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Optical Inversion of (2*R*)- to (2*S*)-Isomers of 2-[4-(2-Oxocyclopentylmethyl)-phenyl]propionic Acid (Loxoprofen), a New Anti-inflammatory Agent, and Its Monohydroxy Metabolites in the Rat

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Enantiomer ratios of 2-[4-(2-oxocyclopentylmethyl)phenyl]propionic acid (loxoprofen) and its monohydroxy metabolites in plasma of rats were determined by high performance liquid chromatography (HPLC) after derivatization with the chiral reagent, (1*S*)-1-(4-dimethylaminonaphthalen-1-yl)ethylamine. The ratios of (2*S*)- to (2*R*)-isomers of the parent acid and 2-[4-(*trans*-2-hydroxycyclopentylmethyl)phenyl]propionic acid (*trans*-alcohol) increased rapidly with time after oral administration of racemic and (2*R*)-loxoprofen, while the (2*S*)-configuration remained completely intact after dosing with (2*S*)-isomer. The results clearly indicate the occurrence of irreversible optical inversion of (2*R*)- to (2*S*)-loxoprofen in rats.

The administration of *trans*- and *cis*-alcohols showed that: (1) the optical inversion also occurs in these monohydroxy metabolites, (2) the *cis*-alcohol is easily converted to the *trans*-alcohol through the parent acid, but the latter is not converted to the former.

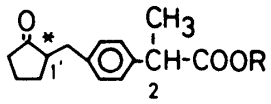
Keywords——anti-inflammatory agent; sodium 2-[4-(2-oxocyclopentylmethyl)phenyl]-propionate dihydrate; loxoprofen sodium; α-arylpropionic acid derivative; optical inversion; HPLC optical resolution

The structural determination of rat urinary metabolites of loxoprofen sodium has established that the active *trans*-alcohol metabolite possesses the (2*S*, 1′*R*, 2′*S*)-configurations[1] as shown in Chart 1. This finding suggested the involvement of two types of stereospecific biotransformation reaction in the formation of this active metabolite: one is the reduction of the α-substituted cyclopentanone to the corresponding (1′*R*, 2′*S*)-*trans*-alcohol and the other is the optical inversion of (2*R*)- to (2*S*)-configuration in the α-substituted propionic acid moiety. In fact, it has been confirmed that the cyclopentanone moiety of loxoprofen is stereospecifically reduced to afford the *trans*-alcohol by the purified loxoprofen-reducing enzyme.[2]

This paper deals with the optical inversion reaction in the propionic acid moiety of loxoprofen. Each enantiomer of loxoprofen and its monohydroxy metabolites in plasma of rats was determined by means of high performance liquid chromatography (HPLC) after oral administration of loxoprofen sodium, its (2*R*)- and (2*S*)-isomers, and the *trans*- and *cis*-alcohols.

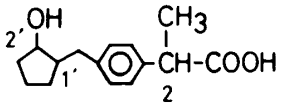
Experimental

Materials——The samples of (2*R*)- and (2*S*)-loxoprofen (optically pure in the α-substituted propionic acid moiety but a racemic mixture of the α-substituted cyclopentanone of loxoprofen), the *trans*- and *cis*-alcohols and their (2*S*)- and (2*R*)-isomers were synthesized by Naruto *et al.* at the Chemical Research Laboratories of Sankyo Co., Ltd., and their chemical structures are shown in Chart 1. The optical purities of (2*R*)- and (2*S*)-loxoprofen were more than 98%. *N*,*N*′-Dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole used for derivatization were obtained from Tokyo Kasei (Tokyo). (1*S*)-(+)-1-Naphthylethylamine (optical purity more than 99%) was purchased from Aldrich



R	C-2	
$Na \cdot 2H_2O$	(*RS*)	loxoprofen sodium
H	(*RS*)	loxoprofen
H	(*R*)	(2*R*)-loxoprofen
H	(*S*)	(2*S*)-loxoprofen

*(*RS*)



C-2	(C-1′, C-2′)		
(*RS*)	(1′*R*,2′*R*)+(1′*S*,2′*S*)		*cis*-alcohol
(*RS*)	(1′*R*,2′*S*)+(1′*S*,2′*R*)		*trans*-alcohol
(*S*)	(*R*)	(*S*)	(2*S*,1′*R*,2′*S*)-*trans*-alcohol (active metabolite)

Chart 1. Chemical Structures of Loxoprofen Sodium, the *trans*- and *cis*-Alcohols and Their Stereoisomers

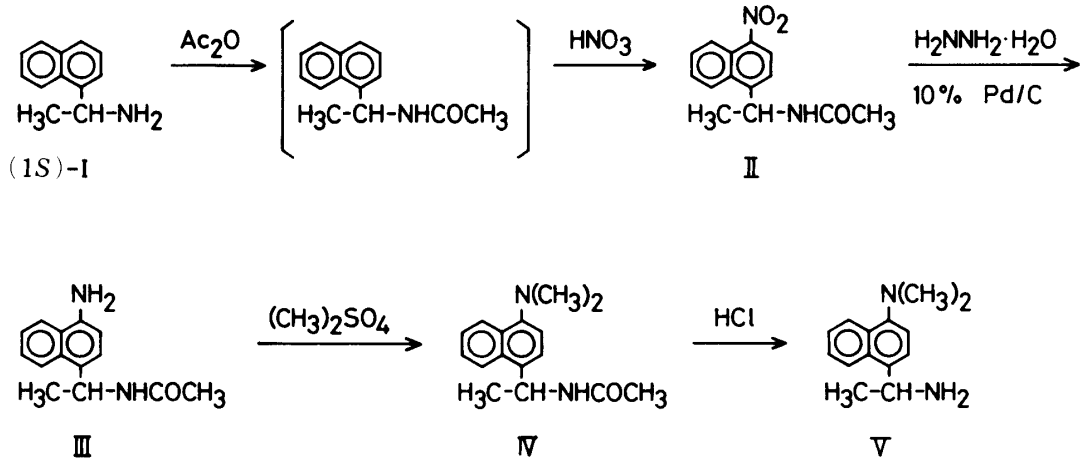


Chart 2. Synthetic Route to (1*S*)-1-(4-Dimethylaminonaphthalen-1-yl)ethylamine (V)

(Milwaukee, U.S.A.). All other reagents were of analytical-reagent grade.

Equipment——Nuclear magnetic resonance (NMR) spectra were recorded on a Varian EM-360L spectrometer (Palo Alto, U.S.A.) at 60 MHz using tetramethylsilane as an internal standard. Optical rotations were measured on a Perkin-Elmer 241 polarimeter (Norwalk, U.S.A.). Abbreviations: s=singlet, d=doublet and m=multiplet. A TWINCLE high performance liquid chromatograph (JASCO, Tokyo) equipped with a μPorasil column (3.9 mm × 30 cm) (Waters Assoc., Milford, U.S.A.) and FP-110 spectrofluorometer (JASCO, λ_{ex} 313 nm, λ_{em} 420 nm) was used. Samples were applied using a Waters Model U6K sample loop injector. Hexane–EtOAc (68:32) was employed as a mobile phase at a flow rate of 1.7 ml/min.

Synthesis of (1*S*)-1-(4-Dimethylaminonaphthalen-1-yl)ethylamine——The synthetic route to the title compound is shown in Chart 2.

(1*S*)-1-(4-Nitronaphthalen-1-yl)-*N*-acetylethylamine (II): (1*S*)-1-(Naphthalen-1-yl)ethylamine ((1*S*)-I, 9.6 g) was added to Ac_2O (37 ml) and the reaction mixture was allowed to stand for 30 min at room temperature. After dropwise addition of conc. HNO_3 (7.1 ml) to the solution at 12 °C, the reaction mixture was stirred for 1 h at room temperature. The mixture was adjusted to pH 9 with 1 N NaOH and extracted with EtOAc. The extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on a silica gel column with EtOAc–benzene (10:1). Evaporation of the eluate *in vacuo* gave II (8 g) as colorless needles. NMR ($CDCl_3$) δ: 1.62 (3H, d, J=8 Hz, $>CHCH_3$), 1.92 (3H, s, $-COCH_3$), 5.68—6.15 (1H, m, $-CH<$), 6.90—8.55 (6H, m, aromatic H).

(1*S*)-1-(4-Aminonaphthalen-1-yl)-*N*-acetylethylamine (III): A 10% Pd-C catalyst (0.1 g) was added to a stirred solution of II (2.5 g) in EtOH (15 ml), then hydrazine hydrate (1.5 ml) was added in portions. After further addition of 10% Pd-C (0.1 g), the reaction mixture was refluxed for 1 h, then cooled and filtered. The filtrate was concentrated *in vacuo*. The residue obtained was chromatographed on a silica gel column with EtOAc. Evaporation of the eluate *in vacuo* gave III (1.8 g) as a colorless powder. NMR ($CDCl_3$) δ: 1.62 (3H, d, J=8 Hz, $>CHCH_3$), 1.91 (3H, s, $-COCH_3$), 5.50—6.00 (1H, m, $-CH<$), 6.58—8.20 (6H, m, aromatic H).

(1*S*)-1-(4-Dimethylaminonaphthalen-1-yl)-*N*-acetylethylamine (IV): Dimethyl sulfate (2.0 ml) was added to a stirred mixture of III (1.5 g) and $NaHCO_3$ (1.7 g) in water (5 ml) at 0 °C. Stirring at room temperature was continued for 3 h to obtain a clear solution. The excess reagent was removed by distillation and the aqueous layer was

concentrated *in vacuo*. The residue was subjected to preparative thin-layer chromatography (TLC) using EtOAc. A fraction of *Rf* 0.30 gave IV (1.2 g) as a colorless powder. NMR ($CDCl_3$) δ: 1.67 (3H, d, J=8 Hz, $>CHCH_3$), 1.96 (3H, s, $-COCH_3$), 2.90 (6H, s, $-N(CH_3)_2$), 5.40—6.10 (1H, m, $-CH<$), 6.80—8.50 (6H, m, aromatic H).

(1*S*)-1-(4-Dimethylaminonaphthalen-1-yl)ethylamine (V): A solution of IV (1.2 g) dissolved in conc. HCl (15 ml) was refluxed for 35 h. The solution was adjusted to pH 9 with 2 N NaOH and extracted with EtOAc. The extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to preparative TLC using EtOAc as a developing solvent. Compound V was obtained as a slightly yellow oil (0.41 g). NMR ($CDCl_3$) δ: 1.65 (3H, d, J= 8 Hz, $>CHCH_3$), 1.96 (2H, br, $-NH_2$), 2.96 (6H, s, $-N(CH_3)_2$), 4.70—5.20 (1H, m, $-CH<$), 6.90—8.60 (6H, m, aromatic H). $[\alpha]_D$ +19.75° (c=2.0, EtOH).

Drug Administration——Wistar-Imamichi male rats weighing *ca.* 200—230 g were used after being starved for 18 h. A solution of loxoprofen sodium in distilled water was administered orally at a dose of 10 mg/kg. The suspensions in 0.5% tragacanth solution were administered at a dose of 10 mg/kg for the *trans*- and *cis*-alcohols, and a dose of 5 mg/kg for (2*R*)- and (2*S*)-loxoprofen. At 5, 15 and 30 min, and 1, 3 and 6 h after dosing, rats were anesthetized with chloroform and blood was obtained by heart puncture with a heparinized syringe. Plasma samples were obtained by centrifugation at 2500 rpm for 10 min at 4 °C. Under these conditions, reduction of loxoprofen by erythrocytes was negligible.

Sample Preparation——Plasma samples (1.0 ml) were acidified with 1 N HCl (0.1 ml) and extracted with hexane–EtOAc (3 : 1, 5 ml). The organic layer was washed with 0.1 N HCl (1.0 ml) and a 4-ml aliquot was concentrated *in vacuo*. (1*S*)-1-(4-Dimethylaminonaphthalene-1-yl)ethylamine (100 μg, 0.1 ml), 1-hydroxybenzotriazole (200 μg, 20 μl) and DCC (200 μg, 0.1 ml), each in CH_2Cl_2–pyridine (10 : 1) solution, were added to the residue and the reaction mixture was allowed to stand at room temperature for 1 h. After evaporation of the solvent *in vacuo*, hexane–EtOAc (3 : 1, 0.5 ml) and 0.1 N HCl (0.5 ml) were added and the mixture was stirred on a Vortex mixer for about 1 min. The organic layer was dried over Na_2SO_4 and 20 μl of the reaction mixture was injected into the HPLC column.

Calibration Curve and Recovery Test——Each enantiomer in plasma was quantitated by the use of a calibration curve prepared by assaying standards containing 2, 5, 10 and 20 μg of loxoprofen, *trans*- and *cis*-alcohols per tube. The calibration curve obtained by plotting peak area *vs.* concentration of the enantiomer showed good linearity and passed through the origin. The detection limit was 1 ng for loxoprofen, and 2 ng for *trans*- and *cis*-alcohols.

These three authentic compounds were added to plasma at concentrations of 2, 5, 10 and 20 μg/ml. The plasma sample was extracted, derivatized and analyzed as mentioned above. The recoveries of the compounds from plasma were more than 95%.

Results

Synthesis of (1*S*)-1-(4-Dimethylaminonaphthalen-1-yl)ethylamine

(1*S*)-1-(4-Dimethylaminonaphthalen-1-yl)ethylamine was introduced by Goto *et al.*[3] as

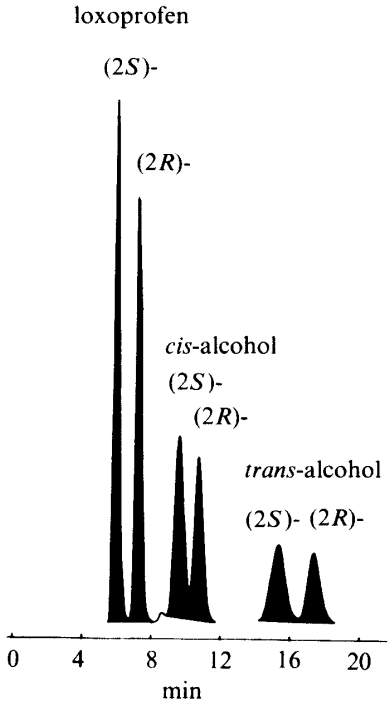


Fig. 1. HPLC Separation of a Mixture of Diastereomeric Amides of Loxoprofen, and *trans*- and *cis*-Alcohols

a chiral derivatization reagent for carboxylic acids. Since the attempted optical resolution of 1-(4-dimethylaminonaphthalen-1-yl)ethylamine by the use of D- or L-α-methoxy-α-methyl-1-naphthaleneacetic acid[3] was unsuccessful, the reagent was synthesized by a new method starting with the optically active (1*S*)-1-(naphthalen-1-yl)ethylamine. The optical purity of the synthesized reagent was more than 99.0% as judged by the usual criteria.

HPLC

Each enantiomer of loxoprofen, *trans*-alcohol and *cis*-alcohol was reacted with the chiral reagent and the products were analyzed by HPLC. The structure of each product was confirmed by mass spectrum (MS) of the pooled sample obtained by HPLC. Figure 1 shows a high performance liquid chromatogram of a mixture of these diastereomers, which are well separated under the chromatographic conditions mentioned above. There was no interference peak due to the rat plasma.

Administration of Loxoprofen Sodium

Total concentrations and enantiomer ratios of the parent compound, and *trans*- and *cis*-alcohols in rat plasma were determined by the HPLC method after oral administration of 10 mg/kg dose of loxoprofen. The results are shown in Figs. 2a and 3a. The maximal concentration of the unchanged compound appeared at 15 min after oral administration, thus suggesting the occurrence of rapid gastrointestinal absorption of loxoprofen sodium. The plasma main metabolite, the *trans*-alcohol, showed a similar pattern, indicating the rapid reduction of loxoprofen. These results are in good agreement with the results from radioisotope studies.[4] The ratios of (2*S*)- to (2*R*)-isomers of the parent acid and *trans*-alcohol increased rapidly with time after administration and reached almost 100% (2*S*)-isomer after 3 h (*trans*-alcohol)—6 h (the parent acid) (Fig. 3a). Unexpectedly, the *cis*-alcohol showed an enantiomer content of nearly 100% as early as 5 min after administration.

Administration of (2*R*)- and (2*S*)-Loxoprofen

Figures 2b, 2c and Figs. 3b, 3c show the concentrations and the enantiomer ratios of the parent acid and its main metabolites in plasma after oral administration of 5 mg/kg doses of (2*R*)- and (2*S*)-loxoprofen to rats. The enantiomers showed no significant differences in peak

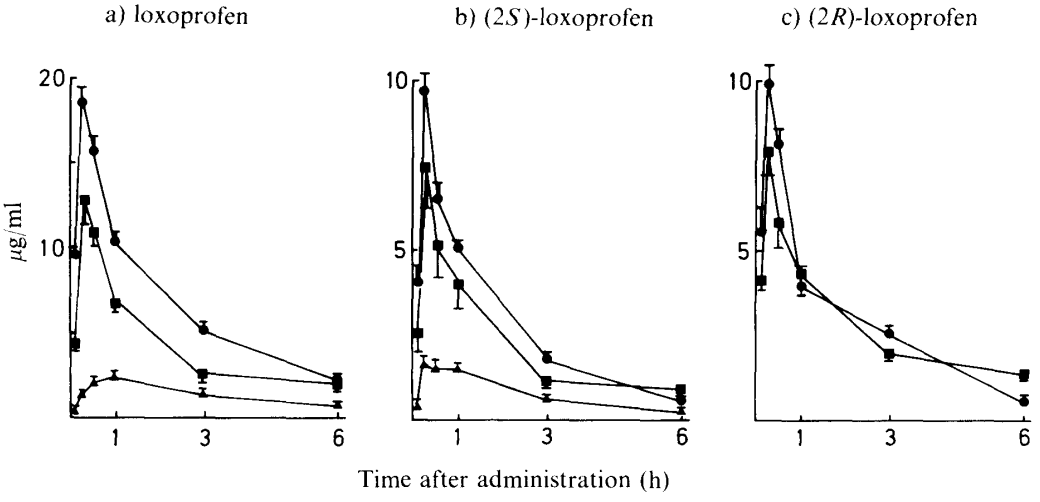


Fig. 2. Concentrations of Loxoprofen and Its Monohydroxy Metabolites in Plasma after Oral Administration of Loxoprofen Sodium, and (2*S*)- and (2*R*)-Loxoprofen to Rats

The doses were 10 mg/kg for loxoprofen and 5 mg/kg for each isomer. Each point represents the mean ± S.E. of three rats.
—●—, loxoprofen; —■—, *trans*-alcohol; —▲—, *cis*-alcohol.

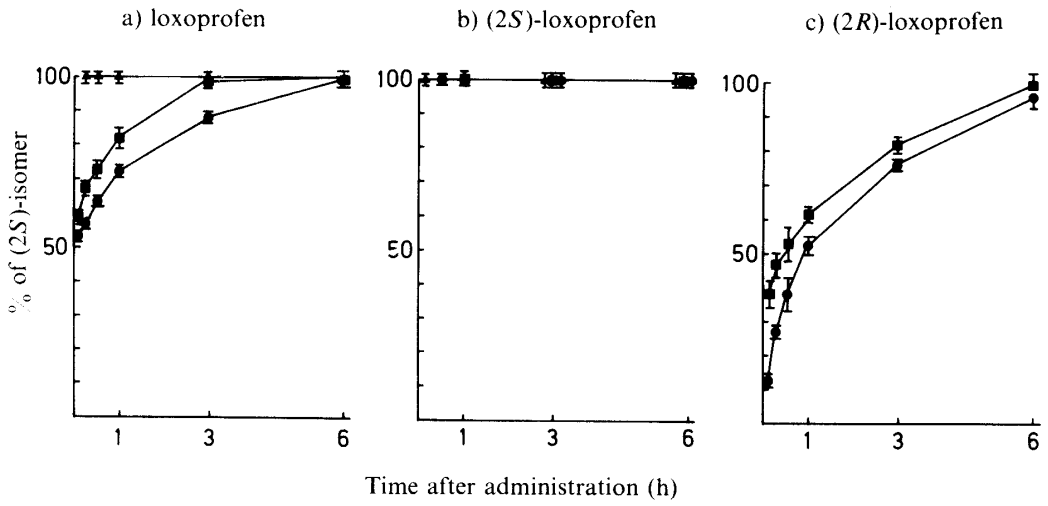


Fig. 3. Percentages of (2*S*)-Isomers of Loxoprofen and Its Monohydroxy Metabolites in Plasma after Oral Administration of Loxoprofen Sodium, and (2*S*)- and (2*R*)-Loxoprofen to Rats

The doses were 10 mg/kg for loxoprofen and 5 mg/kg for each isomer. Each point represents the mean ± S.E. of three rats.
—●—, loxoprofen; —■—, *trans*-alcohol; —▲—, *cis*-alcohol.

times and peak levels of the parent acid and the *trans*-alcohol. However, a marked difference was observed in the formation of the *cis*-alcohol, which was formed only from the (2*S*)-enantiomer.

After oral administration of (2*R*)-loxoprofen, the enantiomeric (2*S*)-isomer was detected at as early as 5 min (12.6% for the parent acid and 38.4% for *trans*-alcohol) and the contents increased rapidly in a similar manner to that seen after loxoprofen sodium administration. The (2*S*)-enantiomer ratios of the *trans*-alcohol were much higher than those of the parent acid. In contrast, administration of the (2*S*)-isomer gave the parent acid and the monohydroxy metabolites with the stereochemically intact (2*S*)-configuration. These results unambiguously established the occurrence of irreversible optical inversion of (2*R*)- to (2*S*)-loxoprofen in the rat.

The plasma half-life of (2*S*)-loxoprofen was 5.7 h after administration of racemic loxoprofen, whereas it was 3.2 h after dosing of (2*S*)-loxoprofen. This prolonged half-life of the (2*S*)-isomer observed in the racemate dosing may be due to the successive formation of the (2*S*)-isomer by inversion of the (2*R*)-isomer. The dose-normalized areas under the plasma concentration–time curve (AUC) of the (2*S*)-*trans*-alcohol up to 6 h were 10.91, 13.00 and 12.16 $\mu g \cdot h^{-1} \cdot ml^{-1}$ after oral administration of racemic, (2*S*)- and (2*R*)-loxoprofen, respectively. Thus, there was no significant difference in the AUC values of the active metabolite formed from the three stereoisomers.

Administration of *trans*- and *cis*-Alcohols

Figure 4 and Fig. 5 show the concentrations and enantiomer ratios of the unchanged compound and the metabolites after oral administration of the *trans*- and *cis*-alcohols. Maximum concentrations were attained at 15—30 min after oral administration of the monohydroxy metabolites, indicating the rapid absorption of these compounds. Administration of the *cis*-alcohol afforded loxoprofen at two to three times higher levels than those seen after dosing of the *trans*-alcohol, and formed the *trans*-alcohol at high concentrations. This was in sharp contrast to the result that the *trans*-alcohol gave no detectable amount of *cis*-alcohol.

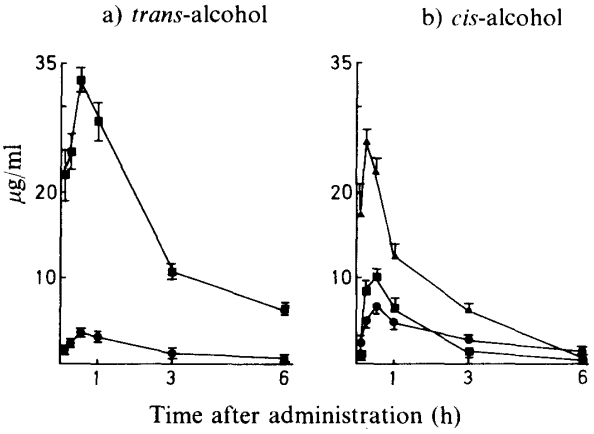


Fig. 4. Concentrations of Loxoprofen and Its Monohydroxy Metabolites in Plasma after Oral Administration of the *trans*- and *cis*-Alcohols to Rats

The doses of both compounds were 10 mg/kg. Each point represents the mean ± S.E. of three rats. —●—, loxoprofen; —■—, *trans*-alcohol; —▲—, *cis*-alcohol.

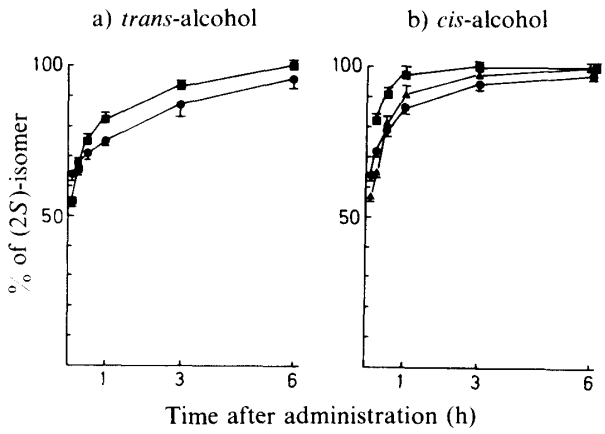


Fig. 5. Percentages of (2*S*)-Isomers of Loxoprofen and Its Monohydroxy Metabolites in Plasma after Oral Administration of the *trans*- and *cis*-Alcohols to Rats

The doses of both compounds were 10 mg/kg. Each point represents the mean ± S.E. of three rats. —●—, loxoprofen; —■—, *trans*-alcohol; —▲—, *cis*-alcohol.

TABLE I. Percentages of the Metabolites[a] with Respect to the Total Concentrations in Plasma after Oral Administration of *trans*- and *cis*-Alcohols to Rats

Administered compound	% of total concentration					
	min			h		
	5	15	30	1	3	6
trans-Alcohol	7.2 ± 1.6	9.0 ± 0.7	9.7 ± 0.2	10.3 ± 1.4	10.5 ± 0.3	12.2 ± 0.3
cis-Alcohol	18.9 ± 1.1	34.3 ± 1.2	42.6 ± 0.4	46.8 ± 2.1	40.2 ± 1.9	56.8 ± 0.5

The doses were 10 mg/kg. Each value represents the mean ± S.E. of three rats.
a) Loxoprofen after *trans*-alcohol dosing. Loxoprofen plus *trans*-alcohol after *cis*-alcohol dosing.

Table I shows the percentages of the combined metabolites to the total amount in plasma. The ratios of loxoprofen plus *trans*-alcohol increased gradually to about 57% at 6 h after administration of the *cis*-alcohol, while loxoprofen formed from the *trans*-alcohol remained at a low level of *ca.* 10%. These results are in accord with the finding that large amounts of *trans*-alcohol-derived dihydroxy metabolites were excreted in the urine of rats dosed with ^{14}C-labeled *cis*-alcohol.[5]

The (*S*/*R*) ratios of the unchanged compound and the metabolites increased with time after administration of *trans*- and *cis*-alcohols, and the (2*S*)-isomer was exclusively found in 6 h plasma. It is clear that the (2*R*)-isomers of the *trans*- and *cis*-alcohols were optically inverted to the (2*S*)-isomers in the same manner as loxoprofen.

Discussion

Previous metabolic studies of loxoprofen sodium in rats revealed that the urinary main metabolites have the (*S*)-configuration of the α-phenylpropionic acid moiety, and consequently indicated the possible occurrence of optical inversion of (2*R*)- to (2*S*)-isomers in loxoprofen.[1] The present results on enantiomer determination after administrations of (2*R*)-

and (2*S*)-loxoprofen confirmed that rapid inversion does occur. The pharmacologically equal potency of the (2*R*)- and (2*S*)-isomers of loxoprofen may be interpreted as a result of this *in vivo* rapid inversion reaction, as in the cases of ibuprofen,[6] 2-(2-isopropylindan-5-yl)propionic acid[7] and others.[8-10]

In previous studies,[5] administration of *trans*-alcohol-^{14}C to rats afforded the corresponding diol metabolites in the urine, formed by direct hydroxylation at the cyclopentanol moiety of the *trans*-alcohol. Administration of *cis*-alcohol-^{14}C resulted in the formation of the same diols as the main urinary metabolites, together with another diol derivable by simple hydroxylation of the *cis*-alcohol. This formation of *trans*-alcohol-originated diols from the *cis*-alcohol is explained by the present finding that the *cis*-alcohol is rapidly converted to the *trans*-alcohol through the intermediate parent ketone (loxoprofen). On the other hand, the *trans*-alcohol seems to be oxidized only at a limited rate and be in *in vivo* equilibrium with a small amount of the parent ketone.

Administration of (2*S*)-loxoprofen afforded the *cis*-alcohol but (2*R*)-loxoprofen gave no significant amount of *cis*-alcohol in spite of the occurrence of rapid inversion of (2*R*)- to (2*S*)-isomers. Further, the *cis*-alcohol formed from racemic loxoprofen had a (2*S*)-isomer ratio of nearly 100% as early as 5 min after dosing. These combined results suggest that the *cis*-alcohol might be stereospecifically produced from (2*S*)-loxoprofen only. This problem is now under investigation in *in vitro* systems.

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