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Structural Determination of Rat Urinary Metabolites of Sodium 2-[4-(2-Oxocyclopentylmethyl)phenyl]propionate Dihydrate (Loxoprofen Sodium), a New Anti-inflammatory Agent

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The main metabolites of loxoprofen sodium were isolated from rat urine by column chromatography. Their chemical structures were determined on the basis of spectral data and by comparison of the metabolites with authentic samples to be as follows: the parent acid (M-0), two reduction products, *i.e.*, the *cis*-alcohol (M-1) and the *trans*-alcohol (M-2), the α-hydroxy ketone (M-3) and three diol metabolites (M-4, M-5 and M-6). The established metabolite structures all indicated that metabolic reactions of loxoprofen in rats occur only at the cyclopentanone moiety.

The *trans*-alcohol metabolite, which has a high inhibitory activity on prostaglandin (PG)-synthetase, was determined to be optically pure, with (2*S*, 1′*R*, 2′*S*)-configurations, by high performance liquid chromatography (HPLC) analysis after derivatization to the diastereomeric amide of the carboxy group with (−)-α-phenylethylamine reagent, and subsequently to the ester of the hydroxy function using (−)-α-methoxy-α-trifluoromethylphenylacetyl chloride.

Keywords——anti-inflammatory agent; sodium 2-[4-(2-oxocyclopentylmethyl)phenyl]-propionate dihydrate; loxoprofen sodium; α-arylpropionic acid derivative; rat urinary metabolite; structural determination; absolute configuration; ^{13}C-NMR analysis

Sodium 2-[4-(2-oxocyclopentylmethyl)phenyl]propionate dihydrate (loxoprofen sodium), is a newly synthesized α-arylpropionic acid derivative[1] having potent anti-inflammatory and analgesic activities with a relatively weak gastrointestinal ulcerogenicity.[2] Metabolic reactions such as carbonyl reduction and/or hydroxylation can be expected to occur on the α-substituted cyclopentanone moiety of the loxoprofen molecule. The metabolites formed are of stereochemical interest in view of the stereospecificity of the enzyme reaction.

This paper describes the isolation of the main urinary metablites of rats and their structural determination with emphasis on the establishment of the stereochemistry of the monohydroxy metabolites, which contain three asymmetric carbons.

Experimental

Chemicals——The authentic samples used for the identification of the urinary metabolites were prepared by Naruto *et al.*[1] Sodium cyanoborohydride ($NaCNBH_3$), potassium tri-*sec*-butyl borohidride (K-selectride), (−)-α-phenylethylamine, (−)-α-methoxy-α-trifluoromethylphenylacetyl chloride ((−)-MTPA Cl) and diethyl azodicarboxylate were obtained from Aldrich (Richmond, U.S.A.). All other reagents were of analytical-reagent grade.

Instruments——A GPC/ALC 202/401 high-performance liquid chromatograph (Waters Assoc., Milwaukee, U.S.A.) equipped with a model 71 ultraviolet (UV) detector (Waters) and an R401 differential refractometer (Waters) were used. An Si-60 Lobar column (Sizes A and B, Merck) and a Semi Prep μPorasil column (7.8 mm × 30 cm, Waters) were employed for the preparative separation and an μPorasil column (3.9 mm × 30 cm, Waters) for the analysis of urinary metabolites. Hexane–EtOAc (9 : 1—5 : 5) was employed as a mobile phase at a flow rate of 3.0 ml/min. Samples were applied with a U6K sample loop injector (Waters). Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian EM-360L spectrometer at 60 MHz using tetramethylsilane as an internal

standard. Abbreviations used: s=singlet, d=doublet, t=triplet, q=quartet and m=multiplet. Carbon 13 nuclear magnetic resonance (^{13}C-NMR) spectra were measured with a Varian XL-100 spectrometer at 25.2 MHz Mass spectra (MS) were obtained on a Hitachi D-100 spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter and optical rotatory dispersion spectra were taken on a JASCO J-20 spectrometer (Tokyo). Melting points were determined on a micro hot-stage apparatus and are uncorrected.

Administration of Loxoprofen Sodium——Loxoprofen sodium dissolved in distilled water was administered orally at a daily dose of 70 mg/kg to male Donryu rats weighing *ca.* 400 g for 2 d and, after an interval of 5 d, two further doses of 100 mg/kg/d (total dose of loxoprofen sodium, 10.0 g) were given. The 24 h urine was collected in the presence of a few drops of toluene (total urine volume, 2.7 l).

Isolation of Metabolites——The isolation procedures for urinary metabolites are shown in Chart 1. The urine

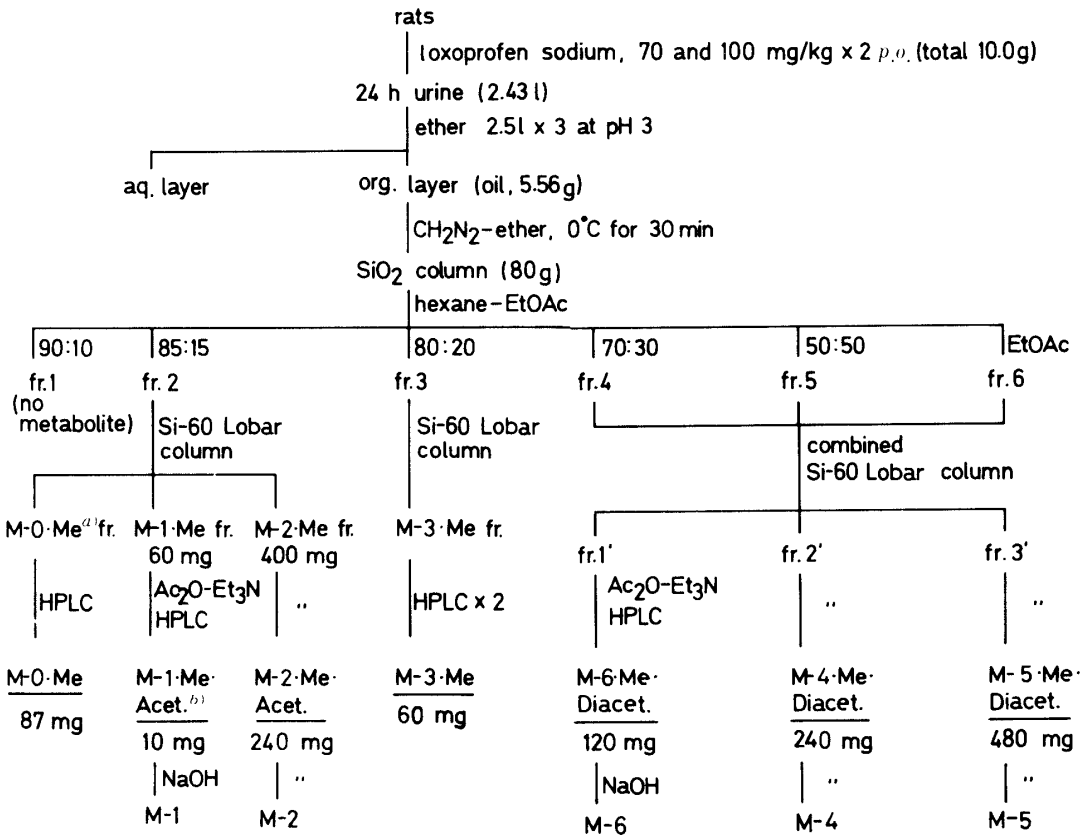


Chart 1. Isolation Procedures for Urinary Metabolites of Loxoprofen Sodium in Rats

a) Me=methyl ester.
b) Me Acet.=methyl ester acetate.

sample (2.43 l) was adjusted to pH 3 with 4 N HCl and extracted with ether (2.5 l×3). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. CH_2N_2–ether solution (50 ml) was added to the residue (5.56 g) in EtOAc (100 ml) and the mixture was allowed to stand for 30 min under ice-cooling. After decomposition of the excess reagent with AcOH, the reaction mixture was concentrated *in vacuo* and the residue was subjected to column chromatography on silica gel (80 g). Six fractions were eluted with the following hexane–EtOAc solvent systems: fr. 1 (90 : 10, 200 ml), fr. 2 (85 : 15, 100 ml), fr. 3 (80 : 20, 100 ml), fr. 4 (70 : 30, 200 ml), fr. 5 (50 : 50, 200 ml) and fr. 6 (EtOAc only, 200 ml). Fraction 1 contained no metabolite.

High performance liquid chromatography (HPLC) analysis of fr. 2 using a μPorasil column and a hexane–EtOAc (85 : 15) mobile phase showed the presence of three metabolite peaks of the methyl esters of M-0 (t_R 3.0 min), M-1 (t_R 5.1 min) and M-2 (t_R 9.3 min). Preparative chromatography of fr. 2 on an Si-60 Lobar column with the same solvent system successfully separated these three compounds, affording M-0 methyl ester (87 mg, after re-chromatography under the same conditions), M-1 methyl ester (60 mg) and M-2 methyl ester (400 mg).

M-0 methyl ester: $[\alpha]_D$ +17° (c=0.79%, EtOH). NMR ($CDCl_3$) δ: 1.43 (3H, d, J=7 Hz), 1.5—3.1 (9H, m), 3.56 (1H, q, J=7 Hz), 3.58 (3H, s), 6.99 (2H, d, J=9 Hz), 7.10 (2H, d, J=9 Hz). MS m/z: 260 (M^+), 201 (base), 177.

The M-1 methyl ester obtained above was treated overnight with acetic anhydride (1 ml) and triethylamine

(0.2 ml) at room temperature. The solution was concentrated *in vacuo* and chromatographed on an Si-60 Lobar column with hexane–EtOAc (90 : 10) to give pure M-1 methyl ester acetate as an oil (10 mg). $[\alpha]_D$ +50.3° (c=0.18%, EtOH). NMR ($CDCl_3$) δ: 1.43 (3H, d, J=7 Hz), 1.4—2.2 (7H, m), 1.96 (3H, s), 2.4—2.8 (2H, m), 3.60 (1H, s), 3.66 (1H, q, J=7 Hz), 5.06 (1H, m), 7.10 (4H, s). MS m/z: 304 (M^+), 244, 185 (base).

The acetate was hydrolyzed in MeOH (5 ml) and 10% aq. NaOH (2 ml) at room temperature overnight. The reaction mixture was concentrated *in vacuo*, acidified with dil. HCl and extracted with ether. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give M-1 as a solid compound. NMR ($CDCl_3$) δ: 1.48 (3H, d, J=7 Hz), 1.5—2.2 (7H, m), 2.5—2.95 (2H, m), 3.70 (1H, q, J=7 Hz), 4.10 (1H, br s), 6.15 (2H, br s, OH), 7.20 (4H, s). MS m/z: 248 (M^+), 230, 186, 91 (base).

M-2 methyl ester was acetylated in acetic anhydride (20 ml) and triethylamine (0.5 ml) and subsequent chromatographic purification in the same manner as described for M-1 methyl ester afforded the corresponding acetate as an oil (240 mg). NMR ($CDCl_3$) δ: 1.43 (3H, d, J=7 Hz), 1.4—2.9 (9H, m), 1.92 (3H, s), 3.60 (3H, s), 3.63 (1H, q, J=7 Hz), 4.8 (1H, m), 7.10 (4H, s). MS m/z: 304 (M^+), 244, 185 (base).

The acetate (120 mg) was hydrolyzed overnight in a mixture of MeOH (10 ml) and 10% aq. NaOH (1 ml) at room temperature. The hydrolyzed product was precipitated after concentration *in vacuo* and acidification with dil. HCl. Recrystallization from hexane–ether gave M-2 as colorless needles (52 mg). mp 82—84 °C, $[\alpha]_D$ +64.6° (c=0.15%, MeOH). NMR ($CDCl_3$) δ: 1.48 (3H, d, J=7 Hz), 1.5—2.2 (7H, m), 2.3—2.9 (2H, m), 3.70 (1H, q, J=7 Hz), 3.85 (1H, m), 5.95 (2H, br s, OH), 7.20 (4H, q, J=9 Hz). MZ m/z: 248 (M^+), 230, 186, 91 (base).

HPLC analysis of fr. 3 using a μPorasil column and hexane–EtOAc (70 : 30) as a mobile phase showed the presence of M-3 methyl ester at t_R 5.5 min, accompanied by many peaks of urinary components. This fraction of t_R 5.5 min was collected by Lobar column chromatography and chromatographic purification was repeated twice more under the same conditions. Pure M-3 methyl ester (60 mg) was obtained as an oil. $[\alpha]_D$ +32.0° (c=0.053%, MeOH). MS m/z: 276 (M^+), 258, 248, 217, 205, 178, 145, 117, 99 (base).

M-3 methyl ester (20 mg) was acetylated with acetic anhydride and triethylamine in the same manner as described above. The Lobar column chromatography with hexane–EtOAc (90 : 10) separated the corresponding acetates into two components in relative amounts of 2 : 1.

Fraction 4, fr. 5 and fr. 6 were combined and subjected to Lobar column chromatography using hexane–EtOAc (50 : 50) as the mobile phase to afford the methyl ester fractions of M-4, M-5 and M-6. These three metabolites had similar mass spectra; MS m/z: 278 (M^+), 260, 242, 219, 201, 177, 117 (base).

Each methyl ester fraction was acetylated with acetic anhydride and triethylamine in the same manner as described above and again chromatographed on a Lobar column using hexane–EtOAc (85 : 15) as the mobile phase.

M-4 methyl ester diacetate was obtained from fr. 2′ as an oil (240 mg). Hydrolysis of this compound (60 mg) as described above and recrystallization from hexane–ether gave M-4 as colorless needles (32 mg). mp 146—148 °C. $[\alpha]_D$ +80.9° (c=0.063%, MeOH). MS m/z: 264 (M^+), 246, 228, 205, 118, 91 (base).

M-5 methyl ester diacetate was obtained from fr. 3′ as a colorless oil (480 mg). $[\alpha]_D$ +71.9° (c=0.29%, EtOH). NMR ($CDCl_3$) δ: 1.48 (3H, d, J=7 Hz), 1.92 (3H, s), 2.01 (3H, s), 1.7—2.9 (7H, m), 3.66 (3H, s), 3.70 (1H, q, J=7H), 4.82 (1H, br s), 5.10 (1H, br s), 7.15 (4H, s). MS m/z: 362 (M^+), 302, 242, 184 (base).

Alkaline hydrolysis of this compound (120 mg) and recrystallization from hexane–ether gave M-5 as colorless needles (55 mg). mp 91—93 °C. $[\alpha]_D$ +29.6° (c=0.064%, MeOH). MS m/z: 264 (M^+), 246, 228, 205, 118, 91 (base).

M-6 methyl ester diacetate was obtained from fr. 1′ as a colorless oil (480 mg). $[\alpha]_D$ +61.3° (c=0.12%, EtOH). NMR ($CDCl_3$) δ: 1.46 (3H, d, J=7 Hz), 1.90 (3H, s), 1.99 (3H, s), 1.7—2.9 (7H, m), 3.60 (3H, s), 3.63 (1H, q, J=7 Hz), 5.00 (2H, m), 7.10 (4H, s). MS m/z: 362 (M^+), 302, 242, 184 (base).

Alkaline hydrolysis of this compound (100 mg) and recrystallization from hexane–ether gave M-6 as colorless needles (60 mg). mp 86—87 °C. $[\alpha]_D$ +55.2° (c=0.074%, MeOH). MS m/z: 264 (M^+), 246, 228, 205, 118, 91 (base).

Enantiomer Ratio Analysis of M-0, M-1 and M-2——Chart 2 shows the derivatization procedures for determination of the enantiomer ratios of the asymmetric carbons in both the propionic acid side chain and the cyclopentane ring of M-0, M-1 and M-2.

1) M-0 (parent acid) methyl ester (31 mg) in tetrahydrofuran (THF) (1 ml) was treated with K-selectride (1 ml) at 0 °C for 40 min. The reaction mixture was diluted with water and extracted with ether. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed on an Si-60 Lobar column with hexane–EtOAc (85 : 15) to afford the reduction product (*cis*-alcohol methyl ester) as an oil (23 mg). The oil in MeOH (5 ml) was treated with 10% aq. NaOH (1 ml) at room temperature overnight. The hydrolyzed product was precipitated after concentration *in vacuo* followed by acidification with dil. HCl. Recrystallization from hexane–ether gave the free acid (*cis*-alcohol) as colorless needles (14 mg).

This *cis*-alcohol in CH_2Cl_2 (5 ml) was mixed with 2,2′-dipyridyldisulfide (22 mg), triphenylphosphine (26 mg) and (−)-α-phenylethylamine (12 mg) and kept at 0 °C for 30 min. The reaction mixture was concentrated *in vacuo* and chromatography of the residue on an Si-60 Lobar column with hexane–EtOAc (70 : 30) separated the corresponding major diastereomeric amide from the more slowly eluted minor one. The major amide purified above was then treated with (−)-MTPA Cl in pyridine (1 ml) at 0 °C for 24 h. After evaporation of the solvent, the residue was analyzed by HPLC using a μPorasil column and a hexane–EtOAc (10 : 90) mobile phase at a flow rate of 3 ml/min.

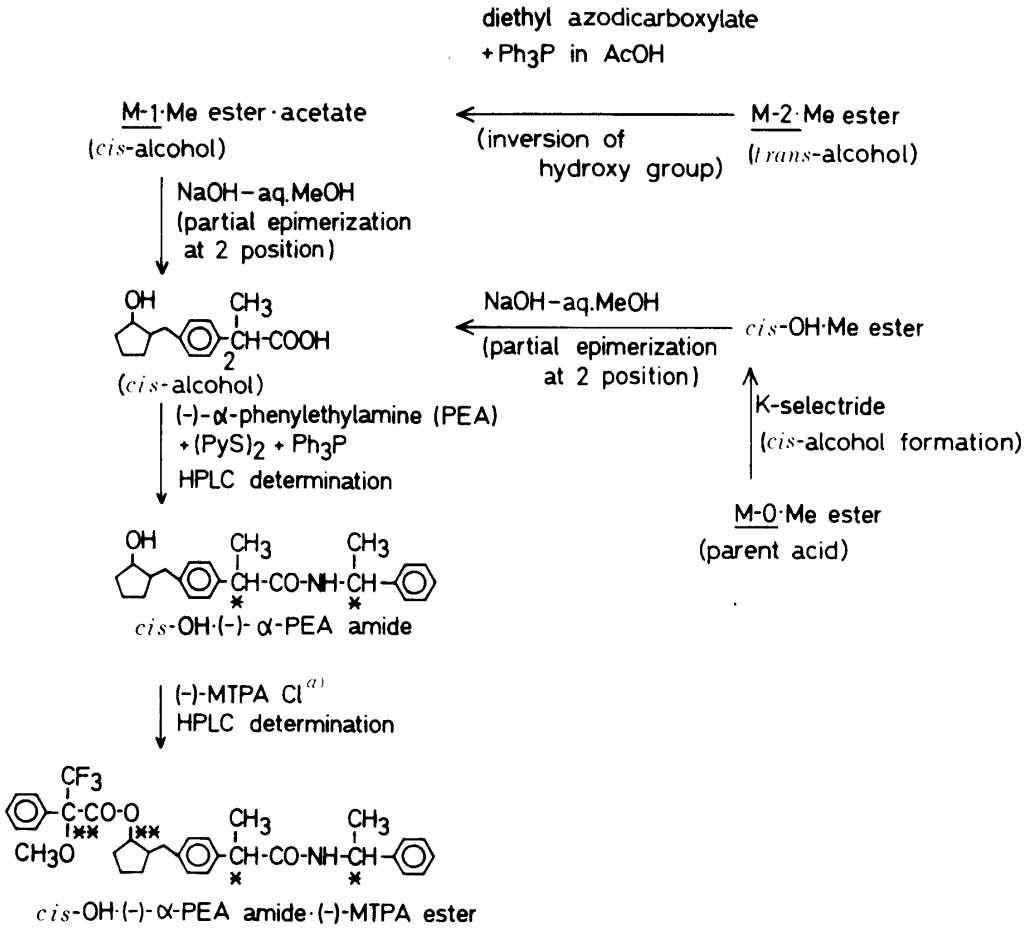


Chart 2. Derivatization Procedures for Enantiomer Ratio Analysis of M-0, M-1 and M-2

a) (−)-α-Methoxy-α-trifluoromethylphenylacetyl chloride.

2) The hydrolysis product (*cis*-alcohol, 10 mg) from M-1 methyl ester acetate was dissolved in CH_2Cl_2 (1 ml) and treated with 2,2′-dipyridyldisulfide (11 mg), triphenylphosphine (13 mg) and (−)-α-phenylethylamine (6 mg) at 0 °C for 30 min. The reaction mixture was worked up as described for M-0 (parent acid) methyl ester. Chromatographic purification afforded an equal amount (6 mg) of the corresponding two diastereomeric amides. The more rapidly eluted diastereomeric amide (5 mg) was treated with (−)-MTPA Cl (5 mg) in pyridine (0.2 ml) at 0 °C for 24 h. The reaction mixture was concentrated *in vacuo* and analyzed by HPLC under the same chromatographic conditions as described above.

3) Pure M-2 (*trans*-alcohol) in MeOH–ether (1 : 3, 10 ml) was treated with freshly prepared CH_2N_2–ether solution (5 ml) under ice-cooling. The excess reagent was decomposed with AcOH and the solvent was evaporated off *in vacuo*. Triphenylphosphine (130 mg), diethyl azodicarboxylate (80 mg) and acetic acid (30 ml) were added to a solution of the residue (M-2 methyl ester) in THF (3 ml), and the mixture was allowed to stand at 0 °C for 1 h. The solvent was evaporated off *in vacuo* and the residue was chromatographed on an Si-60 Lobar column. Elution with hexane–EtOAc (85 : 15) gave the acetate of the *cis*-alcohol methyl ester (as a result of inversion of the hydroxy group).

This compound was successively hydrolyzed, condensed with (−)-α-phenylethylamine, esterified with (−)-MTPA Cl and analyzed by HPLC in the same manner as mentioned above.

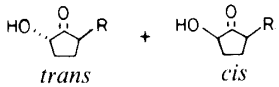
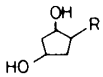
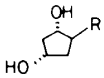
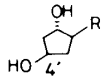
Results

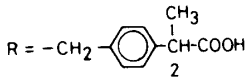
The chemical structures and some properties of the isolated urinary metabolites are summarized in Table I.

M-0

The MS of M-0 methyl ester showed a molecular ion peak at *m/z* 260, indicating that M-0

TABLE I. Chemical Structures and Stereochemical Properties of the Isolated Urinary Metabolites of Loxoprofen Sodium in Rats

Metabolite No.	Isolated as	mp	$[\alpha]_D$	Metabolite structure	Stereochemistry at 2-Carbon	Stereochemistry at 1′- and 2′-Carbons
M-0 (Parent acid)	Methyl ester	Oil	+17.0		(*S*) (57% *e.e.*[a])	(1′*S*) (29% *e.e.*)
M-1 (*cis*-Alcohol)	Methyl ester acet.	Oil	+50.3		(*S*)	Racemic
M-2 (*trans*-Alcohol)	Methyl ester acet.	Oil	+53.6		(*S*)	(1′*R*,2′*S*)
	Free	82—84				
M-3 (α-Ketol)	Methyl ester	Oil		 *trans* + *cis*		
M-4 (Diol)	Methyl ester diacet.	Powder	+71.9			
	Free	91—93				
M-5 (Diol)	Methyl ester diacet.	Powder	+61.3			
	Free	86—87				
M-6 (Diol)	Methyl ester diacet.	Powder	+51.0			
	Free	146—148				



a) Enantiomer excess.

is the unchanged acid. Indeed, M-0 methyl ester was confirmed to be identical with an authentic sample by comparison of the NMR and MS and t_R value on HPLC.

The absorbances at 226 and 295 nm in the circular dichroism (CD) spectrum of M-0 methyl ester, due to the α-phenylpropionic acid side chain and the α-substituted cyclopentanone moiety, respectively, were compared with those of the authentic (2*S*, 1′*S*)-loxoprofen methyl ester (Fig. 1).[3] The enantiomer excess values were 57 and 29% for the 2- and 1′-carbons, respectively, in the M-0 methyl ester.

M-0 methyl ester was reduced stereoselectively with K-selectride to the *cis*-alcohol methyl

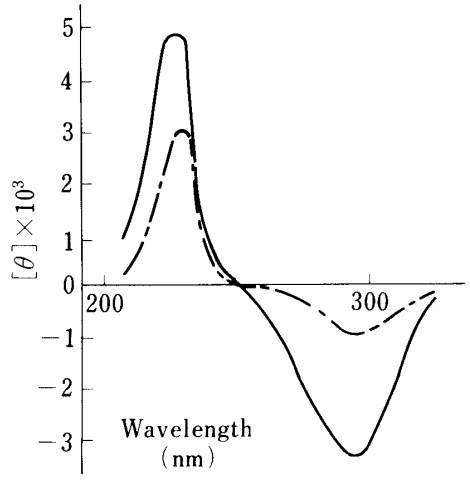


Fig. 1. CD Spectra of M-0 (Parent Acid) Methyl Ester and Authentic (2*S*, 1′*S*)-Loxoprofen Methyl Ester

——, authentic sample; — - —, isolated M-0 methyl ester.

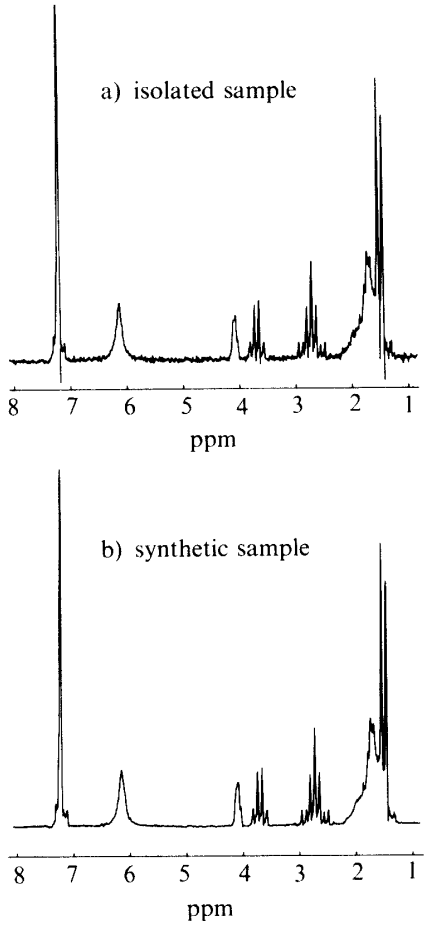


Fig. 2. ^{1}H-NMR Spectra of Isolated M-1 and Synthetic *cis*-Alcohol

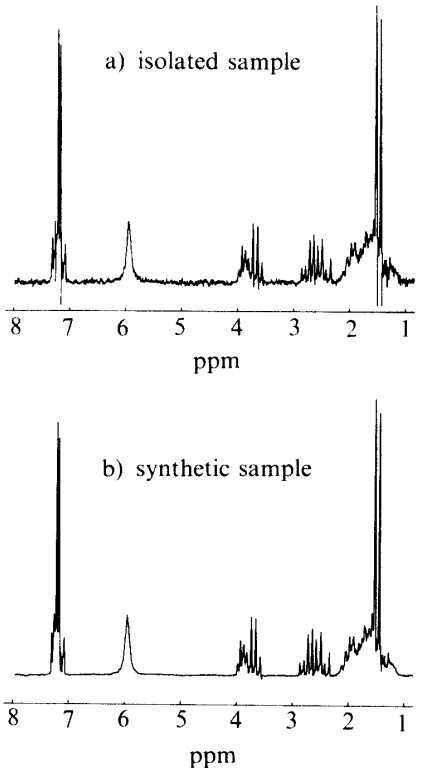


Fig. 3. ^{1}H-NMR Spectra of Isolated M-2 and Synthetic *trans*-Alcohol

ester, which was hydrolyzed and treated with (−)-α-phenylethylamine to give the corresponding diastereomeric amides. The amides mixture was separated by HPLC into the major (less polar) and minor diastereomers. Treatment of the major diastereomeric amide with (−)-MTPA Cl afforded the corresponding newly formed diastereomeric (−)-MTPA esters. HPLC analysis of the esters showed two peaks in the relative ratio of 63 : 37. The major peak was identical with the corresponding authentic sample derived from the (2*S*, 1′*S*, 2′*S*)-*cis*-alcohol by comparison of the t_R value on HPLC. Therefore, the 1′-asymmetric carbon of the *cis*-alcohol derived from M-0 methyl ester was found to have the (*S*)-configuration with 26% enantiomer excess. This value was in good agreement with that from the CD analysis mentioned above. Consequently, the isolated M-0 was established to be predominantly composed of (2*S*, 1′*S*)-isomer.

M-1

The MS of M-1 methyl ester acetate showed a molecular ion peak at *m/z* 304, suggesting that M-1 was a reduction product of the parent ketone. The NMR spectrum of M-1 was in good agreement with that of synthetic 2-[4-(*cis*-2-hydroxycyclopentylmethyl)phenyl]propionic acid (Fig. 2). Further, the mass and NMR spectra and the t_R value on HPLC of M-1 methyl ester acetate were identical with those of the authentic sample of methyl 2-[4-(*cis*-2-acetoxycyclopentylmethyl)phenyl]propionate.

The CD spectrum of the M-1 methyl ester acetate showed a positive peak (at 226 nm, due to the α-phenylpropionic acid side chain) having the same magnitude as that of the authentic (2*S*)-*cis*-alcohol, thus indicating that the asymmetric 2-carbon in the α-phenylpropionic acid

moiety of this metabolite has optically pure (*S*)-configuration. On the other hand, the high-performance liquid chromatogram of the diastereomeric amide of M-1 showed two peaks. This discrepancy can be interpreted as being a result of partial racemization at the 2-carbon atom during alkaline hydrolysis of the methyl ester acetate prior to the derivatization with (−)-α-phenylethylamine. HPLC analysis of the (−)-MTPA ester of the less polar diastereomeric amide revealed the presence of two peaks with the same intensity. Therefore, it is apparent that M-1 (*cis*-alcohol) is stereochemically a racemic mixture of the α-substituted *cis*-cyclopentanol, and its positive optical rotation (the methyl ester acetate, +50.3°) is due to the essentially pure (*S*)-enantiomer in the α-arylpropionic acid moiety.

M-2

The MS of M-2 methyl ester acetate was similar to that of M-1 methyl ester acetate mentioned above, suggesting that M-2 is also a reduction product. The NMR spectrum of M-2 coincided with that of a synthetic sample of 2-[4-(*trans*-2-hydroxycyclopentylmethyl)-phenyl]propionic acid (Fig. 3). Both the spectral data and the t_R value on HPLC of the M-2 methyl ester acetate were in good agreement with those of the authentic methyl 2-[4-(*trans*-2-acetoxycyclopentylmethyl)phenyl]propionate.

An attempt was made to estimate the enantiomer ratio of the α-substituted *trans*-cyclopentanol in M-2, but HPLC separation of a diastereomeric mixture of the (−)-MTPA ester derived from the chemically synthesized *trans*-alcohol failed. Therefore, the isolated M-2 (*trans*-alcohol) methyl ester was converted to the *cis*-alcohol methyl ester by inversion reaction with triphenylphosphine and diethyl azodicarboxylate in acetic acid. The high-performance liquid chromatogram of the derivative of the inverted product obtained by treatment with (−)-α-phenylethylamine followed by (−)-MTPA Cl showed a single peak having the same t_R value as that of the corresponding compound derived from the authentic (1′*R*,2′*R*)-*cis*-alcohol synthesized chemically. Therefore, M-2 was proved to be the *trans*-alcohol having (1′*R*,2′*S*)-configurations in the α-substituted cyclopentanol ring. The sign and magnitude of the optical rotatory dispersion (ORD) and CD spectra of the isolated metabolite were in good agreement with those of the authentic (2*S*,1′*R*,2′*S*)-*trans*-alcohol, thus indicating essentially pure (2*S*)-configuration in the α-arylpropionic acid moiety. These combined results clearly demonstrate that the isolated M-2 (*trans*-alcohol) methyl ester possesses stereochemically pure (2*S*,1′*R*,2′*S*)-configurations.

M-3

The MS of the isolated M-3 methyl ester (Fig. 4) showed the molecular ion at *m/z* 276, suggesting the introduction of one oxygen atom into the parent ketone. The newly introduced hydroxy group was assumed to be at the α-position to the carbonyl function due to the weak dehydration peak at *m/z* 258 in the MS.

M-3 methyl ester acetates were separated into two components (relative amount 2 : 1) by HPLC. The mass and NMR spectra and the t_R values of major and minor acetates were in agreement with those of the authentic methyl 2-[4-(*trans*-3-acetoxy-2-oxocyclopentyl-methyl)phenyl]propionate and methyl 2-[4-(*cis*-3-acetoxy-2-oxocyclopentylmethyl)phenyl]-propionate,[4] respectively.

M-4, M-5 and M-6

The MS of these methyl ester metabolites showed the same molecular ion peak at *m/z* 278, indicating that they are all dihydroxycyclopentane derivatives. The newly introduced hydroxy group in each metabolite was assigned to be at the same 4′-position on the cyclopentanol ring based on the following ^{13}C-NMR signals: at 75.5 and 73.4 ppm in M-4 methyl ester diacetate (Fig. 5), at 78.1 and 73.3 ppm in M-5 methyl ester diacetate (Fig. 6) and at 77.9 and 73.1 ppm in M-6 methyl ester diacetate (Fig. 7).

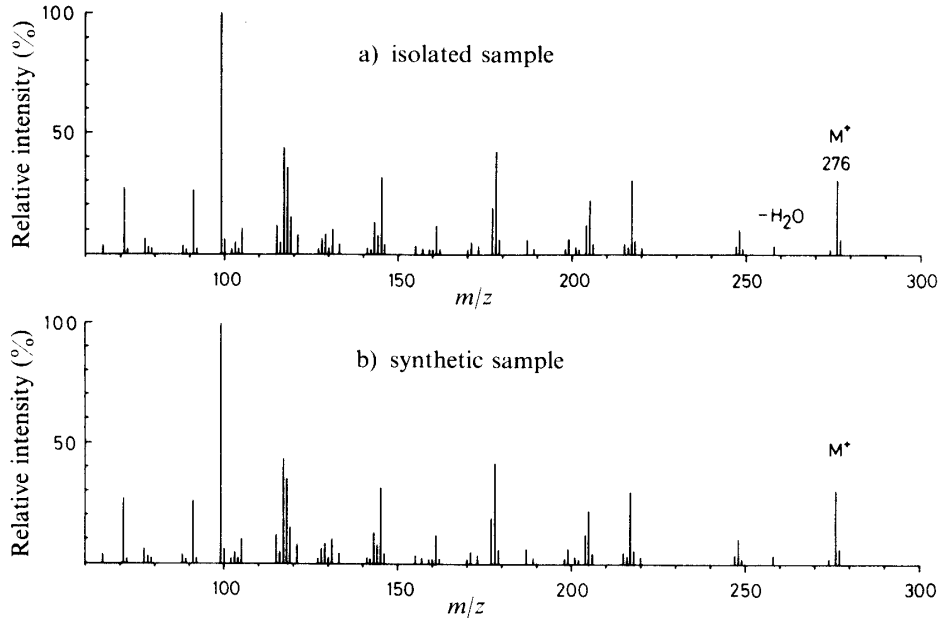


Fig. 4. MS of the Methyl Ester of Isolated M-3 and Synthetic α-Ketol

The relative configurations of the 2′-hydroxy group to the 1′-benzyl substitutent in these diol derivatives were assigned by examination of the ^{13}C-NMR signal due to the benzyl carbon in the methyl ester diacetates: *cis* in M-4 (34.9 ppm), *trans* in M-5 (38.4 ppm) and *trans* in M-6 (38.3 ppm).

These three diol methyl ester diacetates were finally identified by comparison of the ^{13}C-NMR, ^{1}H-NMR and MS, and t_R values on HPLC with those of the synthetic authentic diol derivatives as follows: M-4 methyl ester diacetate as (±)-methyl 2-[4-(1β,2β,4β)-2,4-diacetoxycyclopentylmethyl)phenyl]propionate,[5] M-5 methyl ester diacetate as (±)-methyl 2-[4-(1β,2α,4α)-2,4-diacetoxycyclopentylmethyl)phenyl]propionate[5] and M-6 methyl ester diacetate as (±)-methyl 2-[4-(1β,2α,4β)-2,4-diacetoxycyclopentylmethyl)phenyl]propionate.[5]

Discussion

Preliminary GC-MS examination revealed that the main urinary metabolites of loxoprofen sodium in rats consisted of two monohydroxy compounds, a hydroxy ketone and three major diols, all of which were formed by reduction and/or hydroxylation reactions occurring at the cyclopentanone moiety of the loxoprofen molecule. Establishment of the stereochemistry of these metabolites seemed to be of importance in view of the stereospecific enzyme reactions concerned and also in order to achieve an understanding of the pharmacological action of loxoprofen sodium.

The isolated *cis*-alcohol (M-1) and *trans*-alcohol (M-2) were found to have the optically pure (2*S*)-configuration in the α-arylpropionic acid moeity. The recovered parent acid (M-0) also consisted predominantly of the (2*S*)-isomer. These findings suggested the occurrence of optical inversion of (2*R*)- to (2*S*)-configuration in both loxoprofen and the monohydroxy metabolites, as already reported in the cases of ibuprofen,[6] 2-(2-isopropylindan-5-yl)-propionic acid[7] and other α-arylpropionic acid derivatives.[8-10] In fact, the occurrence of this stereospecific inversion reaction has been confirmed by HPLC determination of each enantiomer in rat plasma following administration of the (2*R*)-stereoisomer of loxoprofen.[11]

It has been established that loxoprofen sodium, when administered to rats, exhibits a strong inhibitory effect on PG synthetase (as strong as that of indomethacin) but it has only a

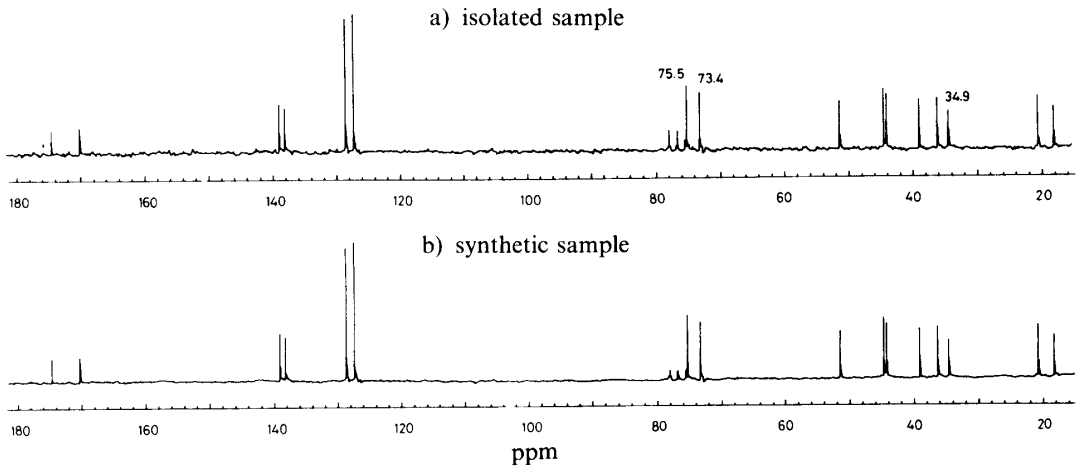


Fig. 5. ^{13}C-NMR Spectra of the Methyl Ester Diacetate of Isolated M-4 and Synthetic 2′β,4′β-Diol

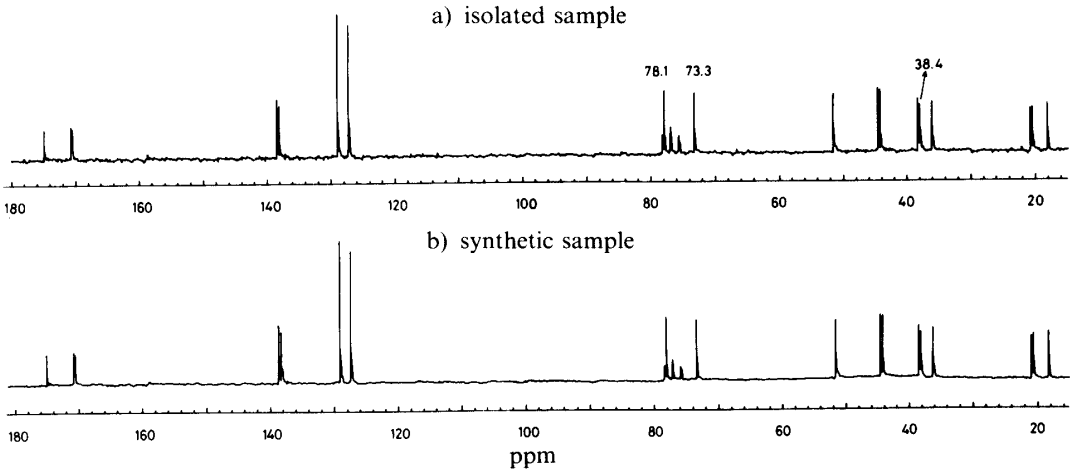


Fig. 6. ^{13}C-NMR Spectra of the Methyl Ester Diacetate of Isolated M-5 and Synthetic 2′α,4′α-Diol

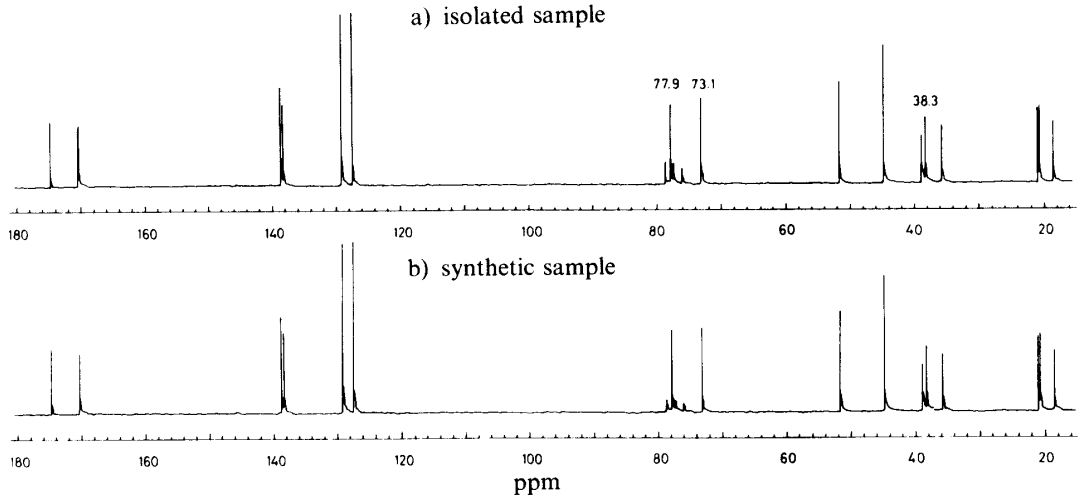


Fig. 7. ^{13}C-NMR Spectra of the Methyl Ester Diacetate of Isolated M-6 and Synthetic 2′α,4′β-Diol

weak inhibitory effect on the bovine seminal microsomal enzyme system.[12] The isolated *trans*-alcohol having the stereochemically pure (2*S*, 1′*R*, 2′*S*)-configurations was shown to be highly active for *in vitro* prostaglandin (PG) synthetase inhibition, but synthetic stereoisomers of the *trans*-alcohol other than the (2*S*, 1′*R*, 2′*S*)-compound and the other urinary metabolites all showed only weak or essentially no inhibitory activity in *in vitro* assay.[12] Therefore, the potent pharmacological activity of loxoprofen sodium due to PG synthetase inhibition was proved to be elicited by the formation of the (2*S*, 1′*R*, 2′*S*)-*trans*-alcohol metabolite.

On the basis of the established structures of the urinary metabolites together with the amounts isolated, the main metabolic pathways of loxoprofen sodium in rats may be postulated to be as follows: initial reduction of the α-substituted cyclopentanone moiety to give the major *trans*-alcohol with rapid inversion of the (2*R*)- to the (2*S*)-configuration in the α-arylpropionic acid side chain and subsequent hydroxylation of the monohydroxy metabolites to the corresponding 2′,4′-diol compounds as the major urinary metabolites in rats.

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References and Notes

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3) (2*S*, 1′*S*)-Loxoprofen denotes the optically pure (2*S*, 1′*S*)-stereoisomer of loxoprofen.
4) The terms *trans* and *cis* for the 3′-acetoxy group refer to the 1′-benzyl substituent. See Table I.
5) As the absolute configurations of the isolated diol metabolites have not been determined, α and β represent only the relative configurations.
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