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THE METABOLIC CHIRAL INVERSION OF 2-PHENYLPROPIONIC ACID IN RAT, MOUSE AND RABBIT

SYLVIE FOURNEL* and JOHN CALDWELL†
Department of Pharmacology, St. Mary's Hospital Medical School, London W2 1PG, U.K.

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Abstract—The metabolic chiral inversion of the 2-arylpropionic acids has been investigated in laboratory animals, using the simplest congener, 2-phenylpropionic acid, as a model compound. The chiral inversion was found to occur after administration of the racemate to the rat and rabbit, but not in the mouse. The formation of the ester glucuronide was enantioselective for the *R*-(−)-isomer in the rat and rabbit, but was preferential for the *S*-(+)-form in the mouse. In the rat, the extent of inversion from *R*-(−) to *S*-(+) was greater at a dose of 30 mg/kg than at 150 or 300 mg/kg. The enantiomeric composition of the acid in urine was the same when the racemate was given orally or by i.p. injection. When the *R*-(−)isomer was given to rats, some 30% of the excreted acid was in the *S*-(+)-form, but when the *S*-(+)-isomer was given, the inversion was much less evident. In this case, the *S*/*R* ratio of the excreted phenylproprionic acid was *ca* 9:1. Following the administration of the racemate to rats, the plasma elimination half-life of the *R*-(−)-form was shorter (3.0 vs 4.8 hr for the *S*-(−)-isomer); this was due to its considerably greater plasma clearance (65.9 vs 43.6 μg/ml hr), since the volumes of distribution of the enantiomers were the same. The *S*/*R* ratio of 2-phenylpropionic acid in plasma rose progressively with time, from 1:1 in the dose solution to 2.1:1 at 8 hr.

There is increasing interest in the stereochemical aspects of drug metabolism and disposition, both in terms of the differential handling in the body of the individual enantiomers of racemic drugs, and of the preferential formation of enantiomers of chiral metabolites [1, 2]. A fascinating situation occurs with the 2-arylpropionic acids, or 'profens', currently an important group of non-steroidal anti-inflammatory drugs (NSAIDs). These drugs have within their structures a chiral centre which undergoes a unique and biologically fortuitous inversion from the inactive *R*-isomer to its biologically active *S*-antipode, without any other change to the molecule [3]. This chiral inversion is a general feature of the fate of 'profen' NSAIDs, although there do occur variations between species and drug in its extent (see the review by Hutt and Caldwell [3]).

Various reasons have been put forward for the importance of this reaction [3–5], which indicate a need for a fuller consideration of its occurrence, mechanism, etc. Most of the 'profen' NSAIDs present difficulties with such studies, since they undergo various routes of metabolism giving a number of products, often diastereoisomeric, whose stereochemistry must be defined: this is particularly the case with ibuprofen [3, 6]. There is thus a need for studies with a model substrate with which these problems are minimized. We have chosen the simplest congener, 2-phenylpropionic (hydratropic) acid (Fig. 1), since its metabolism in rodent species is known to involve only glucuronic acid conjugation [7]. Evidence from early studies of its fate suggested that it underwent inversion of its chiral centre [3], and this has been confirmed by the present study and other, concurrent, reports in the literature [8–10]. Although 2-phenylpropionic acid (Fig. 1) does not possess anti-inflammatory activity, it is of interest to note that, like a number of arylacetic and aryloxyacetic acids, it is an inducer of bilirubin UDP-glucuronyltransferase and cytochrome P-450 dependent lauric acid 12-hydroxylation in rat liver [11]. This induction is apparently due principally to the *S*-(+)-enantiomer [12].

In this paper we present an account of the stereochemical aspects of the disposition of 2-phenylproprionic acid in three laboratory species, and the effect of dose size and route of administration in the rat.

MATERIALS AND METHODS

Compounds. The following compounds were purchased: [carboxyl-^{14}C]-*RS*-2-phenylpropionic acid,

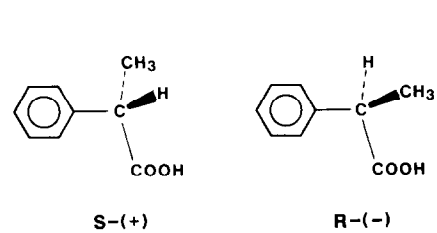

Fig. 1. Flying wedge diagrams depicting the three-dimensional structures of the enantiomers of 2-phenylpropionic acid.

* Present Address: Centre du Medicament, Université de Nancy, 30 rue Lionnois, F-54001 Nancy Cedex, France.
† To whom correspondence should be addressed.

specific radioactivity 388 μCi/mg, radiochemical purity >98% by paper chromatography, TLC and HPLC (Amersham International, Aylesbury, U.K.); *R*-(−)- and *S*-(+)-2-phenylpropionic acids, both of 99% optical purity (Lancaster Synthesis, Lancaster, U.K.); *RS*-2-phenylpropionic acid, 1-naphthylacetic acid, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (DEC) and *R*-(+)-1-[naphthen-1-yl]ethylamine (NEA), optical purity 99% (Aldrich Chemical Co., Gillingham, Dorset, U.K.). HPLC solvents were purchased from Fisons plc (Loughborough, U.K.).

Animals and dosing. Male and female Wistar albino rats (body wt 200–250 g; Oxford Laboratory Animal Company, Oxford, U.K.), male CBA mice (body wt 25 g; from a colony maintained by the Animal Department of St. Mary's Hospital Medical School) and female Dutch rabbits (body wt 3 kg; Ranch Rabbits, Crawley Down, Sussex, U.K.) were used in this study, and had free access to pelleted food and water throughout. The animals were housed in cages appropriate to their size, allowing the separate collection of urine and faeces.

2-Phenylpropionic acid was administered dissolved in propane-1,2-diol by i.p. injection to all three species, and also by stomach tube to rats. Excreta were collected daily for two days after dosing, and stored at −20° until analysed.

Other rats were dosed with 2-phenylpropionic acid, decapitated under light ether anaesthesia at various intervals and exsanguinated into tubes containing lithium heparin. Plasma was separated by centrifugation and stored at −20° until analysed.

Radiochemical techniques. ^{14}C in plasma, excreta and solutions was estimated as described by Sangster *et al.* [13] by liquid scintillation spectrometry using a Packard TriCarb Minaxi spectrometer, Model 4450, and Scintran Cocktail T (toluene–Triton-X-100 based; BDH Chemicals, Poole, Dorset, U.K.). Quench correction was achieved by reference to an external standard.

High pressure liquid chromatography (HPLC). This used a Waters Associates (Harrow, Middlesex, U.K.) M-45 pump and M-440 u.v. detector equipped with a 254 nm filter, linked to a Philips (Cambridge, U.K.) PM 8251 chart recorder. The column was housed in a Waters Z-module, and samples were introduced into the system with a Rheodyne 7521 valve loop injector. Two systems were used: A, column: Waters Radial-PAK cartridge 100 × 5 mm i.d. containing 10 μ Porasil; mobile phase hexane: ethyl acetate (4:1 by volume), flow rate 1.6 ml/min. In this system, the *R*-(+)-1-[naphthen-1-yl]ethylamides of *R*-(−)- and *S*-(+)-2-phenylpropionic acid and 1-naphthylacetic acid had retention times 8.5, 5.5 and 20.5 min, respectively. B, column: Waters Radial-PAK cartridge 100 × 5 mm i.d. containing μBondapak C_{18}; mobile phase 30% v/v aqueous methanol containing 3% v/v glacial acetic acid, flow rate 2 ml/min. In this system, the retention time of 2-phenylpropionic acid was 17.9 min.

In some experiments, 0.5 min fractions of the column eluant were collected into scintillation minivials with a LKB 2112 RediRac fraction collector. Cocktail T added and counted for ^{14}C.

Determination of the enantiomeric composition of 2-phenylpropionic acid in plasma and urine. To aliquots (0.1–0.5 ml) of plasma, urine or 1:1 mixtures of urine and 1 M NaOH incubated at 37° for 30 min, was added 100 μg 1-naphthylacetic acid (10 μl of a 10 mg/ml solution), followed by 2 ml 1 M HCl. The whole was extracted with 7 ml benzene on a vortex mixer, the layers separated by centrifugation, the benzene layer carefully dried (anhydrous Na_2SO_4), filtered and evaporated to dryness *in vacuo*. The residue was taken up in 1 ml dichloromethane and treated with DEC and NEA as described by Hutt *et al.* [10] to convert the acids to their respective *R*-(+)-1-[naphthen-1-yl]ethylamides. These were then analysed using HPLC system A.

The enantiomers were quantitated by reference to calibration curves constructed over the range 0–250 μg/ml of each isomer of 2-phenylpropionic acid, at a constant total concentration of 250 μg/ml, relating the ratio of the heights of the peaks of the diastereoisomeric amides of 2-phenylpropionic acid and the amide of the internal standard to the concentration of the enantiomer. These were linear over the concentration range studied, and were established freshly on each occasion the assay was used. In experiments involving the administration of [^{14}C]-*RS*-2-phenylpropionic acid, the enantiomers were also quantitated by counting fractions of column eluant as described.

RESULTS

Elimination of ^{14}C

Table 1 shows the urinary elimination of ^{14}C following the administration of [^{14}C]-*RS*-2-phenylpropionic acid (150 mg/kg i.p.) to rats, rabbits and mice. In each case an acceptable (*ca* 50% of the dose) recovery of ^{14}C was obtained in the urine in 48 hr, with the bulk of the excretion occurring on the first day. No attempt was made to achieve a complete balance of administered ^{14}C; in the present study, recoveries were generally less than those reported previously [7], but allowance must be made for differences in dose size and vehicle and/or route of administration.

Characterization of urinary metabolites

When neat urine was examined by HPLC in system

Table 1. Excretion of ^{14}C by rats, rabbits and mice given *RS*-[^{14}C]-2-phenylpropionic acid

	% administered dose recovered in urine in: 0–24 hr	24–48 hr	0–48 hr
Rat male	33.1 ± 3.7	24.4 ± 9.2	57.5 ± 11.6
female	41.0 ± 5.0	5.9 ± 7.1	46.6 ± 4.9
Rabbit	40.8, 53.5	5.8, 1.4	46.6, 54.9
Mouse	42.5 ± 7.6	8.8 ± 3.6	51.3 ± 5.6

Animals received *RS*[^{14}C]-2-phenylpropionic acid by i.p. injection dissolved in propane-1,2-diol at a dose of 150 mg/kg, and were housed in suitable metabolism cages for 48 hr with free access to food and water throughout. Values quoted are the means ± SD for at least four rats and mice, and individual values for two rabbits. The excretion of ^{14}C was monitored by liquid scintillation counting. For further details, see the text.

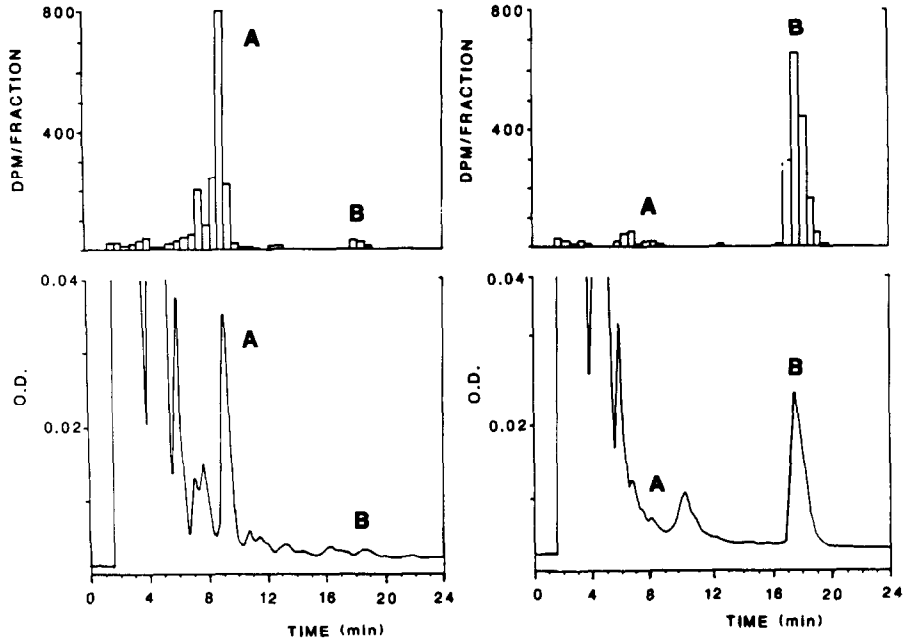

Fig. 2. HPLC traces of the 0–24 hr urine of a male rat given *RS*-[^{14}C]-2-phenylpropionic acid, obtained before and after treatment with NaOH. Dose 150 mg/kg, administration and urine collection, HPLC system B (reversed phase) as described in the text. The left-hand panels show traces for untreated urine, those on the right urine after treatment with an equal volume of 1 M NaOH at 37° for 30 min. Upper panels give histograms of the ^{14}C content of 0.5 min fractions of column eluant, lower panels give the corresponding u.v. absorption trace, detector 254 nm. Peaks labelled 'A' correspond to 2-phenylpropionyl glucuronide, 'B' to 2-phenylpropionic acid.

B, two discrete ^{14}C-containing peaks were seen, a major one (*ca* 90% of applied radioactivity) with retention time 9.0 min and a minor one (<10% of applied radioactivity) with retention time 17.9 min. Treatment of the urine with 1 M NaOH resulted in the virtually complete disappearance of the major peak, and >95% of the ^{14}C applied was recovered in the peak of retention time 17.9 min, which corresponds to 2-phenylpropionic acid. Typical chromatograms of rat urine before and after treatment with NaOH are presented in Fig. 2.

These data are consistent with the presence in urine of 2-phenylpropionic acid both free and as its ester glucuronide, and Table 2 gives quantitative data for the elimination of these two compounds in the three species examined.

Enantiomeric composition of excreted 2-phenylpropionic acid

(a) *Following* [^{14}C]-RS-*2-phenylpropionic acid to various species.* [^{14}C]-2-Phenylpropionic acid was extracted from urine, before and after mild alkali treatment, and converted to its diasteroisomeric naphthylethylamides as described. These were separated and quantitated by HPLC in system A, followed by scintillation counting of fractions of column eluant for ^{14}C. A typical chromatogram is shown in Fig. 3.

Table 3 gives details of the relative proportions of 2-phenylpropionic acid present in the urine of the three species examined as each of its enantiomers, as such and following mild alkaline treatment to cleave its ester glucuronide. In male and female rats and rabbits, it is seen that there is an enrichment of the excreted material with respect to the *S*-(+) enantiomer as compared with the administered racemate (a 50:50 mixture), and this is far more marked in the 24–48 hr than in the 0–24 hr urine. It is also noteworthy that the *S*/*R* ratio for the free acid is smaller than that for the total following alkali treatment, suggesting that there may be slight stereoselectivity in the glucuronidation of the *S*-(+) enantiomer.

Table 3 also shows the results obtained in mice, which are very different from those in the other two species tested. The enantiomeric composition of the total excreted 2-phenylpropionic acid in both the 0–24 hr and 24–48 hr urines is identical with that of

Table 2. Metabolism of *RS*-[^{14}C]-2-phenylpropionic acid by rats, rabbits and mice

	% [^{14}C] dose present as: Free acid	Glucuronide
Rat male	3.0 ± 1.4	30.1 ± 3.7
female	7.4 ± 8.2	37.4 ± 8.2
Rabbit	1.2, 11.2	39.4, 42.1
Mouse	13.4 ± 6.2	29.1 ± 6.2

Animals, dosing and collection of urine as in Table 1. Urinary metabolites were assayed by HPLC as described in the text. Figures are % ^{14}C dose in 0–24 h urine as that metabolite, mean ± SD for rats and mice (N = 4); individual values for two rabbits are presented.

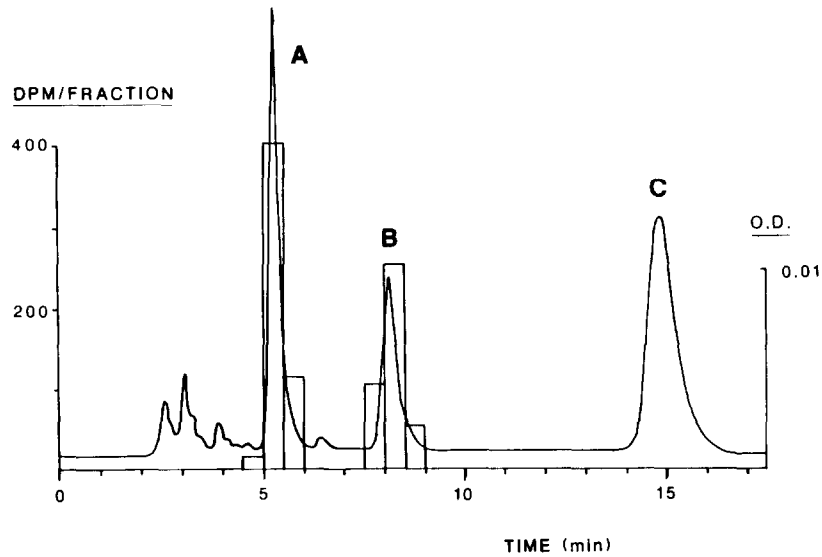

Fig. 3. HPLC determination of the enantiomeric composition of 2-phenylpropionic acid in the urine of a mouse given *RS*-[^{14}C]-2-phenylpropionic acid. Dose 150 mg/kg, administration and urine collection, HPLC system A (normal phase) as described in the text. Urine was treated with NaOH, extracted and derivatized as described in the text. The histogram gives the ^{14}C content of 0.5 min fractions of column eluant and the continuous trace shows u.v. absorption at 254 nm. Peak *A* is the *S*/*R* and peak B the *R*/*R* diastereoisomer; peak C is the naphthylethylamide of 2-naphthylacetic acid, the internal standard.

the administered racemate, i.e. a 50:50 mixture, thus showing that the chiral inversion reaction apparently does not occur in this species. However, the free acid contains an excess of the *R*-(−) enantiomer, suggesting that the glucuronidation is selective for the *S*-(+) antipode. This stereoselectivity in the mouse is the reverse of that seen in the other two species studied.

(b) *Studies with individual enantiomers in the male rat, and the influence of dose size and route of administration.* Table 4 presents results of studies in male rats involving the administration of the individual enantiomers of 2-phenylpropionic acid, and the influence of dose size upon the enantiomeric composition of excreted 2-phenylpropionic acid.

After the administration of *R*-(−)-2-phenylpropionic acid at a dose of 150 mg/kg p.o., some 53% of the dose was recovered in the 0–24 hr urine, made up of 70% *R*-(−) and 30%) *S*-(+) enantiomers. Very similar results were obtained following the administration of 300 mg/kg [68% *R*-(−), 32% *S*-(+), but with a dose of 30 mg/kg, the corresponding values were 55% *R*-(−)/45% *S*-(+). When rats received *S*-(+)-2-phenylpropionic acid, a total of 65% of the dose was excreted in the 24 hr urine, made up of 10% *R*-(−) and 90% *S*-(+) isomers. As was the case after the administration of racemic 2-phenylpropionic acid (see Table 3), the extent of inversion observed, as indicated by the *S*/*R* ratios, was generally greater on the second day after dosing than on the first, and that the ratios for the free acid are less than those for the total acid (free plus glucuronide), again suggesting a stereoselectivity of conjugation of the *S*-(+) enantiomer.

Table 3. Enantiomeric composition of 2-phenylpropionic acid in urine of rats, rabbits and mice

	% 2-phenylpropionic acid excreted in that form:							
	Male rat		Female rat		Rabbit		Mouse	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
R free	47	37	36	n.d.	27	31	65	65
total	34	22	27	21	29	32	48	50
S free	53	63	64	n.d.	73	69	35	35
total	65	78	73	79	71	68	52	50
Ratio *S*/*R*								
free	1.13	1.70	1.78	—	2.70	2.23	0.54	0.54
total	1.91	3.54	2.70	3.76	2.45	2.12	1.08	1.00

n.d. = not detected, — = not calculated.

Animals, dosing and collection of urine as in Table 1. The enantiomeric composition of 2-phenylpropionic acid in urine was determined by HPLC before and after treatment with mild alkali, as described in the text. Figures quoted are the means of four rats and mice or two rabbits: in no case was the variability >6%, and thus indications of standard error are omitted here.

Table 4. Enantiomeric composition of 2-phenylpropionic acid in rat urine following the administration of different doses of *R*, *S* and *RS*-2-phenylpropionic acid to male rats

	% 2-phenylpropionic acid excreted in that form after administration of:									
	30 mg/kg		150 mg/kg				300 mg/kg			
	R		*R*		*S*		*R*		*S*	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
R free	56	n.d.	75	42	9	19	67	n.d.	14	n.d.
total	55	37	70	43	7	14	68	35	9	11
S free	44	n.d	25	58	91	80	33	n.d.	86	n.d.
total	45	63	30	57	93	86	32	65	91	89
Ratio *S*/*R*										
free	0.78	—	0.33	1.38	10.1	4.2	0.49	—	6.1	—
total	0.82	1.70	0.43	1.33	13.2	6.1	0.47	1.85	10.1	8.1

n.d. = not detected, — = not calculated.

Male rats received the individual enantiomers of 2-phenylpropionic acid dissolved in propane-1,2-diol by i.p. injection, at the doses stated in the table. The enantiomeric composition of 2-phenylpropionic acid in urine was determined by HPLC before and after treatment with mild alkali, as described in the text. Figures quoted are the means of 4 rats: in no case was the variability >6%, and thus indications of standard error are omitted here.

The enantiomeric composition of 2-phenylpropionic acid in the urine of rats given the racemic acid i.p. or p.o. was essentially independent of the route of administration, as shown by the data presented in Table 5.

Plasma pharmacokinetics of the enantiomers of 2-phenylpropionic acid in male rats given the racemate p.o.

Figure 4 shows the plasma level–time curves for total 2-phenylpropionic acid and its individual enantiomers following the oral administration of *RS*-[^{14}C]-2-phenylpropionic acid, and Table 6 presents pharmacokinetic parameters describing these curves. It is apparent that the *R* isomer is eliminated far faster than the *S* antipode, although their volumes of distribution are very similar. The *S* enantiomer thus has a greater AUC than the *R* isomer. The faster elimination of the *R* isomer is due to its loss both by glucuronidation and/or excretion and by inversion to the *S* antipode: by contrast only the first of these fates is a major option for the *S* isomer. There occurs a progressive enrichment of plasma 2-phenylpropionic acid with respect to the *S* enantiomer and this is shown in Fig. 5, which plots the *S*/*R* ratio of plasma 2-phenylpropionic acid against time.

Table 5. Influence of route of administration upon the metabolic chiral inversion of 2-phenylpropionic acid in male rats

	% Urinary 2-phenylpropionic acid present as that isomer:			
	p.o.		i.p.	
	Day 1	Day 2	Day 1	Day 2
R free	41	24	47	37
total	38	22	34	22
S free	59	75	53	63
total	62	78	65	78
Ratio *S*/*R*				
free	1.43	3.12	1.13	1.70
total	1.63	3.54	1.91	3.54
% Administered dose recovered as *R* and *S* enantiomers				
Mean	46.9	4.9	33.1	24.4
S.D.	3.3	1.0	3.7	9.2
	(N = 4)			

Male rats received *RS*-2-phenylpropionic acid dissolved in propane-1,2-diol at a dose of 150 mg/kg, by i.p. injection or by stomach tube, and their 0–24 and 24–48 hr urines collected. The enantiomeric composition of 2-phenylpropionic acid in urine was determined by HPLC before and after treatment with mild alkali, as described in the text. Figures quoted are the means of four rats: in no case was the variability >6%, and thus indications of standard error are omitted here.

Table 6. Pharmacokinetic parameters describing the plasma concentrations of *R*, *S*, and *RS*-2-phenylpropionic acid in male rats receiving *RS*-[^{14}C]-2-phenylpropionic acid

	Isomer		
	RS	*R*	*S*
Cp_0 (μg/ml)	121.3	62.3	60.2
$t_{\frac{1}{2}}$ (hr)	3.83	3.00	4.78
k_{el} (hr^{-1})	0.1811	0.2313	0.1451
AUC (μg/ml hr)			
0–8 hr	486.5	214.5	272.0
0–inf	699.1	284.5	429.7
Vd (ml)	309.2	301.1	311.3
CL (ml/hr)	53.6	65.9	43.6

Dose 150 mg/kg i.p., administration as described in the text. Groups of rats were sacrificed and exsanguinated at various times up to 8 hr following dosage, and the plasma levels of the enantiomers of 2-phenylpropionic acid determined by HPLC as described in the text. Pharmacokinetic parameters were calculated from the mean data, since separate animals were used for each of the time points.

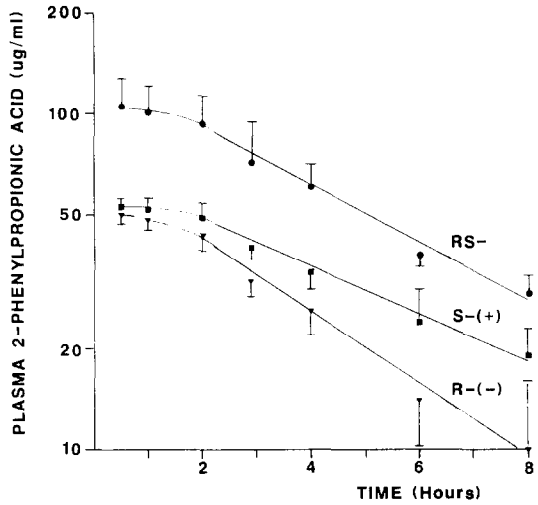

Fig. 4. Plasma level–time curves for total (*RS*), *R*- and *S*-2-phenylpropionic acid in male rats given *RS*-[^{14}C]-2-phenylpropionic acid. Dose 150 mg/kg, administration as described in the text. Pairs of rats were sacrificed and exsanguinated at various times up to 8 hr following dosage, and plasma levels of the enantiomers of 2-phenylpropionic acid determined by HPLC system A as described in the text. Data are presented as means with error bars to indicate ranges.

DISCUSSION

The metabolic fate of 2-phenylpropionic (hydratropic) acid was first investigated by Kay and Raper [14], who found that it was converted to its ester glucuronide in rabbit and dog, and also gave rise to a glycine conjugate in the latter species. Subsequently, the ester glucuronide was isolated from rabbit urine and characterized [15]. The first studies with [^{14}C]-labelled material were those of Dixon *et al.* [7], who confirmed the earlier findings of ester glucuronidation. This was the sole route of metabolism in laboratory animals and primate species. These authors also found the glycine and taurine conjugates in ferret and cat, and the latter species was unable to glucuronidate the acid [16].

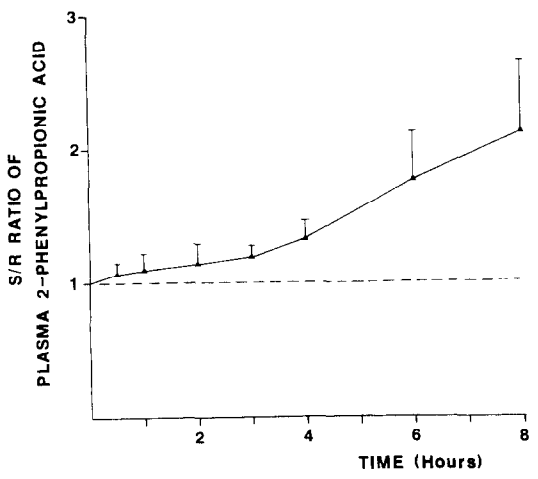

Fig. 5. Enantiomeric ratio (*S*/*R*) of 2-phenylpropionic acid as a function of time in the plasma of male rats given *RS*-[^{14}C]-2-phenylpropionic acid. For details of the experiment, see the legend of Fig. 4 and the text. Data are presented as means with error bars to indicate ranges.

These various studies did not consider the stereochemistry of the metabolism of 2-phenylpropionic acid, although Robinson *et al.* [15] did administer the individual enantiomers: they were able to isolate the glucuronide of the *S*-(+) isomer in a crystalline form, but not that of the *R*-(−) antipode. This is no doubt due to the differential solubilities of these diastereoisomeric conjugates (glucuronic acid is, of course, chiral).

Recent interest in the stereochemical aspects of the metabolism of 2-phenylpropionic acid arises from its use as a model for the 'profen' NSAIDs (see Introduction) and, additionally, from the stereoselectivity of its enzyme-inducing properties [11, 12]. In the last year, in addition to the present work, Yamaguchi and Nakamura [9] in the rat, Tanaka (personal communication, 1985) in the dog, and Meffin *et al.* [17] in the rabbit, have each reported that, like many other 'profens', 2-phenylpropionic acid undergoes a chiral inversion from the *R* enantiomer to the *S* form *in vivo*.

The present report confirms and extends the findings of these workers with respect to the rat and rabbit, and allows comparisons to be made. The reaction is apparently more rapid in the rabbit than the rat, since the *S*/*R* ratios are the same on days 1 and 2 in this species, unlike the rat, where the extent of inversion is greater on day 2. Furthermore, the female rat is more active than the male (Table 3). This is the first report concerning the mouse, which apparently does not effect the chiral inversion reaction, at least with respect to 2-phenylpropionic acid. Other examples of combinations of drug and animal species where this reaction is not extant include clidanac in the rat and mouse and indoprofen in man [5].

The results after the administration of *S*-(+)-2-phenylpropionic acid to rats are noteworthy. The metabolic chiral inversion is usually thought of as unidirectional, from *R*-(−) to *S*-(+), but it is clear from Table 4 that, at doses of 150 and 300 mg/kg, small but significant amounts of the *S*-(+) enantiomer are inverted *in vivo*. The quantities of the *R*-(−) isomer found in urine after the administration of its *S*-(+) antipode are readily measurable, i.e. well in excess of the limits of detection, with the assay method used [10]. Lee *et al.* [6] have tentatively suggested that this reverse reaction may occur with ibuprofen: it may have been overlooked in other cases due to the complications introduced by the metabolism of the substrate by competing routes, which may introduce their own stereochemical complications by giving rise to diastereoisomeric metabolites. Examples of this include ibuprofen [3] and loxoprofen [18].

Studies in the rat have shown that this reaction is unaffected by route of administration (p.o. or i.p.) and thus is unlikely to exhibit a 'first-pass effect'. The extent of inversion is less at higher doses (150 and 300 mg/kg) than low (30 mg/kg), but the dif-

ferences are small. In all cases examined, the extent of inversion is greater on day 2 than day 1.

Both enantiomers of 2-phenylpropionic acid undergo ester glucuronidation, and this reaction exhibits a stereoselectivity for the *S*-(+) enantiomer in rat and mouse. Indeed, in the mouse, although the enantiomeric composition of urinary total 2-phenylpropionic acid (free + glucuronide) is unaltered from that of the administered racemate, i.e. no inversion had occurred, the free fraction is predominantly (65%) the *R* isomer, showing the preferential conjugation of the *S*. However, in the rabbit, the enantiomeric composition of free and conjugated fractions is the same (Table 3), showing that glucuronidation is not stereoselective in this case. Taken together, the evidence shows that the processes of glucuronidation and chiral inversion are entirely independent, as has been shown for ibuprofen in humans [6].

Plasma pharmacokinetic studies in the rat have shown that the *R* isomer is more rapidly cleared than its *S* antipode. This is an observation which has been made in other species with other compounds [4]. The faster elimination of the *R* isomer is due to its loss both by glucuronidation and/or excretion and by inversion to the *S* antipode; by contrast only the first of these fates is open to the *S* isomer.

The mechanism of the chiral inversion of the 2-arylpropionates is the subject of speculation, and the various pathways proposed have been reviewed by Hutt and Caldwell [3]. Available experimental evidence supports the idea that the critical intermediates are acyl CoA thioesters of the acids [19], but this is not proven at present. The demonstration of the occurrence of the inversion reaction with this model compound may permit further insight into this problem, by allowing the design of mechanistic experiments not compromised by the multiplicity of competing metabolic routes open to the substrate.

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Note added in proof: Readers' attention is drawn to the recent paper of Meffin *et al.*, describing in detail the inversion of 2-phenylpropionic acid in the rabbit (P. J. Meffin, B. C. Sallustio, Y. J. Purdie and M. E. Jones, *J. Pharmac. exp. Ther.* **238**, 280 (1986)).

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