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Species Differences in Metabolism of Sodium 2-[4-(2-Oxocyclopentylmethyl)-phenyl]propionate Dihydrate (Loxoprofen Sodium), a New Anti-Inflammatory Agent

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Urinary metabolites of loxoprofen-^{14}C sodium were determined in rats, mice, dogs and monkeys after oral administration of the drug. In all these animal species, the cyclopentanone moiety of loxoprofen was predominantly reduced to *trans*-OH (an active principle), together with *cis*-OH (minor product). The monohydroxy metabolites were further hydroxylated in rats to the diols (M-4, M-5 and M-6), which were excreted in urine as the main metabolites. Mice excreted the monohydroxy metabolites mainly as their free forms. In dogs, the monohydroxy metabolites were conjugated with taurine and glucuronic acid, and monkeys furnished the ester glucuronides of the monohydroxy metabolites. Thus, species differences were observed both in the hydroxylation and in the conjugation reactions of the monohydroxy metabolites produced by the reduction of loxoprofen.

Keywords——anti-inflammatory agent; sodium 2-[4-(2-oxocyclopentylmethyl)phenyl]-propionate dihydrate; loxoprofen sodium; α-arylpropionic acid derivative; taurine conjugate; species difference; hydroxylation; conjugation; radio-HPLC

Sodium 2-[4-(2-oxocyclopentylmethyl)phenyl]propionate dihydrate (loxoprofen sodium), is a new nonsteroidal anti-inflammatory agent with high potency comparable to that of indomethacin after oral dosing.[1] *In vitro* studies have established that the principal metabolite in plasma of various animals, the *trans*-OH derivative, is a highly effective inhibitor of prostaglandin (PG) synthetase, thus strongly suggesting that the *trans*-OH derivative is an active metabolite of loxoprofen sodium, which itself has only a weak inhibitory activity.[2] This active metabolite formation may account for the relatively low incidence of gastro-intestinal lesion after oral administration of loxoprofen sodium.[3]

It has been established that the active *trans*-OH metabolite is formed by the stereospecific reduction of the cyclopentanone moiety of the loxoprofen molecule by an aromatic aldehyde–ketone reductase,[4] and it has the (2*S*, 1′*R*, 2′*S*)-configurations.[2]

Structural elucidation of the main metabolites was accomplished by spectral analysis of samples isolated from rat urine and by chemical synthesis, which will be reported elsewhere. The present paper is concerned with species differences in the urinary metabolites of loxoprofen in the rat, mouse, dog and monkey. Possible metabolic pathways are presented.

Experimental

Compounds——Loxoprofen-^{14}C sodium labeled at the methyl carbon of the propionic acid side chain and other related authentic compounds were all synthesized by Naruto *et al.* in the Chemical Research Laboratories, Sankyo Co., Ltd. The radiochemical purity of loxoprofen-^{14}C sodium was more than 97% as determined by thin-layer chromatography (TLC) on a Kieselgel 60 F_{254} plate (Merck, thickness 0.25 mm) using a solvent system of benzene–EtOAc–AcOH (20 : 10 : 1), and the specific radioactivity was 43.1 μCi/mg.

Preparation of *trans*-OH-^{14}C and *cis*-OH-^{14}C Derivatives——A solution of loxoprofen-^{14}C sodium (50 mg) in 90% EtOH (10 ml) was treated with $NaBH_4$ (10 mg) in aqueous EtOH (2 ml), and the mixture was stirred under ice-

cooling for 30 min. The excess reagent was decomposed with AcOH and the reaction mixture was concentrated *in vacuo*. The residue was extracted with ether. The combined extracts were washed with water and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the oily residue was subjected to preparative TLC on a Kieselgel 60 F_{254} plate using a solvent system of benzene–EtOAc–AcOH (30 : 16 : 1). The zones at *Rf* 0.60 (*cis*-OH fr.) and 0.40 (*trans*-OH fr.) were each scraped off and extracted with EtOAc. Further chromatography of each extract on a short SiO_2 column with ether gave the *cis*-OH-^{14}C compound (6 mg) and the *trans*-OH-^{14}C compound (23 mg). The radiochemical purities of the *trans*-OH-^{14}C and *cis*-OH-^{14}C derivatives were more than 96% and 92%, respectively.

Synthesis of Taurine and Glycine Conjugate of *trans*-OH——Taurine conjugate: 4-Dimethylaminopyridine (10 mg) and triethylamine (0.5 ml) were added to a solution of the *trans*-OH compound (4 g) in acetic anhydride (50 ml). After standing at room temperature for 3 h, the reaction mixture was poured into ice-water and extracted with ether. The combined extracts were concentrated under reduced pressure and the residue was subjected to column chromatography on silica gel. Elution with EtOAc gave the acetate of the *trans*-OH compound (2.5 g) as a viscous oil. The *trans*-OH acetate (1.2 g) and thionyl chloride (0.7 g) were heated at 80 °C for 2 h. The excess thionyl chloride was evaporated off *in vacuo* and the residue was added to a well stirred solution of taurine (0.6 g) and NaOH (0.2 g) in water (6 ml) at 0 °C. After standing for 15 min, the reaction mixture was extracted with EtOAc and the separated aqueous layer was concentrated *in vacuo*. The residue was dissolved in MeOH (20 ml) and the precipitate was filtered off. NaOH (0.4 g) was added to the filtrate and the solution was allowed to stand at room temperature for 18 h. After concentration of the reaction mixture *in vacuo*, the residue was dissolved in dil. HCl and the solution was concentrated *in vacuo*. The residue was taken up in MeOH (20 ml) and the undissolved precipitates were filtered off. The filtrate was concentrated and subjected to preparative liquid chromatography (Merck, Lobar column RP-8-B type). Elution with MeOH–H_2O–AcOH (50 : 50 : 2) gave the taurine conjugate (0.6 g) as an amorphous powder. mp 200 °C (dec.). *Anal.* Calcd for $C_{17}H_{25}NO_5 \cdot 2H_2O$: C, 52.15; H, 7.46; N, 3.57. Found: C, 52.23; H, 7.07; N, 3.33.

Glycine Conjugate: *tert*-Butyl chlorocarbonate (1.3 g) was added to a solution of the *trans*-OH acetate (1.3 g) and triethylamine (2.0 g) in tetrahydrofuran (50 ml) at 0 °C and the mixture was allowed to stand at 0 °C for 30 min. Then powdered glycine ethyl ester hydrochloride (1.12 g) was added, and the mixture was allowed to stand in an ice bath for 1 h. After work-up in the usual manner, the reaction mixture was subjected to column chromatography on silica gel. Elution with EtOAc–hexane (1 : 1) gave the amide of glycine ethyl ester (1.1 g) as an oil. A solution of the amide in MeOH (20 ml) was treated with 2.5 N NaOH (20 ml) and the resulting mixture was allowed to stand at room temperature for 18 h, then concentrated *in vacuo*. The concentrate was acidified with dil. HCl and extracted with EtOAc. The organic layer was concentrated *in vacuo* and the residue obtained was subjected to column chromatography on silca gel. Elution with EtOAc–MeOH (5 : 1) gave the glycine conjugate (0.5 g) as an amorphous material. mp 200 °C (dec.). *Anal.* Calcd for $C_{17}H_{23}NO_4 \cdot 1/2H_2O$: C, 64.95; H, 7.70; N, 4.46. Found: C, 64.69; H, 7.52; N, 4.53.

Animal Experiments——Male Wistar-Imamichi rats weighing *ca.* 200 g, male ddY mice weighing *ca.* 20 g, male beagle dogs weighing *ca.* 10 kg and male crab-eating monkeys (*Macaca fascicularis*) weighing *ca.* 4 kg were all employed after being fasted for 16 h prior to the experiments. Loxoprofen-^{14}C sodium dissolved in distilled water was administered orally at a dose of 2 mg/kg to the rats and mice using a stomach tube and to the monkeys through an intranasal tube. The dogs were given the same dose of loxoprofen-^{14}C sodium in a gelatin capsule with water (*ca.* 20 ml). The *trans*- and *cis*-OH-^{14}C compounds dissolved in 1% $NaHCO_3$ solution were administered orally to the rats at a dose of 2 mg/kg. The animals were housed in individual metabolism cages. Urine (under toluene) and feces were collected separately for each 24 h period.

Measurement of Radioactivity——The radioactivity of the urine was measured with a liquid scintillation spectrometer (Packard model 3390, Downers Grove, U.S.A.) in 10 ml of a scintillator solution (2,5-diphenyloxazole (PPO) 8 g, dimethyl 1,4-bis(2-(5-phenyloxazolyl))benzene (POPOP) 0.2 g, toluene 500 ml and EtOH 500 ml). Fecal samples were homogenized in 9 volumes of 50% EtOH with a Polytron homogenizer and the radioactivity was determined by the combustion method using a Packard 306 oxidizer. Radioactive zones on TLC plates were scraped into counting vials. The radioactivities were extracted with MeOH (0.5 ml) and counted in 10 ml of a scintillator solution (PPO 8 g, dimethyl POPOP 0.2 g, toluene 200 ml and dioxane 800 ml). The counting efficiency was corrected by the external standard ratio method.

Identification and Characterization of Metabolites——The Free Metabolites: Radioactive urine samples were directly spotted on a Kieselgel 60 F_{254} plate and developed one- and/or two-dimensionally using the following solvent systems; (A) benzene–acetone–AcOH (80 : 15 : 5), (B) $CHCl_3$–acetone–HCOOH (50 : 15 : 3), (C) $CHCl_3$–MeOH–HCOOH (25 : 2 : 1), (D) BuOH–AcOH–H_2O (4 : 1 : 1), (E) BuOH–AcOH–H_2O-ether (4 : 1 : 1 : 3) and (F) $CHCl_3$–MeOH–AcOH (24 : 8 : 1). The radioactive metabolites were detected by autoradiography using X-ray film and their *Rf* values were compared with those of nonlabeled authentic samples,which were made visible by spraying a solution of vanilin (0.5 g) dissolved in 100 ml sulfuric acid–ethanol (4 : 1), followed by heating. The solvent systems D, E and F were employed for the analysis of the conjugated metabolites.

Further identification of the urinary metabolites of the rats, especially of the stereoisomers of diol metabolites, was accomplished by high performance liquid chromatography (HPLC) of the methyl esters. A urine sample was

adjusted to pH 2 with dil. HCl and extracted with ether. The combined extracts (the free fraction) were dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was dissolved in ether and treated with CH_2N_2–ether solution for 30 min at 0 °C. The excess reagent was decomposed with AcOH and the solvent was evaporated off under a stream of N_2.

A TRIROTAR-II high performance liquid chromatograph (Jasco, Tokyo) equipped with a variable-wavelength ultraviolet (UV) detector (UVIDEC-100-III, Jasco) and a radio liquid chromatograph detector (Aloka, Tokyo) was used with a stationary phase of μPorasil (0.4 × 30 cm, Waters, U.S.A.) and a mobile phase of hexane–EtOAc (4 : 1—1 : 1, flow rate 2 ml/min). The samples were injected through a U6K loop sample injector (Waters).

The Conjugated Metabolites: The conjugated metabolites of both the untreated and the hydrolyzed urine samples were characterized by HPLC and TLC. HPLC was carried out with a μBondapak C_{18} column (0.4 × 30 cm, Waters); 0.05 M Na_2HPO_4–citrate buffer (pH 3)/MeOH (60 : 40, flow rate 1.0 ml/min) was used for the separation of the conjugates and the identification of the taurine conjugate of the *trans*-OH compound in dog urine. Each separated conjugate was pooled to determine the chemical structure of the aglycone. Satisfactory separation of the conjugates in monkey urine was achieved by concave gradient elution (2 mode) (GP-A30, Jasco) with 20 to 30% CH_3CN–H_2O (adjusted to pH 3 with AcOH) for 64 min at a flow rate of 1.0 ml/min. The TLC conditions were the same as described above.

Hydrolysis of the Conjugates——Dog Urine: Urine (50 ml) was adjusted to pH 3 with 4 N HCl and extracted with ether. The residual aqueous layer was applied to an Amberlite XAD-2 column (2 × 20 cm) and after being washed with water, eluted with MeOH. The MeOH eluate was concentrated *in vacuo* to dryness below 40 °C. The residue was dissolved in water (10 ml) (the conjugate fraction).

1) Glusulase Hydrolysis. The conjugate fraction (0.1 ml) was diluted with 0.1 M acetate buffer (pH 5.0, 2.0 ml) and incubated with glusulase (10000 Fishman units of glusulase, Endo Lab., U.S.A.) for 24 h at 37 °C. The reaction mixture was extracted with ether and the combined organic layers were concentrated to dryness under a stream of N_2.

2) Alkaline Hydrolysis. Aliquots (0.1 ml) of the conjugate fraction and pooled conjugate fraction from HPLC were mixed with 0.2 N or 5.0 N NaOH (2.0 ml each) and heated at 120 °C for 1, 3 and 5 h under an N_2 atmosphere. The reaction mixtures were adjusted to pH 3 with 6 N HCl and extracted with ether. The combined organic layers were concentrated to dryness under a stream of N_2.

Monkey Urine: The conjugates in monkey urine were hydrolyzed by mild alkaline treatment. A mixture of the urine sample (0.5 ml) and 0.4 N NaOH (0.5 ml) was allowed to stand at room temperature for 24 h, then it was analyzed by TLC.

Mouse Urine: The conjugates in mouse urine were hydrolyzed with glusulase as described for the dog urinary conjugates.

Analysis of Metabolites in Feces——Homogenates of feces in 50% EtOH were extracted 3 times with 80% EtOH and the combined extracts were concentrated to dryness *in vacuo*. The residue was dissolved in a small volume of 80% EtOH for analysis by TLC.

Quantitative Determination of the Metabolites——The free metabolites in urine of various animals were determined by TLC using solvent system A. The conjugated metabolites were analyzed by TLC after hydrolysis of mouse urine with glusulase and after mild alkaline treatment of monkey urine. The conjugates in dog urine were determined directly by radio-HPLC.

Results

Urinary and Fecal Excretion

The radioactivities in 24 h urine and feces of various animals after oral administration of loxoprofen-^{14}C sodium are summarized in Table I. The orally administered loxoprofen

Table I. Urinary and Fecal Excretions of Radioactivity after Oral Administration of Loxoprofen-^{14}C Sodium to Various Animals

Animals		% of dose		
		Urine	Feces	Total
Rat	(5)	85.08	9.52	95.60
Mouse	(4)	81.57	10.03	91.60
Dog	(3)	64.48	34.05	97.83
Monkey	(2)	81.83	5.15	86.98

The dose of loxoprofen-^{14}C sodium was 2 mg/kg. Numbers of animals used are given in parentheses.

sodium was absorbed largely from the gastro-intestinal tract and excreted mainly in the urine. The relatively higher radioactivity of dog feces might be due to species differences in the metabolism as mentioned later. Excretions of radioactivity following oral administration (2 mg/kg) of the *trans*-OH-^{14}C and *cis*-OH-^{14}C compounds were 81.1 and 81.9% in 24 h urine, and 10.4 and 8.3% in 24 h feces, respectively. These values were in close agreement with those for loxoprofen-^{14}C sodium.

Identification of the Free Metabolites

The representative two-dimensional thin-layer autoradiogram of rat urine shown in Fig. 1 revealed that most of the metabolites consist of the free forms. The major metabolites of M-4, M-5 and M-6 were relatively polar. M-1 was identified as the *cis*-OH compound, M-2 as the *trans*-OH compound, M-3 as the α-ketol, M-4 as the 2′β, 4′β-diol, M-5 as the 2′α, 4′α-diol and M-6 as the 2′α, 4′β-diol, by comparisons with authentic samples. The t_R values on HPLC of the methyl esters of these diol metabolites, M-4, M-5 and M-6, were also in good agreement with those for the authentic samples (Fig. 2). The free metabolites in urine and feces of other animals were identified in a similar manner. These identifications were further confirmed by gass chromatography-mass spectrum (GC-MS) measurement of the urinary metabolites of rats and monkeys after derivatization of the separated samples with CH_2N_2–ether followed by BSTFA reagent.

Characterization and Identification of the Conjugated Metabolites in Urine of Dog and Monkey

Figure 3 shows thin-layer radiochromatograms of urine of various animals with solvent system D or E. Dog and monkey urine contained many conjugates, with three or four main components.

Dog Urine——Both glusulase and 0.5 N alkaline treatment of the conjugate fraction yielded the same amount (25—28%) of ether-extractable radioactivity. TLC of the ether extracts showed the main component to be the *trans*-OH compound as well as the parent acid and *cis*-OH compound (minor products). Therefore, ester glucuronides of these compounds were suggested to occur in dog urine. After strong alkaline treatment (heating with 5 N NaOH at 120 °C for 5 h), 94% of the radioactivity of the conjugate fraction was transferred to the

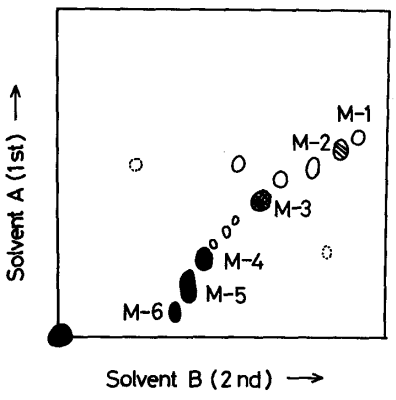

Fig. 1. Two-Dimensional Thin-Layer Radiochromatogram of Metabolites in Rat Urine after Oral Administration of Loxoprofen-^{14}C Sodium

Solvent systems: A, benzene–acetone–AcOH (80:15:5); B, $CHCl_3$–acetone–HCOOH (50:15:3).

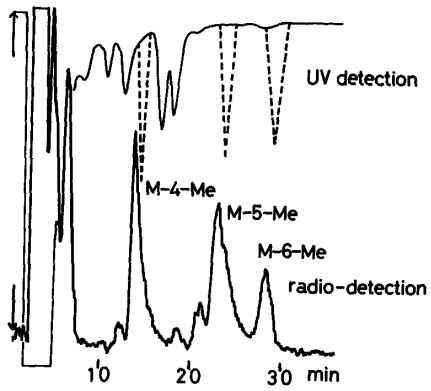

Fig. 2. High-Performance Liquid Chromatogram of the Methyl Esters of Main Diol Metabolites in the Ether Extract of Rat Urine after Oral Administration of Loxoprofen-^{14}C Sodium

A μPorasil column was eluted with hexane–EtOAc (1:1) at a flow rate of 2 ml/min. Metabolites were monitored with (a) an UV detector (UVIDEC-100-III, 230 nm) and (b) a radio liquid chromatograph detector (Aloka). The dotted lines indicate peaks of authentic samples.

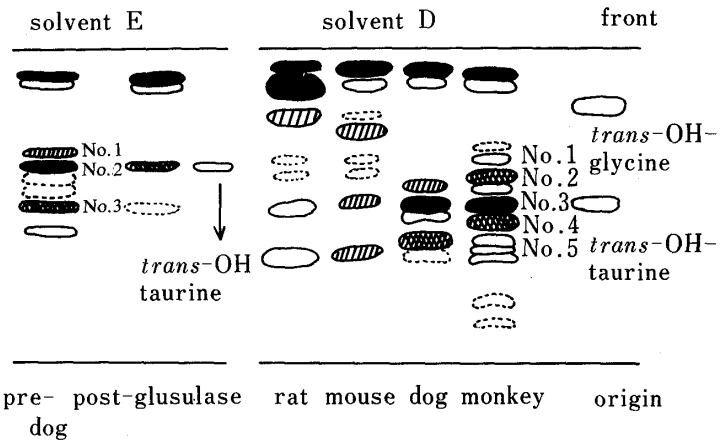

Fig. 3. Thin-Layer Radiochromatograms of the Conjugated Metabolites in Urine of Rat, Mouse, Dog and Monkey after Oral Administration of Loxoprofen-^{14}C Sodium

Solvent systems: D, BuOH–AcOH–H_2O (4:1:1); E, BuOH–AcOH–H_2O–ether (4:1:1:3).

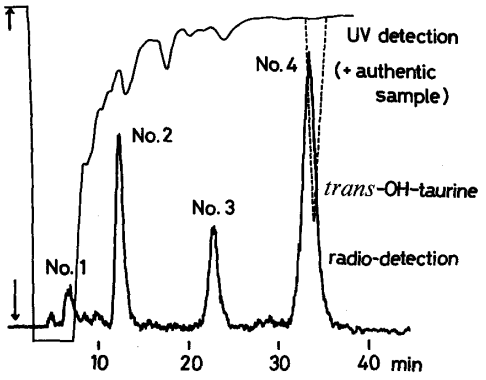

Fig. 4. High-Performance Liquid Chromatogram of the Conjugated Metabolites in Dog Urine after Oral Administration of Loxoprofen-^{14}C Sodium

A μBondapak C_{18} column was eluted with 0.05 M Na_2HPO_4–citrate buffer (pH 3)/MeOH (60:40) at a flow rate of 1.0 ml/min. Metabolites were monitored with (a) an UV detector (UVIDEC-100-III, 230 nm) and (b) a radio liquid chromatograph detector (Aloka). The dotted lines indicate peaks of authentic samples.

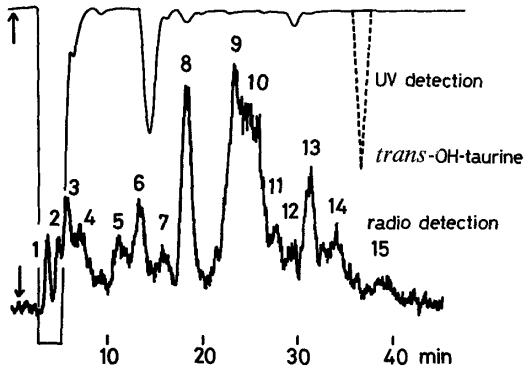

Fig. 5. High-Performance Liquid Chromatogram of the Conjugated Metabolites in Monkey Urine after Oral Administration of Loxoprofen-^{14}C Sodium

A μBondapak C_{18} column was eluted with concave gradient from 20 to 30% CH_3CN–H_2O (adjusted to pH 3) for 64 min at a flow rate of 1.0 ml/min. Metabolites were monitored with (a) an UV detector (UVIDEC-100-III, 230 nm) and (b) a radio liquid chromatograph detector (Aloka).

ether extract, which again consisted of the same components as mentioned above on TLC. These results indicated the occurrence of some amino acid conjugates as the major component(s) together with the minor ester glucuronides. The main spot (No. 2) in Fig. 3 had the same *Rf* values as those of the authentic taurine conjugate of the *trans*-OH compound with several solvent systems. HPLC confirmed that the main peak (No. 4) in Fig. 4 was identical to the authentic sample of the taurine conjugate. The free forms released from the moderate peaks No. 2 and No. 3 on mild alkaline treatment were the *trans*-OH compound and parent acid, respectively. The minor peak No. 1 gave the *cis*-OH compound as the aglycone.

Monkey Urine——The chromatographic patterns of the conjugated metabolites in monkey urine are shown in Fig. 3 (TLC) and in Fig. 5 (HPLC). These are apparently more complex than those of dog urine mentioned above. Satisfactory separation of the monkey

urine conjugates was not obtained under the same HPLC conditions as used for the dog urine conjugates, and was finally accomplished by gradient elution with 20 to 30% CH_3CN–H_2O (pH 3). Spot No. 3 in Fig. 3 had an *Rf* value similar to that of the *trans*-OH taurine conjugate, but HPLC examination revealed the absence of taurine conjugates in monkey urine.

Mild alkaline treatment smoothly hydrolyzed the conjugated metabolites, amounting to 85% of the conjugates. Glusulase hydrolysis also gave similar results. Thus, the majority of the conjugates in monkey urine are thought to be ester glucuronides. TLC analysis of the liberated metabolites revealed the *trans*-OH compound and the parent compound to be major components, while the *cis*-OH compound was present in a moderate amount together with the minor diols.

Metabolites Profiles in Urine of Various Animals

The metabolites in 24 h urine were determined by the TLC method in rats, mice, dogs and monkeys after oral administration of 2 mg/kg of loxoprofen-^{14}C, and the results are shown in Table II. Most of the metabolites in rat urine were present as the free forms, mainly as the diol metabolites M-5 (36.1%), M-4 (16.3%) and M-6 (9.1%), the conjugates amounting to only 9.0%. On the other hand, dog and monkey urine contained conjugate fractions amounting to *ca.* 65% of the urinary activity. The conjugates in mouse urine accounted for 27% of the urinary metabolites. Based on the structural resemblance, the diols M-5 and M-6 were assumed to be derived from the *trans*-OH compound by further hydroxylation and M-4, from the *cis*-OH compound. The α-ketol metabolite (M-3) found in rat urine was not detected in other animals. Dogs excreted the *trans*-OH compound mainly as the taurine and ester glucuronic acid conjugates (29.2 and 13.6%, respectively). Monkeys furnished the ester glucuronides of the *trans*-OH compound (26.3%), *cis*-OH compound (8.9%) and the parent compound. The glycine conjugate of the *trans*-OH compound was not detected in any animal

TABLE II. Metabolites in 24 h Urine of Various Animals after Oral Administration of Loxoprofen-^{14}C Sodium

Metabolites	% of urinary radioactivity			
	Rat (5)[a]	Mouse (4)	Dog (3)	Monkey (2)
Loxoprofen	0.9	9.2	1.6	1.9
M-1: *cis*-OH	1.0	12.5	2.8	2.6
M-2: *trans*-OH	3.3	25.6	18.8	9.0
M-3: α-ketol	6.4	0.5	0.5	0.8
M-4: 2′β,4′β-diol	16.3	3.0	2.0	4.3
M-5: 2′α,4′α-diol	36.1	1.6	0.5	2.2
M-6: 2′α,4′β-diol	9.1	0.9	0.5	0.7
Loxoprofen glucuronide	—[b]	n.d.[c]	8.0	9.9
cis-OH glucuronide	—	1.0	3.3	8.9
trans-OH glucuronide	—	9.3	13.6	26.3
trans-OH taurine	—	—	29.2	—
Diol-glucuronide	—	n.d.	n.d.	5.2
Total conjugates[d]	9.0	27.2	64.4	65.0

The dose of loxoprofen-^{14}C sodium was 2 mg/kg. For the determination of conjugated metabolites, mouse urine was hydrolyzed with glusulase and monkey urine by mild alkaline treatment. Conjugates in dog urine were directly analyzed by radio-HPLC.

a) The number of animals used.
b) Not determined.
c) Not detected.
d) Metabolites present at the origin on TLC with solvent A.

TABLE III. Metabolites in 24 h Urine of Rats after Oral Administration of the *trans*-OH-^{14}C and *cis*-OH-^{14}C Compounds

Metabolites	% of urinary radioactivity	
	trans-OH	*cis*-OH
Loxoprofen	1.2	1.8
M-1: *cis*-OH	0.6	2.7
M-2: *trans*-OH	3.8	0.9
M-3: α-ketol	0.6	2.1
M-4: 2′β, 4′β-diol	3.3	29.1
M-5: 2′α, 4′α-diol	58.4	30.6
M-6: 2′α, 4′β-diol	13.8	6.2
Total conjugates[a]	9.4	10.1

The dose of *trans*- or *cis*-OH-^{14}C compound was 2 mg/kg. Values are the means of three rats.
a) Metabolites present at the origin on TLC with solvent A.

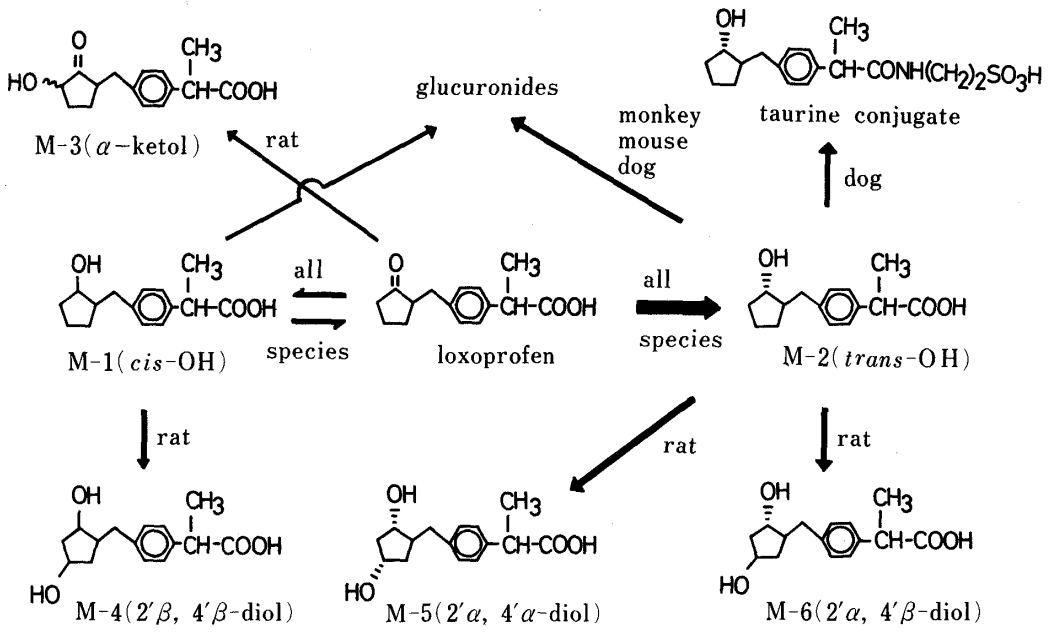

Fig. 6. Possible Metabolic Pathways of Loxoprofen in Animals

species examined.

In order to examine the further transformation of the primary monohydroxy metabolites and to establish possible metabolic pathways of loxoprofen, urinary metabolites were analyzed in rats after oral administration of the *trans*- or *cis*-OH-^{14}C compounds. Table III shows that administration of the *trans*-OH compound furnished M-5 (58.4%) and M-6 (13.8%) as the main metabolites, while M-4 (29.1%) and M-5 (30.6%) were formed from *cis*-OH. Thus, diol metabolites were shown to be formed by hydroxylation of the monohydroxy intermediates. M-4 was formed from *cis*-OH only.

Discussion

On the basis of the determination of the urinary metabolites, the main metabolic pathways of loxoprofen are presented in Fig. 6. In all animal species examined, loxoprofen is

predominantly reduced to afford the *trans*-OH compound together with the *cis*-OH compound as a minor product. The monohydroxy metabolites are hydroxylated further at the cyclopentanol moiety in rats, giving rise to the diols M-4, M-5 and M-6 as the main urinary metabolites. Mice excrete the monohydroxy metabolites and parent acid as the free forms together with the *trans*-OH glucuronide. In dogs and monkeys, the monohydroxy metabolites formed are mainly converted to conjugated forms. Thus, the taurine conjugate of the *trans*-OH compound is the major component in dog urine, together with minor ester glucuronides. Monkeys excrete the parent acid and monohydroxy metabolites as the major ester glucuronides. Human studies on loxoprofen sodium have revealed that the urinary metabolites are largely ester glucuronides of the parent acid, the *trans*-OH compound and *cis*-OH compound.[5] Therefore, the metabolism of loxoprofen in human subjects appears to have a close similarity to that of monkeys.

After oral administration to rats, the *trans*-OH compound was converted to the diols M-5 and M-6, while the *cis*-OH compound afforded M-4 and M-5, the latter (M-5) being the diol derived from the *trans*-OH compound. This finding suggested the occurrence of *in vivo* oxidation of the *cis*-OH compound back to the parent ketone, which is in turn reduced to the thermodynamically more stable *trans*-OH compound. This *in vivo* conversion reaction of the *cis*-OH compound to the *trans*-OH compound is thought to favor the pharmacological potency of loxoprofen sodium, since the *trans*-OH compound is an active principle with strong inhibitory activity on PG synthetase.

The principal metabolic pathways of phenylacetic acid derivatives are known to be conjugation reactions of the carboxylic acid group with glucuronic acid and/or with several amino acids.[6] The amino acids involved vary with both the chemical structures of the carboxylic acids and the animal species examined. There is no general rule regarding the relationship between the chemical structures of the carboxylic acids and the types of conjugation, but hitherto obtained findings may be summarized as follows: phenylacetic acid and its simple derivatives are conjugated with glycine in many cases, while the more complex derivatives with condensed heterocyclic rings, such as indomethacin[7] and the α-methyl-substituted derivatives, *e.g.* benoxaprofen[8] and cicloprofen,[9] are mainly conjugated with glucuronic acid. The presence of the α-methyl group or the complex aryl moiety seems to exert some inhibitory effect on either the initial activation step of the carboxylic acid or the subsequent transfer of the amino acid. Although taurine conjugation is a rather minor process in general drug metabolism, carnivorous species appear to form taurine conjugates of relatively simple phenylacetic acid derivatives.[6] Recently, the pyrrole metabolite of pirprofen was reported to undergo taurine conjugation in the rat and mouse.[10] In the metabolism of loxoprofen, the primary reduction product, the *trans*-OH derivative, was shown to be conjugated mainly with taurine in dogs. This is thought to be the first example of the taurine conjugation of an α-methylpropionic acid derivative in dogs.

Excretion patterns of loxoprofen in experimental animals appear to have some similarities to those of *p*-(cyclopropylcarbonyl)phenylacetic acid (SQ 20650),[11] in which the parent keto acid and its carbonyl reduction product are excreted as the free acids in rats, but as the taurine conjugate in dogs and as the glucuronides in monkeys.

Nakamura *et al.* have reported the interesting finding that only the *l*-enantiomer can be converted to the corresponding acyl coenzyme A (CoA) thioester in the optical inversion reaction of *l*- to *d*-ibuprofen in rats.[12] Accordingly, it can be reasoned that the formation of the amino acid conjugates of α-arylpropionic acid derivatives might occur only with the *l*-isomer through the activated thioester intermediate. In this connection, the problem of taurine conjugate formation from *d*- or *l*-enantiomer (at the propionic acid moiety) of the *trans*-OH compound is now under investigation in dogs.

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