in the structures of a wide variety of drugs and environmental chemicals. There has been a resurgence of interest recently, notably from the viewpoint of their toxicity, in such compounds e.g. clofibrate, 2,4-D and 2,4,5-T and the carboxylic acid NSAIDs, with attention focusing upon aspirin, zomepirac and benoxaprofen.

In the light of this current interest in NSAIDs, the metabolic chiral inversion of the 2-arylpropionic acids assumes considerable significance. The chiral inversion always involves the conversion of the inactive *R*(−)-isomers to their pharmacologically active *S*(+)-enantiomers, and the rate of this process varies according to substrate and species. In virtually every instance, biological testing is with the racemates of such compounds and few metabolic studies define the enantiomeric composition of the drugs and their metabolites in body fluids. The occurrence of this chiral inversion suggests that any investigations failing to take it into account are of limited value. It is the purpose of this review to describe the occurrence and mechanism of the chiral inversion of

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the 2-arylpropionates, and to comment upon its significance and implications for their pharmacology and toxicology.

The nomenclature used

The general formula of these acids is Ar.αCH(CH_3)COOH. Unless otherwise stated, configurational designations in the text refer to the configuration about the carbon α- to the carboxyl group of the 2-arylpropionates and their metabolites. α-Methyl refers to the methyl group and α-methine to the –CH– moiety in the propionic acid group.

The absolute configurations using the sequence rule (*RS*) nomenclature have been determined for the compounds whose metabolic inversion is documented here. These configurations have been determined either by an absolute method, such as x-ray crystallography, or by comparison of circular dichroism spectra with a 2-arylpropionate of known configuration, generally one of the isomers of hydratropic acid. In each case, the *R* and *S* symbols refer to the same spatial arrangement (same sequence around the chiral centre). Fig. 1 shows representative three-dimensional structures of the 2-arylpropionic acid enantiomers. Although the *RS*

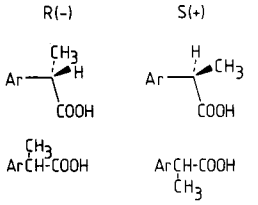


FIG. 1. Stereochemical representations of the *R*(−)- and *S*(+)-enantiomers of the 2-arylpropionic acids. The upper flying wedge diagrams show three-dimensional structures of the enantiomers according to Shen (1979). The lower two-dimensional representations are those used here for equations 3–8.

nomenclature for absolute configuration has no relation to the observed rotations obtained by polarimetry, designated here as (+) or (−), for the compounds considered here, the enantiomers are either *R*(−) or *S*(+). In this review, the *RS* nomenclature is used when justified by the literature cited, and the observed direction of rotation added as indicated by the literature. In some cases, the observed rotations alone are indicated: for these examples, the literature cited has no reference to absolute configuration.

The figures present two-dimensional representations of the compounds, since they are generally used as the racemates (which cannot be drawn conveniently in three dimensions) and it is desired to present the molecular formulae only. Where stereochemical considerations are covered in the figures, absolute configuration has been indicated by the *R*(−)- and *S*(+)-nomenclature as appropriate.

2. Metabolism of hydratropic acid and related compounds

The first report of stereospecific metabolism of the α-methyl substituted phenylacetic acids was that of Kay & Raper (1922), and arose from studies on the metabolism of α-methyl substituted fatty acids. The oxidation of these was proposed to proceed via oxidation of the α-methyl group to an aldehyde, which decomposed with elimination of carbon dioxide to yield a straight chain aldehyde, which was then rapidly oxidized to the corresponding carboxylic acid (eqn 1). As there were at that time few data available on the metabolism of branched chain fatty acids, Kay & Raper extended their studies to include the corresponding phenyl substituted compounds, the simplest being hydratropic acid (2-phenylpropionic acid; compound I in Fig. 2).

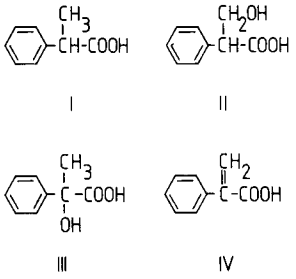
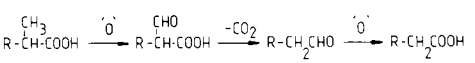


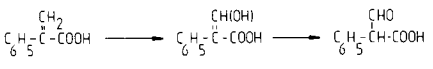
FIG. 2. Structures of hydratropic acid and analogues I: hydratropic acid (2-phenylpropionic acid), II: tropic acid, III: atrolactic acid, IV: atropic acid.

Following administration of racemic hydratropic acid (I) to dogs, a total of approximately one-third of the dose was excreted in the urine, as the free acid and its glycine conjugate. Both (−)- and (+)- forms were present, but the (+)-isomer was present in excess (Kay & Raper 1922).

Equation 1



Equation 2



Kay & Raper (1922) also studied the metabolism of three analogues of hydratropic acid, namely tropic, atrolactic and atropic acids (II–IV; Fig. 2) and the results of these studies are of significance. Racemic tropic (II) and atrolactic (III) acids were

excreted mainly in the urine of dogs, with greater than 90 and 80% respectively being recovered unchanged. Both excretion products were optically inactive. Similar results were obtained after an intraperitoneal dose of tropic acid (II, Fig 2) to rats and mice, ca 95% being rapidly excreted unchanged in urine (Gosselin et al 1955). Atropic acid (IV), however, could not be detected in urine following its administration to dogs, and was reported to be 'completely oxidized' (Kay & Raper 1922). As the extent of oxidation of atrolactic (III) and tropic (II) acids was much less than that of hydratropic acid (I), these authors considered that the ability to form a double bond between the α-carbon atom and the methyl group carbon was a factor in the oxidation. They believed that hydratropic acid (I) could be easily converted to the metabolically labile atropic acid (IV) by oxidation, but the hydroxy acids could not. A reaction mechanism was postulated, by analogy with the β-oxidation of cinnamic acid (eqn 2), the formylphenylacetic acid intermediate being proposed to breakdown to carbon dioxide and phenylacetaldehyde (eqn 1).

In a series of studies on the metabolism of alkylbenzenes in the rabbit, Robinson et al (1955) investigated the metabolism of cumene (isopropylbenzene). This hydrocarbon was oxidized to yield 2-phenylpropionic acid (hydratropic acid), and two alcohols, 2-phenylpropan-2-ol and 2-phenylpropan-1-ol (hydratropyl alcohol), all of which were excreted in the urine as their glucuronides. Both optical isomers of hydratropic acid (I) were formed by metabolic oxidation of cumene, but the (+)-isomer was present in excess and its ester glucuronic acid conjugate was isolated. On administration of racemic hydratropic aldehyde, (+)-hydratropoyl glucuronide was obtained in crystalline form. The authors concluded that the glucuronide of the (−)-isomer was formed but was resistant to isolation.

The metabolism of racemic [^{14}C]hydratropic acid (I) has recently been investigated in man, rhesus monkey, rabbit, cat and rat (Dixon et al 1977a). Most (58–100% of dose) of the administered radioactivity was excreted in the urine within 24 h. The major metabolite in all species was the ester glucuronic acid conjugate, and very small quantities of the glycine conjugate were detected in rat urine. Both taurine and glycine conjugates were detected in the cat. In addition, all five species excreted varying quantities of unchanged acid (2–17% of urinary ^{14}C in 24 h). No attempt was made in that study to examine the stereochemistry of the products isolated.

During the development of ibuprofen (compound VI in Fig. 3), the fate of racemic 2-(4-phenylphenoxy)propionic acid (V, Fig. 3) was exam-

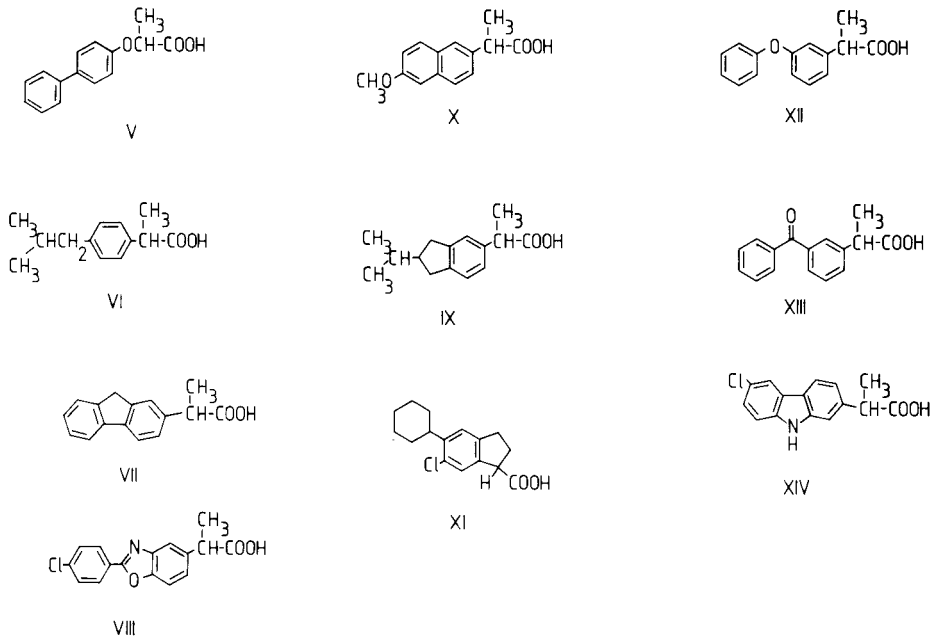


FIG. 3. Structures of 2-arylpropionic acids shown to or suspected to undergo chiral inversion in-vivo V: 2-(4-phenylphenoxy)propionic acid, VI: ibuprofen, VII: cicloprofen, VIII: benoxaprofen, IX: 2-(2-isopropylindan-5-yl)propionic acid, X: naproxen, XI: clidanac, XII: fenoprofen, XIII: ketoprofen, XIV: carprofen.

ined. Its major urinary metabolite in rabbit was found to be the 4'-hydroxy derivative, which was found to be dextrorotatory (Nicholson 1982). The same metabolite, was found in man, but in this case it was 'only slightly' dextrorotatory. These findings indicated the possibilities of species-dependent stereoselective metabolism within this series of compounds (Nicholson 1982).

3. *Ibuprofen (R,S-2-(4-isobutylphenyl)propionic acid, VI)*

Ibuprofen was the first substituted 2-arylpropionic acid shown to undergo metabolic chiral inversion and has subsequently become the most studied compound of this group.

The metabolism of ibuprofen in mammals is shown in Fig. 4. The drug is oxidized to 2-[4-(2-hydroxy-2-methylpropyl)phenyl]propionic acid (VIa) and 2-[4-(2-carboxylpropyl)phenyl]propionic acid (VIc) in rat, dog, baboon and man, both metabolites being dextrorotatory in man (Adams et al 1967; Mills et al 1973).

Mills et al (1973) examined the fate of the optical isomers of ibuprofen given separately to man and found that metabolites VIa and VIc were dextrorotatory, regardless of whether (+)- or (−)-ibuprofen was administered. The change in optical rotation of metabolite VIc relative to the parent compound could arise simply from the introduction of a second asymmetric centre into the molecule (in the isobutyl side chain), but as the formation of metabolite VIa does not involve formation of a new chiral centre it was proposed that structural inversion from the *R*(−)- to the *S*(+)-enantiomer occurred in the body. The optical rotation of the metabolites VIa and VIc was lower after administration of (−)-ibuprofen, suggesting that the inversion of structure was incomplete (Mills et al 1973).

Brooks & Gilbert (1974) developed a g.l.c. method for the separate assay of the stereoisomers of ibuprofen, based on the separation of the diastereoisomeric amides formed on derivatization with *R*(+)-α-methylbenzylamine, on an achiral stationary phase (Table 1). Using this assay, those workers demonstrated that the unchanged ibuprofen present in human urine was predominantly (80%) the (+)-enantiomer. The significance of the increased proportion of the *S*(+)-enantiomer could not be assessed as the racemate had been administered and the stereochemistry of the metabolic products was not investigated.

Conclusive evidence for the chiral inversion of *R*(−)-ibuprofen in the body was obtained by Wechter et al (1974) using the compound deuterium labelled in the α-methyl and α-methine groups i.e. d_4-substituted. Examination by mass spectrometry of the products obtained on administration to man,

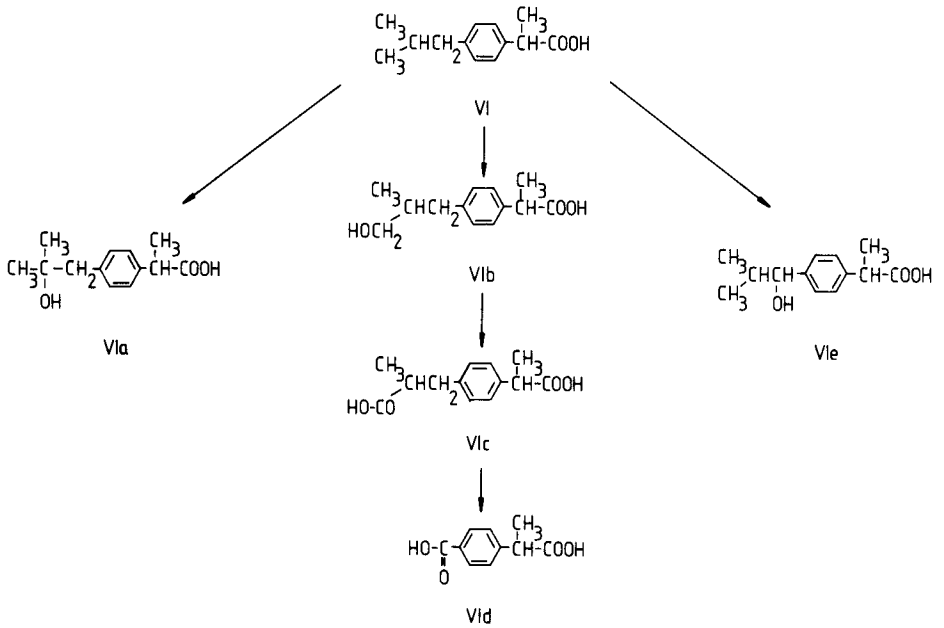


FIG. 4. Metabolism of ibuprofen (VI) in man.

Table 1. Summary of analytical methods used for the determination of enantiomeric composition of various 2-arylpropionic acids in biological samples

Drug/ Metabolite	Derivatized with (Derivatization Method)	Chromatographic Method	System	Reference
Benoxaprofen	*S*-(−)-α-Methylbenzylamine (using 1,1′-carbonyldiimidazole)	g.l.c.	1·02 m × 3 mm i.d. 2% OV17 (80–100 mesh) on Gas Chrom Q Oven temp. 280°, carrier gas He flow rate 60 ml min^{-1}. Retention time: *S*(+) 3·2 min, *R*(−) 3·6 min†	Bopp et al (1979)
Benoxaprofen	*R*(+)-α-Methylbenzylamine (via acylchloride using thionyl chloride)	h.p.l.c.	250 × 4 mm i.d. 10 μm Partisil Solvent: dichloromethane: 2,2,4-trimethylpentane: methanol (100 : 100 : 1·35 by vol) flow rate 1 ml min^{-1}. Detector: uv 309 nm. Retention time: *S*(+) 6·4 min, *R*(−) 8·4 min	Simmonds et al (1980)
	S-(−)-α-Methylbenzylamine (via acylchloride using thionyl chloride)	h.p.l.c.	12·5 cm × 5 mm i.d. 5 μm Hypersil APS Solvent: isooctane–methylene chloride–methanol (55 : 44·8 : 0·2 by vol) flow rate 1 ml min^{-1} Detector: uv 308 nm. Resolution: R_S = 1·59 (Assay time under 5 min, order of elution not stated)	McKay et al (1979)*
^{14}C-Carprofen	*S*-(−)-α-Methylbenzylamine (using 1,1′-carbonyldiimidazole)	t.l.c.	0·2 mm thick silica gel. 60F plates solvent: benzene–ether–methanol (60 : 35 : 5) R_F values: *S*(+) 0·36; *R*(−), 0·41 (Carprofen 0·18)	Kemmerer et al (1979)
Carprofen	As above	h.p.l.c.	25 cm × 4·6 mm i.d. 10 μm silica gel. Solvent: 0·75% methanol in dichloromethane flow rate 2·0 ml min^{-1}. Detector: uv 254 nm (or fluorescence: λ = 240 nm excitation; λ > 350 nm emission). Retention time: *R*(−), 6·4 min (K′ = 5·1) *S*(+), 7·6 min (K′ = 6·7)	Stoltenborg et al (1981)
[^{14}C]Cicloprofen	L-Leucine (via acylchloride using oxalylchloride)	t.l.c.	250 μm thick, Q-1-F-silica gel plates. Solvent: benzene–diethyl ether–methanol (8 : 3 : 1 by vol). R_F values: *R*(−)	Lan et al (1976)
Cicloprofen	[^{14}C]-L-Leucine	t.l.c.	0·27; *S*(+) 0·39 (unreacted cicloprofen 0·77)	
Clidanac	*R*-(+)-α-Methylbenzylamine (using 1-ethyl-3-(3-dimethylamino proply)carbodiimide)	t.l.c.	0·25 mm thick silica gel 60F_{254} plates. Solvent: benzene–acetonitrile (100 : 13 v/v). R_F: *S*(+) 0·56; *R*(−) 0·45. Densitometer reflectometric technique. Light absorbing λ280 nm, reference λ360 nm	Tamura et al (1981)
Ibuprofen	*R*-(+)-α-Methylbenzylamine (via acylchloride using thionyl chloride)	g.l.c.	5 m × 3 mm i.d. 1% OV17 on Gas Chrom Q (100–120 mesh). Oven temp. 220°; carrier gas N_2; I: *S*(+) 2660; *R*(−) 2625	Brooks & Gilbert (1974)
	S(−)-α-Methylbenzylamine (using 1,1′-carbonyldiimidazole)	g.l.c.	1·5 m × 3 mm i.d. 3% OV-17 on Gas Chrom Q (60–80 mesh). Oven temp. 220°; carrier gas He flow rate 90 ml min^{-1}. Retention times: *S*(+) 8·0 min, *R*(−) 9·0 min	Vangiessen & Kaiser (1975)
2-[4-(2-Hydroxy-2-methylpropyl) phenyl]propionic acid	As above	g.l.c.	As above Retention times: *S*(+) 16·9 min, *R*(−) 19·1 min	Kaiser et al (1976)
2-[4-(2-Carboxypropyl)phenyl] propionic acid	As above (Diamide derivatives formed)	g.l.c.	Column as above, oven temp. 285°. Retention times: *S*(+)/*S*(+) 25·2 min; *R*(−)/*S*(+), *S*(+)/*R*(−) 28·4 min *R*(−)/*R*(−) 30·8 min	Kaiser et al (1976)
Naproxen	L-1-(4-Dimethylamino-1-naphthyl) ethylamine (using 1-ethyl-3-(3-dimethylamino propyl)carbodiimide and 1-hydroxybenzotriazole)	h.p.l.c.	1 ft × ¼ in. i.d. μ Porasil. Solvent: n-hexane: tetrahydrofuran (80 : 26 by vol.), flow rate 0·6 ml min^{-1}. Detector: fluorescence (excitation λ320 nm, emission λ 410 nm). Retention time: *R*(−) 11·0 min, *S*(+) 13·0 min	Goto et al (1982)

* Ketoprofen enantiomers also separated using this system, solvent as for benoxaprofen, resolution R_S = 1·66; Fenoprofen enantiomers separated, but solvent changed to 75 : 24 : 1, resolution R_S = 1·37.

† Configuration refers to the chiral centre at the α-carbon atom in the propionic acid moiety.

showed that the *S*(+)-enantiomers of both ibuprofen and metabolite VIa, 2-[4-(2-hydroxy-2-methylpropyl)phenyl]propionic acid, contained only 2 deuterium atoms, both being present in the α-methyl group adjacent to the -COOH. These workers also showed, using a g.l.c. assay, that following administration of the *R*(−)-enantiomer some 80% of the total ibuprofen and 54% of the total metabolite VIa in urine were present as their *S*(+)-enantiomers at 72 h post dosing. The *S*(+)-enantiomer of ibuprofen was found to be excreted unchanged, in terms of configuration, and after administration of the racemate, 71% of the total amounts of both ibuprofen and metabolite VIa were present as the *S*(+)-enantiomers. The loss of two deuterium atoms from the metabolites of *R*(−)-d_4-ibuprofen led Wechter et al (1974) to propose a mechanism for the chiral inversion process (see below). It was also noted, by comparison of the inversion rates of d_0 and d_4 *R*(−)-ibuprofen, that a kinetic isotope effect occurred during the inversion process.

A g.l.c. assay, based on the separation of the diastereoisomers of ibuprofen formed by reaction with *S*(−)-α-methylbenzylamine (Table 1) was developed by Vangiessen & Kaiser (1975). Their method was later shown to be applicable for the separation of the diastereomers formed on derivatization of metabolites VIa and VIc (Kaiser et al 1976), although the diamide of the dicarboxylic acid metabolite, VIc, could not be completely resolved (Table 1). By examination of the urinary excretion products after administration of both the individual enantiomers and of racemic ibuprofen, Kaiser et al (1976) concluded that the oxidation of ibuprofen to VIa was not stereoselective.

The anti-inflammatory activities of racemic, *R*(−)- and *S*(+)-ibuprofen were compared by Adams et al (1976) in three species. It was found that whilst the *S*(+)-enantiomer was approximately 160 times more potent that the *R*(−)-enantiomer in the inhibition of a bovine seminal vesicle prostaglandin synthetase preparation in-vitro, there was no significant difference in activity in-vivo between the racemate and the individual enantiomers in various models of inflammatory disease in the mouse, rat and guinea-pig. The authors ascribed this discrepancy to the virtually quantitative conversion of the *R*(−)- to the *S*(+)-enantiomer in all three species.

The mechanism of the chiral inversion of ibuprofen in-vivo and in-vitro in the rat, using deuterium labelled compounds, has recently been the subject of a brief report by Nakamura et al (1981).

4. *Cicloprofen* (R,S-α-*methylfluorene-2-acetic acid VII; Fig. 3)*

The chiral inversion of cicloprofen was first reported in the dog by Kripalani et al (1976). It was found that the drug isolated from dog blood 16 days after administration of the racemic drug consisted entirely of the *S*(+)-enantiomer. Further experiments showed that the optical rotation of the drug isolated increased from +11° at 3 h post dosing, to +58° after 16 days (specific rotation of *S*(+)-cicloprofen $[\alpha]_D^{25}$ = +58°). The percentage of the *S*(+)-enantiomer in blood thus increased from ca 60% at 3 h to 80% at 24 h and 100% at 16 days after administration.

To investigate the possibility of stereospecific elimination and/or chiral inversion, the enantiomers were individually administered to dogs and it was found that the kinetics of absorption, metabolism and excretion and the urinary metabolites of the enantiomers were similar to those of the racemate. Cicloprofen isolated from dog blood following administration of the *R*(−)-enantiomer showed a progressive change in specific rotation from −58° (100% *R*(−)-enantiomer) to +55° (>97% *S*(+)-enantiomer), over a period of 14 days after a single dose. Chiral inversion occurred in rats, monkeys and man, and the inversion rate varied between species.

These studies were extended by the development of a t.l.c. separation of the diastereoisomers formed by reaction of cicloprofen acyl chloride with L-leucine (Lan et al 1976; Table 1). In addition, the metabolism of two postulated intermediates in the inversion process was also examined.

5. *Benoxaprofen* (R,S-*2-(4-chlorophenyl)-α-methyl-5-benzoxazoylacetic acid, compound VIII; Fig. 3)*

Benoxaprofen is metabolized in man to yield the acyl glucuronide, both free acid and glucuronide being excreted in urine (Chatfield & Green 1978). In common laboratory species marked differences are observed in the route of excretion, both the drug and unidentified polar metabolites being detected in both urine and faeces (Chatfield & Green 1978). The stereoselective inversion of the *R*(−)-enantiomer to the *S*(+)-enantiomer was demonstrated by Bopp et al (1979) using a g.l.c. separation of the diastereoisomeric amides formed by reaction with *S*(−)-α-methylbenzylamine (Table 1). An h.p.l.c. separation of the same derivatives has also been reported (McKay et al 1979; Table 1).

On administration of *R*(−)-benoxaprofen (containing 2·4% of the *S*(+)-enantiomer) to man, the plasma concentration of the *S*(+)-enantiomer increased with time. Some 42% of the dose was

excreted in the urine in 72 h, of which 15·8% was present as the *S*(+)-enantiomer, indicating ca 14% chiral inversion (Bopp et al 1979). Plasma samples taken 72 and 84 h after administration of the racemate to two volunteers contained the *S*(+)-enantiomer only and 75–79% of the material present in their urine was dextrorotatory. Species variability in the rate of chiral inversion in rats and man has been shown by Simmonds et al (1980) with the half-life for the conversion of *R*(−) to *S*(+) being ca 2·5 h in the rat and 108 h in man, following oral administration. The elimination t½ of oral benoxaprofen in man is some 30 h. The inversion process, following i.v. administration of the *R*-(−)-enantiomer, to rats, was essentially complete within 6 h with a half-life of 1·2 h. The inversion could not be demonstrated using either a rat liver 10 000*g* supernatant preparation or gut microflora; some inversion was observed using an everted rat intestine preparation, but the amount of the *S*-(+)-enantiomer formed was small compared with the in-vivo results (Simmonds et al 1980).

6. R,S-*2-(2-Isopropylindan-5-yl)propionic acid (Compound IX; Fig. 3)*

2-(2-Isopropylindan-5-yl)propionic acid is an anti-inflammatory agent with stereoisomers that are known to be biologically equivalent (Teulon et al 1978). Metabolic studies in the rat have shown that the *R*(−)-enantiomer rapidly undergoes chiral inversion (Tanaka et al unpublished observations, see Tanaka & Hayashi 1980). Its major route of metabolic oxidation is shown in Fig. 5, and the stereo-

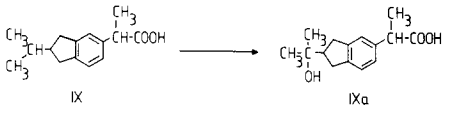


FIG. 5. Metabolic oxidation of 2-(2-isopropylindan-5-yl) propionic acid (IX) in the rat.

chemistry of the product IXa in the rat has been determined by formation of the diastereomeric amides with *R*(+)-α-methylbenzylamine and g.l.c. resolution (Tanaka & Hayashi 1980). Whilst no details of the g.l.c. system were given, both mass spectral and n.m.r. data were presented on material isolated from rat urine after administration of the α-methyl trideuterated *R*(−)-enantiomer of IX and the α-methine deuterated racemic drug. These results indicated that chiral inversion of the molecule about the α-methine group had occurred.

7. *Naproxen (*R,S-*2-(6-methoxynaphth-2-yl)propionic acid, Compound X; Fig 3)*

Of the 2-arylpropionic acid NSAIDs available in the U.K. at this time, naproxen is the only compound commercially available as the *S*(+)-enantiomer, the others being marketed as the racemates.

The chiral inversion of *R*(−)-naproxen to the *S*(+)-enantiomer has been recently reported in the rabbit by Goto et al (1982). These workers developed an h.p.l.c. assay method for the separation of the diastereoisomers of the amide formed on reaction of naproxen with L-1-(4-dimethylamino-1-naphthyl)ethylamine (Table 1). Using this assay, it was shown that the plasma t½ of *S*(+)-naproxen was greater than that of the *R*(−) enantiomer following i.v. administration of the racemic drug, and also, by the separate administration of the individual enantiomers, that *R*(−)-naproxen was converted to the *S*(+)-enantiomer.

8. *Clidanac (*R,S-*6-chloro-5-cyclohexyl-1-indancarboxylic acid, Compound XI; Fig. 3)*

The chiral inversion of this compound is of particular interest, as unlike the examples previously cited, the chiral centre is part of a fused ring system.

The metabolism of clidanac has been reported by Tanayama & Kanai (1977) and involves the formation of a series of isomeric oxidation products of the cyclohexane ring.

The inhibitory activity of clidanac on prostaglandin synthetase is due entirely to its *S*(+)-enantiomer (Tamura et al 1981). The *S*(+)-enantiomer is 5 to 12 times more active than the *R*(−)-enantiomer in rats and mice in-vivo, but in guinea-pigs the isomers were equipotent. This observation prompted an examination of the metabolism of *R*(−)-clidanac in guinea-pigs, and it was found that at 3 h post dosing the material present in plasma was greater than 90% the *S*(+)-enantiomer, and that *S*(+)-clidanac could be detected as early as 15 min post dosing (Tamura et al 1981). The individual enantiomers were determined by t.l.c. densitometry of the diastereomeric amides formed by reaction with *R*(+)-α-methylbenzylamine (Table 1).

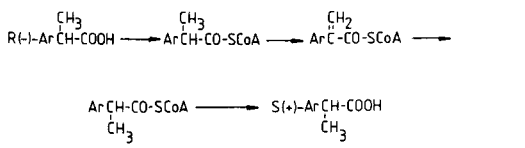
9. *Other 2-arylpropionic acids which may undergo chiral inversion*

*Fenoprofen (*R,S-*2-(3-phenoxyphenyl)propionic acid, Compound XII; Fig. 3).* Whilst there are no reports of the chiral inversion of fenoprofen the enantiomers have been reported to be of equal potency in animal test systems. Fenoprofen is known to undergo incorporation into triglycerides (Fears et

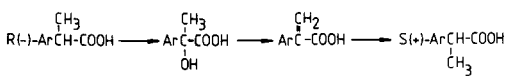
al 1978) which presumably involves formation of an acyl Co A thioester. Evidence to be presented below indicates a role for such thioesters in the chiral inversion process, and thus fenoprofen is a candidate to undergo this reaction.

The resolution of the diastereomeric amides formed by reaction of fenoprofen with $S(-)$-α-methylbenzylamine by h.p.l.c. has been reported by McKay et al (1979).

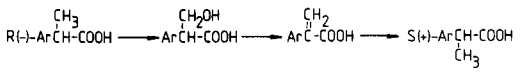
Equation 3



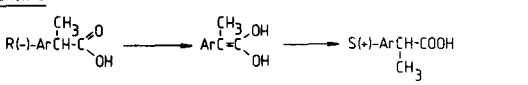
Equation 4



Equation 5



Equation 6



Ketoprofen (R,S-2-(3-benzoylphenyl)propionic acid, Compound XIII; Fig. 3). Ketoprofen, like fenoprofen, has been reported to be incorporated into triglycerides (Fears et al 1978), and, in addition, a study using [^{3}H-α-methyl] drug in man found increasing quantities of circulating radioactivity due to tritiated water. One means of loss of ^{3}H from the α-methyl group would be that proposed for the loss of deuterium from d_4-ibuprofen during the chiral inversion process (Hucker et al 1980; and see below). The resolution by h.p.l.c. of ketoprofen $S(-)$-α-methylbenzylamides has been reported by McKay et al (1979).

Carprofen (R,S-6-chloro-α-methylcarbazole-2-acetic acid, Compound XIV; Fig. 3). In studies on the disposition of *R,S*-carprofen in the rat, the $S(+)$-enantiomer was eliminated more slowly than the $R(-)$-enantiomer, but chiral inversion could not be conclusively demonstrated (Kemmerer et al 1979). In a later study in man, it was found that the stereoselective disposition of carprofen was not as great as in the rat, but the total amount of $S(+)$-carprofen excreted in urine and faeces, exceeded the $R(-)$-enantiomer by between 2–5% of the dose (Stoltenborg et al 1981). As yet the administration of individual enantiomers of carprofen to animals or man has not been reported, but available data point to the possibility of its chiral inversion.

10. *The mechanism of chiral inversion*

Evidence for the chiral inversion of the 2-arylpropionic acids can be obtained by administration of the individual enantiomers to animals or man, followed by analysis of the urinary excretion products. This is easily achieved by formation of a derivative, introducing a second chiral centre, followed by chromatographic separation of the diastereoisomers so formed (see Table 1). The mechanism of the inversion process, however, is much harder to study, and can best be examined by deuterium substitution about the chiral centre, followed by mass spectral examination of the products obtained, and/or by synthesis and metabolism of potential intermediates in the inversion process. All of these methods have been employed in the examination of the inversion process.

Wechter et al (1974) using $R(-)$-d_4-ibuprofen, labelled with 3 deuteriums in the α-methyl group and one at the α-methine carbon atom, found in man that the $S(+)$-enantiomer excreted in urine contained only two deuterium atoms, both of which were in the α-methyl group. In addition metabolite VIa, with the $S(+)$ configuration, also contained only two deuterium atoms, again both in the α-methyl function, whilst the $R(-)$ ibuprofen excreted retained all 4 deuterium atoms. These workers postulated that the chiral inversion process involved formation of an acyl-CoA thioester, followed by dehydrogenation and formation of an α-methylene function via the enzymes of lipid metabolism and speculated on the existence of an $R(-)$-arylpropionic acid isomerase enzyme system, which exerted stereospecificity in the final reduction step (eqn 3). By comparison of the rates of disappearance of $R(-)$-d_4-ibuprofen and $R(-)$-d_0-ibuprofen (rate d_0 : rate $d_4 \sim 2$), the authors suggested that the dehydrogenation was the rate-limiting step (Wechter et al 1974).

The similar chemical natures of ibuprofen and cicloprofen led Lan et al (1976) to propose that the inversion mechanism for the two compounds would be the same. Those workers examined the metabolism of two possible intermediates in the process, α-methylenefluorene-2-acetic acid and α-hydroxy-α-methylfluorene-2-acetic acid (see eqns 3, 4), in-vivo

in rats and in the rat isolated perfused liver. The α-methylene compound, but not the α-hydroxy compound, was converted to a mixture of cicloprofen enantiomers in a ratio of $S(+)$ 55 : $R(-)$ 45, indicating a non-stereoselective reduction. As $R(-)$-cicloprofen undergoes very extensive inversion to the $S(+)$-enantiomer, Lan et al (1976) proposed that dehydrogenation to the methylene intermediate could exert substrate stereoselectivity. In addition, they proposed two other alternative mechanisms but these were not discussed (eqns 5, 6).

Rubio et al (1980) have found α-hydroxycarprofen as a minor metabolite of carprofen in rat, dog and man. Such structures have been postulated as possible intermediates in the chiral inversion of 2-arylpropionates (eqn 4; see Lan et al 1976). In considering this proposed mechanism, and that in equation 5 also, it is necessary to recall the work of Kay & Raper (1922), reviewed above in Section 2. Of the various hydratropic acid analogues in Fig. 2, the α-hydroxy-methyl acid II and the α-hydroxy acid III were recovered quantitatively in urine as the racemates. In contrast the α-methylene acid IV was 'completely destroyed' in-vivo. It is thus unlikely that the mechanisms in equations 4 and 5 contribute to the chiral inversion of the 2-arylpropionates.

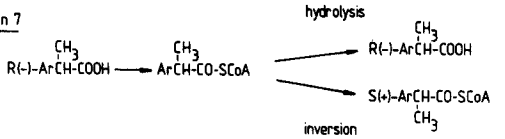
Using α-methyltrideuterated $R(-)$-2-(2-isopropylindan-5-yl) propionic acid (IX), Tanaka & Hayashi (1980) found the tertiary alcohol metabolite, IXa, to be 95% $S(+)$-enantiomer. Examination of this by mass spectrometry and n.m.r. indicated retention of all 3 deuterium atoms. It would thus appear that the chiral inversion reaction of 2-(2-isopropylindan-5-yl) propionic acid does not involve formation of a methylene intermediate as proposed by Wechter et al (1974) since this would cause loss of deuterium from the α-methyl group. Upon administration of α-methine-d_1-racemic IX, its metabolite $S(+)$-IXa was found to comprise an equimolar mixture of deuterated and protonated forms. The inversion of the $R(-)$-enantiomer of IX therefore involved the elimination of the α-methine deuterium substituent.

Mass spectral examination of metabolite IXa following administration of an equimolar mixture of $R(-)$-d_0- and $R(-)$-α-methyltrideuterated IX to rats showed that IXa contained equal amounts of methyl and trideuteromethyl groups, again providing no indication of methylene group formation. This study also failed to reveal a kinetic isotope effect (Tanaka & Hayashi 1980). The authors postulated that the difference between their results and those of Wechter et al (1974) could be due to either the structural differences between the two drugs, or species variability in their metabolism. Tanaka & Hayashi (1980) proposed that the inversion process could involve an enolic form of either the carboxylic acid or an activated thioacyl derivative (eqn 6). A possible mechanism for the inversion of cicloprofen involving an enolic form of the carboxyl group was proposed by Lan et al (1976) (eqn 6).

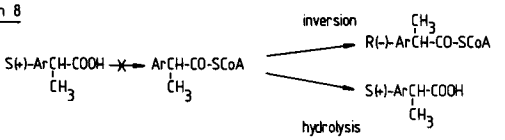
A recent study (Nakamura et al 1981) using α-methine deutero- and α-methyltrideutero-ibuprofen in the rat has extended the observations of Tanaka & Hayashi (1980) discussed above. Following the administration of $R(-)$-α-methine-deuteroibuprofen to rats, the deuterium content of the $S(+)$-enantiomer in plasma was almost zero, and the deuterium content of the $R(-)$-enantiomer also gradually decreased. However, when $R(-)$-α-methyl trideuteroibuprofen was administered, the $S(+)$-enantiomer formed retained all 3 deuterium atoms.

Nakamura et al (1981) found similar results in the rat isolated perfused liver to those obtained in-vivo. Those workers extended their studies using a rat liver homogenate preparation and isolated ibuprofen CoA thioester, the synthesis of which was found to require both Coenzyme A and ATP. Both $R(-)$ and $S(+)$-synthetic ibuprofen-CoA thioesters were found to undergo racemization and hydrolysis on incubation with rat liver homogenates. However, only $R(-)$-ibuprofen was converted to a thioester enzymatically (eqns 7, 8). Thioester synthetase activity was found in both mitochondria and microsomes, whilst isomerase and hydrolase activity were present in cytoplasm, mitochondria and microsomes, although activity was very low in the latter organelle.

Equation 7



Equation 8



The mechanism of the inversion process therefore seems to proceed via an acyl CoA thioester intermediate, which can undergo either chiral inversion about the α-carbon atom or hydrolysis. Nakamura et al (1981) proposed the existence of an acyl-CoA racemase enzyme similar to that proposed by Wechter et al (1974). The nature of the intermediate

in the racemization of the acyl-CoA thioester is presently the subject of speculation.

However, the possibility that the inversion is a chemical, rather than an enzymic reaction must be considered. It is well known that the protons of the carbon α- to the carbonyl group of an acyl CoA thioester are highly acidic (Lynen 1953). This is the basis of the reactivity of acetyl CoA in fatty acid biosynthesis and other reactions. In the case of acyl CoAs with a chiral centre α- to the carbonyl group, the lability of such an α-methine proton due simply to its acidity would result in racemization. If the formation of the acyl CoA were restricted to one enantiomer only, then chiral inversion to the other enantiomer, up to 100%, would logically result from the sequence of reactions shown in equations 7 and 8, without invoking an enzyme to carry out the inversion.

11. *Concluding Remarks*

The chiral inversion of the *R*(−)-2-arylpropionic acids to their *S*(+)-isomers is a novel metabolic route since (a) it is without precedent as the inversion of the single chiral centre of these compounds, and (b) the chiral inversion involves no other change to the acid: the only covalent modification to the acid is the exchange of the α-methine proton, evidenced by studies with specifically deuterated substrates. The inversion in every case involves the conversion of the *R*(−)-isomers to their *S*(+)-antipodes, and the reverse reaction has never been found to occur.

Although knowledge of the mechanism of chiral inversion is incomplete at present, it would appear to be another example of the central role of acyl CoAs in the biochemical pharmacology of carboxylic acids. These high-energy thioesters are well established as the obligatory intermediates in the amino acid conjugations (see Caldwell et al 1980) and are also presumed to be involved in the formation of hybrid fatty acids and hybrid triglycerides of xenobiotic acids (see Caldwell & Marsh 1983), bile salt and bile acid conjugates (Quistad et al 1982), and cholesteryl ester formation (Fears et al 1982). Additionally, acyl CoAs are intimately involved in the hypolipidaemic action of various aryl-acetic and -propionic acids (Fears et al 1978: Fears & Richards 1981). It is of particular interest to note that the 2-arylpropionic acids do not readily give rise to amino acid conjugates (Caldwell 1982), which thus throws emphasis upon chiral inversion as one of the alternative fates of their acyl CoAs. The stereospecific formation of these thioesters from *R*(−)-2-arylpropionates suggests that the structure-metabolism relationships discerned amongst the amino acid conjugations of the various aryl carboxylic acids (Caldwell 1978, 1982) arise from specificity exerted both in acyl CoA formation and by the amino acid *N*-acyl transferases, rather than solely in acyl CoA formation as proposed previously (Dixon et al 1977b; Caldwell 1978, 1982). The various metabolic options open to 2-arylpropionates are illustrated in Fig. 6.

The conversion of the inactive *R*(−)-2-arylpropionic acids to the active *S*(+)-isomers is clearly a reaction of great pharmacological significance. Since the toxicity of NSAIDs to the gastrointestinal tract and actions on blood clotting arise from

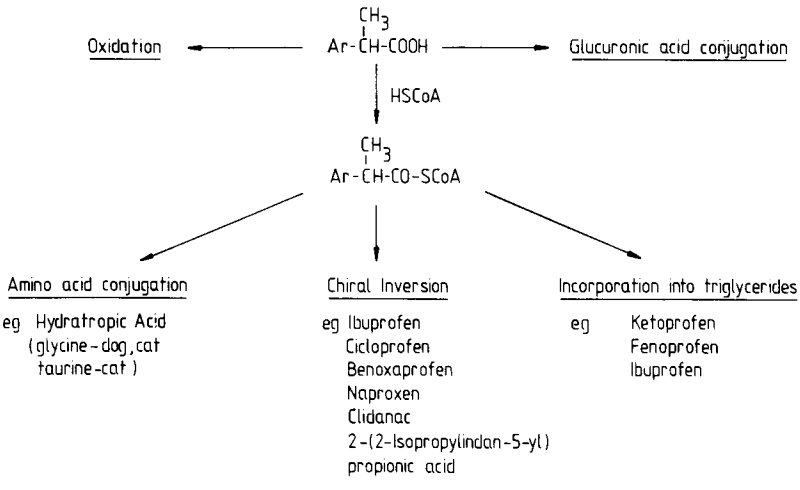


FIG. 6. Summary of metabolic options open to 2-arylpropionic acids in mammals.

the same mechanisms as their anti-inflammatory actions, viz. inhibition of prostaglandin biosynthesis, presumably the chiral inversion of such acids will similarly enhance their toxicity. This influence of the chiral inversion upon the pharmacological and toxic effects of these acids assumes great importance when inter-species variation in its rate and extent are considered (see Table 2). These differences cause considerable difficulty in interpreting inter-species variation in response. Furthermore, when such inter-species variations occur, it is to be expected that there will occur inter-individual variation in man (Williams 1974) with consequent variation in response.

Table 2. Species occurrence of the chiral inversion of 2-arylpropionic acids in mammals

Drug	Species	Reference
Benoxaprofen	Man	Bopp et al (1979)
	Man, Rat	Simmonds et al (1980)
Cicloprofen	Rat, Rhesus monkey, Dog, Man	Lan et al (1976) Kripalani et al (1976)
Clidanac	Guinea-pigs	Tamura et al (1981)*
Ibuprofen	Man	Mills et al (1973) Vangiessen & Kaiser (1975) Kaiser et al (1976) Wechter et al (1974) Brooks & Gilbert (1974)
	Rat	Nakamura et al (1981)
	Mouse, Rat, Guinea-pig	Adams et al (1976)
2-(2-Isopropylindan-5-yl) propionic acid	Rat	Tanaka & Hayashi (1980)
Naproxen	Rabbit	Goto et al (1982)

* Chiral inversion not found in rats and mice.

The information contained in this review is thus of substantial importance to the pharmacology and toxicology of the 2-arylpropionic acids. Indeed, the significance of the chiral inversion lies in its implications not only for metabolic studies with such compounds, but also for their preclinical screening, safety evaluation, clinical pharmacology and even for the form of the compound suitable for drug use. These may be summarized as follows:

1. Metabolic studies must define not only the structures of drug-related materials in blood, tissues and excreta, but also their enantiomeric composition with respect to the acid and species in question and to time (see Table 2 for differences between ibuprofen and clidanac).

2. Stereospecific assays are required to discern relationships between the concentration of an acid and any pharmacological, toxic or therapeutic effects elicited in animals and man. It is essential to assay the amount of the active isomer present, and an assay of the total quantity of active and inactive isomers will be at best of limited value, and may well be highly misleading.

3. It is likely that benefits will be obtained from the use of the *S*(+)-enantiomers of 2-arylpropionates as drugs as opposed to the racemates. This is only found at present in the case of naproxen. In cases of rapid inversion, the inactive *R*(−) isomer serves merely as a prodrug for the active *S*(+)-antipode. Where inversion is slow, the *R*(−)-enantiomer is an unnecessary impurity in the active *S*(+) form. Use of the *S*(+)-enantiomers would permit reduction of the dose given, remove variability in rate and extent of inversion as a source of variability in therapeutic response and would reduce any toxicity arising from non-stereospecific mechanisms.

Acknowledgement

We are grateful to Prof. R. L. Smith for helpful discussion.

Note Added in Proof

The biological activity of the racemic, *S*-(+) and *R*-(−) forms of indoprofen (α-[4-(2-isoindolinyl-1-one)-phenyl] propionic acid) has been reported recently (Buttinoni et al 1983). The *R*-(−)-enantiomer was found to be essentially inactive (>100 times less potent than the *S*-(+)) in the in-vitro inhibition of prostaglandin synthesis, ca 20 times less pharmacologically active in rats and mice in-vivo and 16–17 times less toxic in rats than the *S*-(+)-enantiomer. Buttinoni et al (1983) concluded that chiral inversion of indoprofen occurred to a small extent in these species. A g.l.c. analysis of the individual enantiomers in plasma has been reported (Tosolini et al. 1974). It was found, on oral administration of the racemate to man, that the absorption rates of the enantiomers were similar but the plasma t½ and the area under the plasma concentration time curve of the *R*-(−)-enantiomer was less than that of the *S*-(+)-enantiomer.

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