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Quantitative determination of diol metabolites of CS-670, a new antiinflammatory agent, by capillary column gas chromatography–mass spectrometry

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Abstract

CS-670(I), being developed as a non-steroidal anti-inflammatory agent, is a racemic prodrug. It has been found to be readily metabolized to active metabolites: *trans* and unsaturated mono-ols (*trans*-OH, unsaturated-OH). We report here a method for the quantitative determination of the eight diol stereoisomers excreted in urine after administration I. The diols were well separated and quantitated using capillary column GC–MS after a rather simple derivatization with diazomethane–trifluoroacetic anhydride. Sex differences in rats and species differences between rats and mice were observed in the metabolism of I: the *trans*-diols originating from *trans*-OH were predominantly excreted in male and female rat urine but the excretion rate was greater in the male rats; the *cis*-diols originating from *cis* mono-ol (*cis*-OH) were the major urinary metabolites in mice. The hydroxy groups were mainly introduced at the respective equatorial hydrogen atoms at the 4′-carbon of *trans*-OH and the 5′-carbon of *cis*-OH. The 4′- and 5′-hydroxy groups in the diols were in the *cis* conformation with respect to the original 2′-hydroxy group. As approximately 9% of the *trans*-diols were excreted in urine after administration of *cis*-OH to rats, the chiral inversion from *cis*-OH to *trans*-OH was suggested to occur through the saturated ketone intermediate.

1. Introduction

CS-670 (±)-2-[4-(2-oxocyclohexylidenmethyl) phenyl]propionic acid (I), is a new anti-inflammatory drug. It exerts its pharmacological activity in the body after being transformed to two active metabolites, *trans*-OH and unsaturated-OH [1]. At present, 2-arylpropionic acid non-steroidal anti-inflammatory agents, such as ibuprofen, ketoprofen and loxoprofen, are widely used as racemate drugs in clinical trials [2–4]. It is well-known that the (−)-(*R*) configuration in the propionic acid moiety of these drugs is inverted to (+)-(*S*) in the metabolic process [5,6]. This chiral inversion, by which the inactive (−)-(*R*) enantiomers are converted to the active (+)-(*S*), is also one of the metabolic activation reactions of I [1]. In the initial metabolic steps of this drug, mono-ols, such as unsaturated-OH, *trans*-OH and *cis*-OH, are produced by reduction of the double bond and the cyclohexanone ring. As one or two asymmetric centres are

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generated through this reduction, many stereoisomers exist. Among the metabolites, (2*S*,2'*S*)-unsaturated-OH and (2*S*,1'*R*,2'*S*)-*trans*-OH have been found to be active metabolites from an *in-vitro* study of prostaglandin synthesis (unpublished results). As reported previously, it is considered that the mechanism of action of I in inflammatory tissues is similar to that of loxoprofen [7]. Therefore, the active metabolites of I must be determined to estimate the pharmacological effect. We have already reported the analytical method combined with antibody-mediated extraction and HPLC for determination of the stereoisomeric mono-ols in human and rat plasma [1].

The mono-ol metabolites in plasma have been suggested to be excreted as diol metabolites in rats, because the urinary metabolites were more polar and not hydrolized with glusulase (unpublished results). The most probable eight diol derivatives were prepared, and separation by derivatization with chiral reagents and chiral HPLC were investigated, but not successful.

In this paper, we describe an analytical method for the stereoselective determination of the urinary diol metabolites of I by GC–MS. Furthermore, we report the stereoselective metabolism of I in rats and mice.

2. Experimental

2.1. Instrumentation and chromatographic conditions

A Finnigan MAT 4600 GC–MS system with a pulsed positive-ion and negative-ion chemical ionization accessory was used (CA, USA). A 50% phenyl silicone-coated fused-silica capillary column (DB17) 30 m × 0.32 mm I.D., with a film thickness of 0.25 μm was used (J & W Scientific, Folsom, CA, USA). GC–MS was carried out as follows: The temperature of the injection port and transfer line were kept at 290°C, and the GC oven was maintained at 100°C for 30 s after the splitless injection, and then was programmed to 240°C at 10°C/min and held for 4 min. The electron-impact (EI) mode was used for the ionization, and the ionization voltage and emission current were set at 70 eV and 0.25 mA, and the manifold was maintained at 260°C. For quantitation of the diol metabolites and internal standard (ISTD), both molecular ions, m/z 484 and m/z 470, were measured by selected-ion monitoring (SIM).

2.2. Materials

All standards of I, mono-ols, diol metabolites (diol-1 to diol-8) and internal standard (ISTD, diol metabolite of loxoprofen) for GC–MS analysis were synthesized in the Chemical Research Laboratories of Sankyo (Fig. 1). The deuterium-labelled diols, diol-3-d_3 and diol-8-d_3, were also used as ISTD. These deuterated ISTDs were isolated from urine after administration of the deuterium labelled *trans*-OH(d_3) to rats (Fig. 2). Trifluoroacetic anhydride (TFAA) and N-methyl-N-nitrosamide (MNA) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). MNA was used for the preparation of diazonmethane, and all other reagents and solvents used were of analytical grade.

2.3. Animals, dosing and sample collection

Male and female Wistar rats (250–280 g) and male ddY mice (20 g) were used after being fasted overnight prior to the experiment. Compound I was dissolved in 0.1 *M* phosphate buffer and orally administered at a dose of 2 mg/kg to rats and mice. Animals were kept in a metabolic cage, and urine samples were collected at periods of 0 to 6 h and 6 to 24 h. The samples were pooled and frozen at −20°C until analysis.

2.4. Preparation of the samples for GC–MS

The pooled urine was diluted ten fold with water, and 1 μg of ISTD (50 μl of a 20 μg/ml solution in methanol) was added to 1 ml of the diluted urine. The urine was extracted with 5 ml of ethyl acetate after acidification with 0.1 ml of 1 *M* HCl, and the organic layer was evaporated to dryness. The extract was derivatized to methylester with diazomethane, and the methylester was then reacted with 0.2 ml of TFAA in 0.5 ml

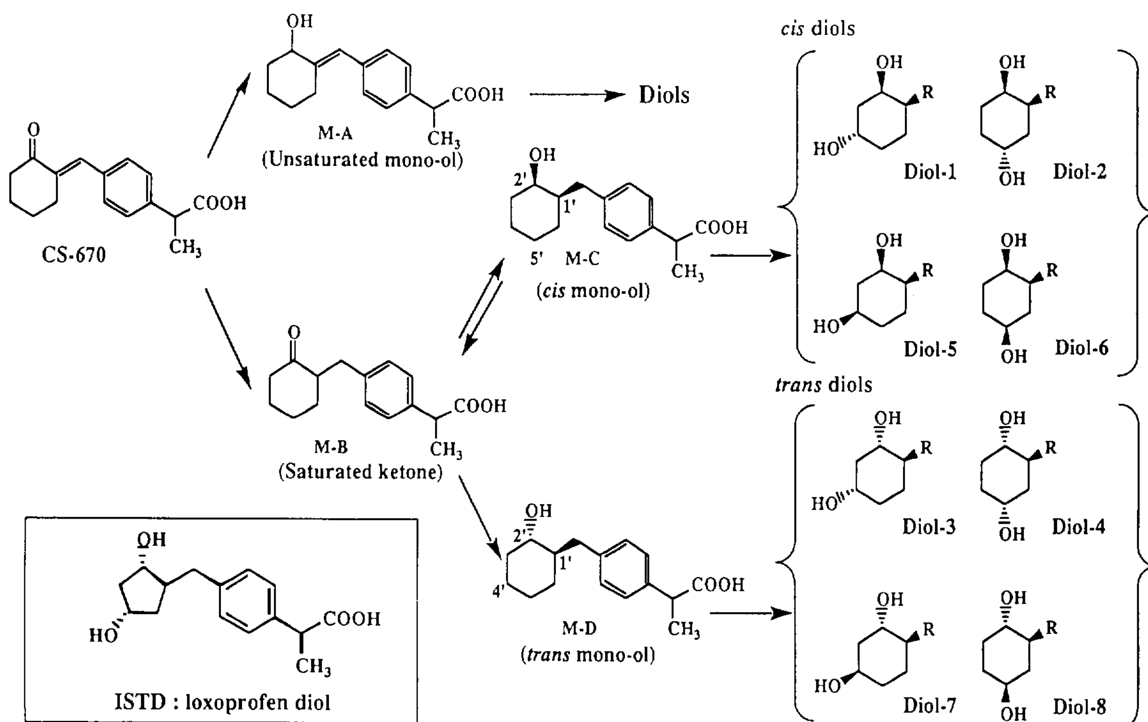

Fig. 1. Metabolic pathway of CS-670 in rats and mice.

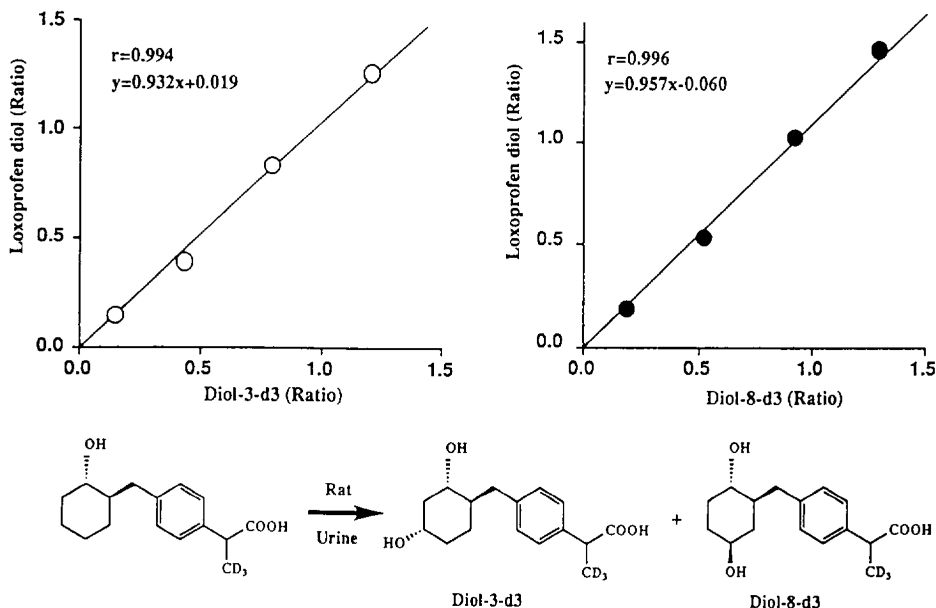

Fig. 2. Relationship between loxoprofen diol and deuterium labeled diols used as the internal standard.

of ethyl acetate for 30 min at room temperature. After evaporation of the reaction mixture, the product was re-dissolved in 0.5 ml of ethyl acetate, and a 1-μl aliquot was injected into the GC–MS system. Also, the glucuronic acid conjugate in the urine was hydrolyzed with 0.2 *M* naOH for 30 min at 60°C. After adjusting the pH to 2–3 using 5 *M* HCl, the sample solution was extracted and derivatized in the same manner as the untreated urine.

3. Results and discussion

3.1. Measurement of diol metabolites

In order to investigate the stereoselective metabolism of I, the separation behaviour of the authentic eight diol standards was examined by GC–MS. The carboxyl and hydroxy moieties of the diols were converted to methylester/trifluoroacetyl groups with diazomethane and TFAA, respectively. This derivatization reaction of the diols was completed quantitatively, and the corresponding difluoroacetylated derivatives were identified by GC–MS (total-ion current mode). It was found that the eight stereoisomers of the diol were well-separated, as shown in Fig. 3. The diol metabolites in urine were quantitatively extracted with ethyl acetate under acidic conditions, and the derivatized diols were determined in the SIM mode using m/z 480 and m/z 470, which are the molecular ions of the diol derivative and ISTD. In the quantitative analysis of the diol metabolites, no interfering peaks derived from the derivatizing reagents and urine were observed on the mass-chromatograms. Furthermore, no diol metabolites other than the eight standards were observed in rat urine after administration of I.

3.2. Calibration curve

The diol of loxoprofen, the structure of which is very similar to that of the diols of I, was used as the ISTD for determination of the urinary diol metabolites. All calibration curves of the 8 diols were linear over the range 0–5000 ng ($r = 0.991$–0.997), and the detection limit of each diol was 500 pg/ml.

Stable isotopes (SI), such as deuterium-,

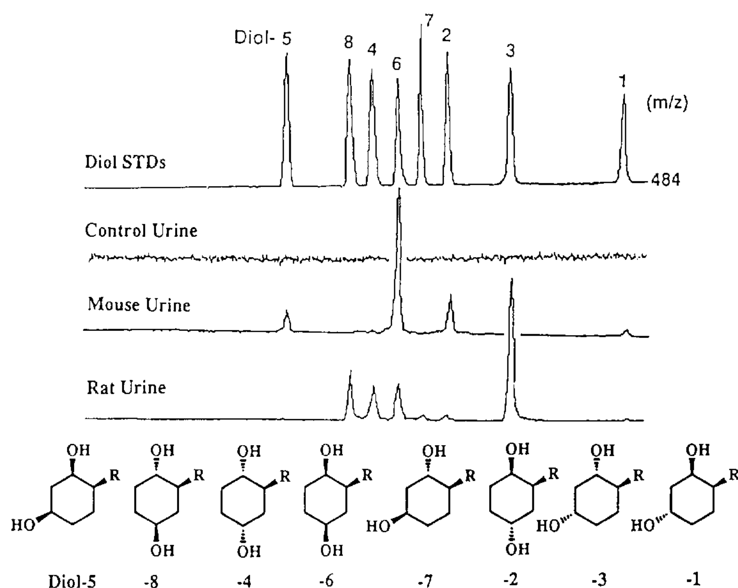

Fig. 3. Typical mass chromatogram of methylester and trifluoroacetylated diol metabolites.

carbon-13-, or mitrogen-15-labelled compounds, are ideal as ISTD for quantitative analysis by GC–MS. However, the synthesis of the eight SI-labelled stereoisomers was rather time-consuming and very difficult in this study. Therefore, in order to justify the use of loxoprofen diol as an ISTD, deuterium-labelled diols were prepared as follows. The main deuterated diol metabolites, diol-3 and diol-8, were isolated from urine after administration of the deuterium-labelled (1'*R*,2'*S*)-*trans*-OH(d_3) to rats, and the relationship between loxoprofen diol and the deuterated diols was examined. As shown in Fig. 2 the slope of the curve for the loxoprofen diol vs. the deuterium-labelled diols was approximately 1, and thus the use of loxoprofen diol as ISTD was justified in the quantitative determination of the eight stereoisomeric diol metabolites.

3.3. Administration of I

We have determined the urinary excretion of radioactivity during a 144-h period after administration of ^{14}C-labelled I to rats (data not shown). The urinary excretion rate in the first 24 h was 92.6%, thereafter only 5.8% until 144 h. Thus, we examined the diol metabolites in the urine from 0 to 24 h in these experiments.

Rat

The excretion of urinary diol metabolites after administration of I at a dose of 2 mg/kg to male and female rats is shown in Table 1 and Fig. 4.

Table 1
Excretion of diol metabolites in urine after oral administration of CS-670 to male and female rats (2 mg/kg; $n = 3$)

Metabolite	Excretion (% of dose)					
	Male			Female		
	0–6 h	6–24 h	0–24 h	0–6 h	6–24 h	0–24 h
Free						
Diol-1	0.82 ± 0.13	0.63 ± 0.32	1.45 ± 0.20	0.81 ± 0.34	2.11 ± 0.06	2.92 ± 0.30
Diol-2	n.d.[a]	n.d.	n.d.	n.d.	n.d.	n.d.
Diol-3	14.55 ± 1.11	15.32 ± 1.30	29.84 ± 0.76	4.04 ± 1.70	20.88 ± 6.39	24.92 ± 4.83
Diol-4	3.71 ± 0.26	4.26 ± 0.29	7.97 ± 0.08	0.73 ± 0.37	3.54 ± 1.75	4.27 ± 1.53
Diol-5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Diol-6	3.32 ± 0.57	2.37 ± 0.40	5.69 ± 0.60	1.29 ± 0.45	2.96 ± 1.49	4.25 ± 1.39
Diol-7	0.72 ± 0.08	0.43 ± 0.22	1.15 ± 0.14	0.64 ± 0.27	1.93 ± 0.28	2.57 ± 0.51
Diol-8	3.65 ± 0.29	3.86 ± 0.34	7.51 ± 0.23	0.61 ± 0.26	2.93 ± 1.42	3.54 ± 1.25
Total	27.77	26.87	53.61	8.12	34.35	42.47
Glucuronide						
Diol-1	0.23 ± 0.12	0.31 ± 0.28	0.55 ± 0.39	0.15 ± 0.15	0.20 ± 0.20	0.35 ± 0.35
Diol-2	n.d.	n.d.	n.d.	0.05 ± 0.04	0.78 ± 0.78	0.83 ± 0.77
Diol-3	1.29 ± 0.67	5.57 ± 2.84	6.87 ± 2.18	0.47 ± 0.47	n.d.	0.47 ± 0.47
Diol-4	0.67 ± 0.44	1.46 ± 0.73	2.12 ± 0.90	0.27 ± 0.20	0.58 ± 0.58	0.85 ± 0.53
Diol-5	n.d.	0.19 ± 0.19	0.19 ± 0.19	0.88 ± 0.52	2.56 ± 1.54	3.41 ± 1.11
Diol-6	2.31 ± 0.95	2.87 ± 1.55	5.18 ± 1.14	0.50 ± 0.27	0.32 ± 0.32	0.82 ± 0.52
Diol-7	n.d.	0.28 ± 0.28	0.28 ± 0.28	0.17 ± 0.14	0.34 ± 0.34	0.51 ± 0.28
Diol-8	0.08 ± 0.07	0.87 ± 0.49	0.95 ± 0.49	0.10 ± 0.10	0.40 ± 0.40	0.49 ± 0.36
Total	4.58	11.55	16.14	2.59	5.18	7.73
Total[b]	32.35	38.42	69.75	10.71	39.53	50.20

[a] Not detected.
[b] Sum of free and glucuronide.

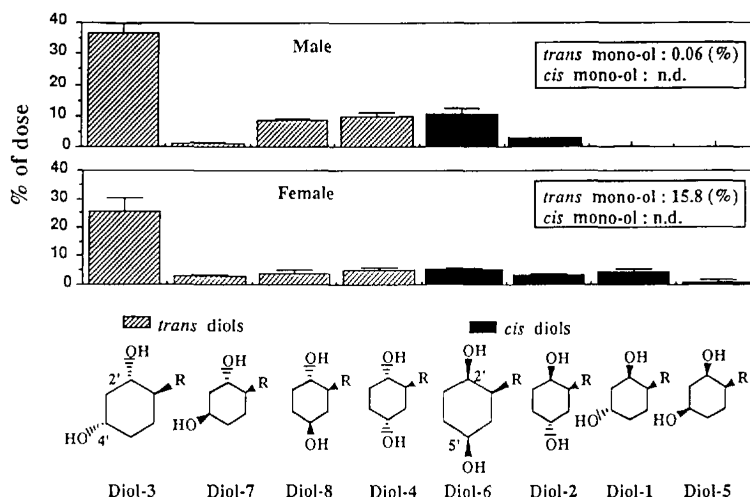

Fig. 4. Urinary excretion of diol metabolites after oral administration of CS-670 to rats.

The diol metabolites were found to be exclusively excreted in urine as their free forms, since the glucuronic acid conjugate hydrolized in the mild alkaline solution was present only in small amounts. In this study, therefore, urinary excretion was taken as the total amount of free and conjugated diols. Seven and eight diol metabolites were detected in the urine of male and female rats, respectively. In male rats, the excretion rate of diol-3 was 36.7% of the original dose; diol-4 (10.1%), diol-6 (10.7%) and diol-8 (8.5%) were excreted to approximately the same extent. Diol-1 and diol-7 constituted less than 2% each of the original dose, and the amounts of diol-2 and diol-5 were either very small or not detected. The total recovery of urinary diol metabolites in female rats was 20% lower than that in male rats, mainly because of a slow excretion rate in the first 0–6 h after dosing. For the main metabolite, diol-3, the differences in excretion rate between 0–6 hr (15.8%) and 6–24 h (20.9%) were not significant in male rats, but excretion was about 5 times higher in the second period (20.9%) than in the first period (4.5%) in female rats. However, the excreted-diol profiles were similar in male and female rats. This result suggests that the sex differences in the excretion rates of the diols are due to cytochrome P-450 levels responsible for the hydroxylation of the mono-ol metabolites. This phenomenon was supported by the urinary excretion rates of mono-ols after administration of I: 0.06 and 15.8% of the dose in male and female rats, respectively (Table 2).

The total excretion of *trans*-diols (male: 56.7%, female: 37.6%) was 4.4 (male) and 2.7 (female) times higher than that of *cis*-diols (male: 12.9%, female: 13.8%), demonstrating the stereoselective formation of *trans*-diol metabolites in both male and female rats. The AUC of the *trans*-OH has been shown to be 10 times higher than that of *cis*-OH after administration of I to rats (unpublished results), indicating that *trans*-OH should be predominantly produced by the stereoselectively reductive metabolism of both the double bond and the cyclohexanone ring of the drug. The existence of a large amount of the *trans*-diols in urine could be a reflection of the stereoselective production of *trans*-OH.

The position of hydroxylation and its conformation were discussed from the amount of diols excreted into the urine of male rats as follows. The ratio of 2′,4′-diol (diol-3, diol-7) and 2′,5′-diol (diol-4, diol-8) was 2 (38.2%/8.5%) in the case of *trans*-configurated diol, while the ratio of 2′,4′-diol (diol-1, diol-5) and 2′,5′-diol (diol-2, diol-5) was 0.2 (2.2%/10.7%) in the *cis*-configurated diol. The results show a preferential hydroxylation at the 4′-carbon for *trans*-OH and at the 5′-carbon for *cis*-OH, and

Table 2
Excretion of mono-ol metabolites in 0–24 h urine after oral administration of CS-670 and its related compounds to rats (2 mg/kg; $n = 3$)

Metabolite	Excretion (% of dose)					
	I		M-A (male)	M-B (male)	M-C (male)	M-D (male)
	Male	Female				
M-A	n.d.[a]	n.d.	0.08 ± 0.06	n.d.	n.d.	n.d.
M-B	0.03 ± 0.01	0.05 ± 0.01	n.d.	0.01 ± 0.01	0.03 ± 0.01	0.01 ± 0.01
M-C	n.d.	n.d.	n.d.	n.d.	0.003 ± 0.003	n.d.
M-D	0.06 ± 0.01	15.76 ± 4.31	n.d.	0.01 ± 0.01	n.d.	0.01 ± 0.01

M-A = unsaturated-OH, M-B = saturated ketone, M-C = *cis*-OH, M-D = *trans*-OH.
[a] Not detected.

the new hydroxy group was predominantly introduced at the respective equatorial hydrogen atom. As a result, a hydroxy group with a *cis* conformation with respect to the 2′-hydroxy group was produced. It was confirmed that the 4′-position of *trans*-OH and the 5′-position of *cis*-OH were preferentially hydroxylated in the *cis* conformation with respect to the original 2′-hydroxy group.

Mouse

In order to investigate the species differences between rats and mice, each stereosiomer of the urinary diols was determined by GC–MS. The results are shown in Table 3. Five diols were detected in the urine of mice, and the total excretion rate was 11.6% of the dose. The main diol metabolite was diol-6 (7.54%) followed by diol-1 (1.8%), and the other diols were less than 1%. The *trans*-diols made up only 0.7% of the total excretion, leading to a very small ratio of *trans* and *cis*-diols (0.06). In contrast with the results found in rats, it was found that *cis*-diols were overwhelmingly produced in mice. This phenomenon could originate from the stereoselective formation of *cis*-OH by reduction of the double bond and the cyclohexanone ring of I, which was obvious from the three-times

Table 3
Excretion of urinary diol metabolites after oral administration of CS-670 and *cis*-OH to mice (2 mg/kg; $n = 3$)

Metabolite	Excretion (% of dose)					
	I			*cis*-OH		
	0–6 h	6–24 h	0–24 h	0–6 h	6–24 h	0–24 h
Free						
Diol-1	0.68 ± 0.17	1.17 ± 0.15	1.84 ± 0.14	0.93 ± 0.13	1.56 ± 0.22	2.49 ± 0.14
Diol-2	0.44 ± 0.09	0.63 ± 0.12	1.06 ± 0.11	0.72 ± 0.07	0.98 ± 0.24	1.70 ± 0.22
Diol-3	0.13 ± 0.07	0.47 ± 0.02	0.66 ± 0.12	n.d.[a]	n.d.	n.d.
Diol-4	0.05 ± 0.05	n.d.	0.05 ± 0.05	n.d.	n.d.	n.d.
Diol-5	0.23 ± 0.09	0.36 ± 0.04	0.05 ± 0.05	0.14 ± 0.08	0.24 ± 0.12	0.38 ± 0.05
Diol-6	2.04 ± 0.54	5.50 ± 1.05	7.54 ± 1.05	2.49 ± 0.32	4.31 ± 0.81	6.79 ± 0.65
Diol-7	0.05 ± 0.05	n.d.	0.05 ± 0.05	n.d.	n.d.	n.d.
Diol-8	0.05 ± 0.05	n.d.	0.05 ± 0.05	n.d.	n.d.	n.d.
Total	3.67	8.13	11.30	4.28	7.09	11.36

[a] Not detected.

larger AUC of *cis*-OH than of *trans*-OH in mice. Just as in rats, the newly-introduced hydroxy group was at the 5′-equatorial positon and was in the *cis* conformation with respect to the 2′-hydroxy group.

3.4. Administration of saturated ketone and unsaturated-OH

The excretion of diol metabolites in urine after administration of synthesized saturated ketone (M-B) and unsaturated-OH (M-A) was determined and compared with the results for I, because these metabolites are thought to be intermediates in the diol metabolism.

As shown in Table 4, diol-6 and diol-3 were excreted as the main urinary metabolites, at rates of 18.8 and 14.1% respectively, after administration of M-B. The ratio of *trans*- to *cis*-diols was 0.9 (22.3%/24.5%), indicating equal production of both diols. This ratio was greatly different from the ratio (4.4) observed after administration of I. For this reason, the administered M-B was racemic with respect to the asymmetry at the 2′-carbon, although an optically active M-B with a 2′-*R* configuration has been considered to be produced by the stereoselective reduction of the double bond when I was administered. To elucidate this problem in the metabolism of I is a subject for the future. Diol-3 (25.6%) was the main metabolite excreted in urine after administration of M-A (Table 4). The large difference in the ratio of *trans*- to *cis*-diols was also observed in this case (39.7%/8.9% = 4.5). The pathway for the biotransformation of M-A to diols could be as

Table 4
Excretion of urinary diol metabolites after oral administration of unsaturated-OH (M-A) and saturated ketone (M-B) to rats (2 mg/kg; $n = 3$)

Metabolite	Excretion (% of dose)					
	Unsaturated-OH (M-A)			Saturated ketone (M-B)		
	0–6 h	6–24 h	0–24 h	0–6 h	6–24 h	0–24 h
Free						
Diol-1	0.02 ± 0.02	0.28 ± 0.14	0.30 ± 0.15	1.68 ± 0.30	0.78 ± 0.07	2.46 ± 0.32
Diol-2	n.d.[a]	n.d.	n.d.	n.d.	0.02 ± 0.02	0.02 ± 0.02
Diol-3	0.97 ± 0.11	10.97 ± 1.10	11.94 ± 1.13	4.47 ± 0.61	7.24 ± 0.77	11.71 ± 1.32
Diol-4	0.29 ± 0.05	4.02 ± 0.53	4.31 ± 0.53	1.16 ± 0.18	1.41 ± 0.62	2.57 ± 0.68
Diol-5	n.d.	n.d.	n.d.	0.14 ± 0.08	0.24 ± 0.12	0.38 ± 0.05
Diol-6	0.24 ± 0.05	1.57 ± 0.76	1.81 ± 0.78	7.80 ± 0.13	4.48 ± 0.40	12.28 ± 0.53
Diol-7	0.03 ± 0.01	0.27 ± 0.14	0.30 ± 0.15	0.24 ± 0.22	0.24 ± 0.14	0.48 ± 0.16
Diol-8	0.24 ± 0.08	2.64 ± 0.08	2.88 ± 0.15	1.08 ± 0.15	1.32 ± 0.47	2.39 ± 0.55
Total	1.79	19.75	21.54	16.57	15.73	32.29
Glucuronide						
Diol-1	0.28 ± 0.15	1.50 ± 0.62	1.78 ± 0.54	1.00 ± 0.21	0.33 ± 0.11	1.33 ± 0.21
Diol-2	n.d.	n.d.	n.d.	0.11 ± 0.11	n.d.	0.11 ± 0.11
Diol-3	2.23 ± 1.26	11.40 ± 4.40	13.63 ± 3.93	2.02 ± 0.40	0.38 ± 0.21	2.41 ± 0.52
Diol-4	0.65 ± 0.33	2.19 ± 1.21	2.84 ± 1.53	0.76 ± 0.15	0.79 ± 0.57	1.54 ± 0.42
Diol-5	n.d.	n.d.	n.d.	1.08 ± 0.31	0.34 ± 0.09	1.42 ± 0.11
Doil-6	1.31 ± 0.82	3.67 ± 2.47	4.99 ± 1.97	5.66 ± 1.26	0.81 ± 0.40	6.47 ± 1.66
Diol-7	0.18 ± 0.12	1.59 ± 0.61	1.76 ± 0.55	n.d.	0.13 ± 0.07	0.13 ± 0.07
Diol-8	1.51 ± 0.27	1.55 ± 0.58	2.07 ± 0.83	1.40 ± 0.12	0.66 ± 0.54	1.30 ± 0.65
Total	6.16	21.90	27.07	12.03	3.44	14.71
Total[b]	7.95	41.65	48.61	28.60	19.17	47.00

[a] Not detected.
[b] Sum of free and glucuronide.

follows: (1) after reoxidation to I by oxidation of the hydroxy group of M-A, *trans* or *cis*-OHs are produced by the reduction of the double bond and the cyclohexanone ring; and then the mono-ols are hydroxylated to diols. (2) The double bond of M-A was first reduced, then hydroxylated. It is difficult to judge which is the case from the experimental results mentioned here.

3.5. Administration of trans and cis mono-ol

The urinary diol metabolites after administration of *trans* and *cis*-OHs, the main metabolites in plasma after dosing with I, were determined. The results are shown in Table 5. The excretion rates of diol-3 and diol-8 were 37.0% and 22.4%, respectively, after administration of *trans*-OH. Diol-6 (41.5%) was detected as the main metabolite after administration of *cis*-OH. These results were similar in pattern to those obtained after administration of I. However, the excretion of diol-8 was larger in the case of *trans*-OH administration compared with that after administration of I. This was explained by assuming that the administered *trans*-OH was a racemate as well as the saturated ketone. The production of *cis*-diols from *trans*-OH was only 0.51% of the dose; on the other hand the formation of *trans*-diols from *cis*-OH was found to be 9.4%. These results suggest that the hydroxy group of *cis*-OH was readily oxidized to the saturated ketone and metabolized to *trans*-configurated diols through *trans*-OH; however, such a metabolic pathway does not exist in the case of *trans*-OH. This is one type of chiral inversion, and similar phenomena were found for loxoprofen [8]. If optically-pure (1′*R*,2′*R*) or (1′*S*,2′*S*)-*cis*-OH were used in this experiment, a clear result might be obtained.

Table 5
Excretion of urinary diol metabolites after oral administration of *cis*-OH and *trans*-OH to rats (2 mg/kg; $n = 3$)

Metabolite	Excretion (% of dose)					
	cis-OH			*trans*-OH		
	0–6 h	6–24 h	0–24 h	0–6 h	6–24 h	0–24 h
Free						
Diol-1	3.76 ± 0.94	2.08 ± 0.41	5.84 ± 0.84	n.d.[a]	n.d.	n.d.
Diol-2	0.18 ± 0.09	0.34 ± 0.34	0.52 ± 0.41	n.d.	n.d.	n.d.
Diol-3	2.26 ± 0.67	2.81 ± 0.64	5.07 ± 1.13	12.39 ± 3.07	16.93 ± 3.53	29.33 ± 0.78
Diol-4	0.54 ± 0.18	0.69 ± 0.16	1.23 ± 0.32	2.04 ± 0.53	3.57 ± 0.48	5.62 ± 0.05
Diol-5	1.77 ± 0.47	1.09 ± 0.17	2.86 ± 0.30	n.d.	n.d.	n.d.
Diol-6	17.36 ± 2.45	9.06 ± 2.27	26.42 ± 3.41	n.d.	0.13 ± 0.13	0.13 ± 0.13
Diol-7	n.d.	n.d.	n.d.	0.89 ± 0.22	1.01 ± 0.22	1.90 ± 0.04
Diol-8	0.84 ± 0.24	1.56 ± 0.09	2.40 ± 0.23	6.89 ± 1.51	9.38 ± 1.74	16.28 ± 0.23
Total	26.71	17.63	44.34	22.21	31.02	53.26
Glucuronide						
Diol-1	1.65 ± 0.66	n.d.	1.65 ± 0.66	n.d.	n.d.	n.d.
Diol-2	0.24 ± 0.12	0.02 ± 0.02	0.24 ± 0.12	n.d.	n.d.	n.d.
Diol-3	0.37 ± 0.25	n.d.	0.37 ± 0.25	6.27 ± 4.56	1.40 ± 1.29	7.67 ± 4.44
Diol-4	0.11 ± 0.09	n.d.	0.11 ± 0.09	0.22 ± 0.11	0.15 ± 0.15	0.36 ± 0.22
Diol-5	1.01 ± 0.25	0.86 ± 0.28	1.87 ± 0.22	n.d.	n.d.	n.d.
Diol-6	12.74 ± 6.23	2.29 ± 1.42	15.03 ± 4.86	0.17 ± 0.17	0.22 ± 0.21	0.40 ± 0.38
Diol-7	n.d.	0.06 ± 0.06	0.06 ± 0.06	0.56 ± 0.51	n.d.	0.56 ± 0.51
Diol-8	0.16 ± 0.11	n.d.	0.16 ± 0.11	5.60 ± 2.94	0.54 ± 0.54	6.09 ± 2.77
Total	16.28	3.23	19.49	12.82	2.31	15.08
Total[b]	42.99	20.86	63.83	35.03	33.33	68.34

[a] Not detected.
[b] Sum of free and glucuronide.

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