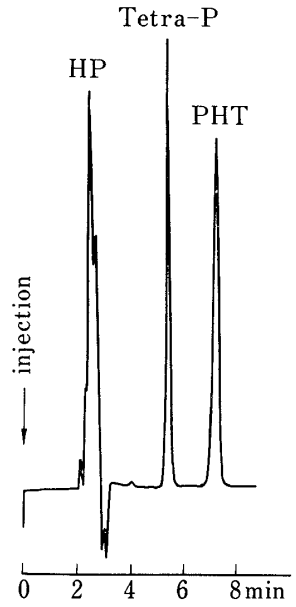

Fig. 1. High-Performance Liquid Chromatogram of the Reaction Mixture of Nitrite Ion (Nitrite Nitrogen: 0.10 ppm) and HP-HCl

conditions: apparatus, HLC-803A (Toyo Soda); detection, UV 228 nm; column, TSK-GEL LS-410 ODS SIL (P-5), 250 mm × 4.6 mm i.d.; mobile phase, 40% acetonitrile in 0.05M phosphate buffer (pH 3.0); flow rate, 1.0 ml/min at ambient temperature; 0.04 a.u.f.s.

Figure 1 shows the HPLC chromatogram of the reaction mixture of nitrite ion (corresponding to 0.10 ppm nitrite nitrogen) and fivefold equivalent of HP–HCl. Under the condition employed, intact HP was eluted together with the reaction solvents 2.5 min after the injection, and the retention times of Tetra–P and PHT were 5.5 and 7.3 min, respectively. Calibration curve was prepared by plotting the peak-area ratio of Tetra–P to PHT against the amounts of nitrite nitrogen. A linear relationship was obtained in range of 0.001 to 0.10 ppm of nitrite nitrogen (nitrite ion: 0.003 to 0.33 ppm) (regression line: $Y=10.10X+0.022$; correlation coefficient: 1.000; recovery: 98.1%).

The present HPLC method using HP is a useful improvement for nitrite determination because of its simplicity, sensitivity, specificity and good reproducibility (coefficients of variation for 0.001, 0.01, 0.02, 0.04, 0.06, 0.08 and 0.10 ppm nitrite nitrogen: 14.4, 2.1, 3.6, 1.4, 0.6, 0.3 and 0.6%; N=5). The studies on nitrite determination from foods and biological fluids are in progress and the details will be reported in the nearest future.

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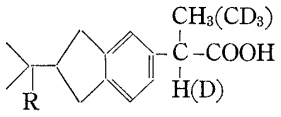
Stereospecific Inversion of Configuration of 2-(2-Isopropylindan-5-yl)-propionic Acid in Rats

The *d*-tertiary hydroxy metabolite, which was isolated from rat urine after oral administration of 3,3,3-d_3-*l*-2-(2-isopropylindan-5-yl)propionic acid (IIPA), was confirmed

to retain the intact CD_3 group on the basis of the MS and NMR spectral analyses. This finding is not compatible with the exomethylene intermediate mechanism proposed for the same epimerization reaction of 2-(4-isobutylphenyl)propionic acid (ibuprofen) in human.

Keywords——anti-inflammatory agent; α-arylpropionic acid derivative; deuterium-labeled compounds; mechanism of inversion of configuration; stereospecific biotransformation; metabolite; rats; MS; NMR

The pharmacological activity of many nonsteroidal anti-inflammatory agents having the substituted 2-arylpropionic acid structures seems to reside predominantly in the *d*-isomer.[1] But in a few examples such as 2-(4-isobutylphenyl)propionic acid (ibuprofen)[2a] and 2-(3-phenoxyphenyl)propionic acid (fenoprofen),[2b] *d*- and *l*-isomers are nearly equal in potency. *S*-(+)-Ibuprofen is reported to be highly active in *in vitro* inhibition of prostaglandin E_2 synthetase, while very little activity occurred in the *R*-(−)-isomer.[2a] Human studies of the agent have revealed that only the *R*-(−)-isomer was epimerized to the *S*-(+)-one[3] and this has been taken as a reason for the *in vivo* equipotency between two enantiomers. A reaction pathway involving the exomethylene intermediate with the side chain structure of $-C(=CH_2)-COOH$ has been proposed for this unique epimerization reaction. 2-(Fluoren-2-yl)propionic acid (cicloprofen) in animals[4] and 2-[2-(4-chlorophenyl)benzoxazol-5-yl]propionic acid (benoxaprofen) in humans[5] are known to undergo the analogous inversion of the *l*- to the *d*-isomer and the concerning mechanism in cicloprofen is supposed to be the same as that for ibuprofen. 2-(2-Isopropylindan-5-yl)propionic acid (IIPA) is also a member of the 2-arylpropionic acid derivatives showing significant anti-inflammatory activity[6] and the stereoisomers were known to be biologically equivalent.[7] The metabolic studies in rats have established that *R*-(−)-IIPA was rapidly epimerized to the *S*-(+)-isomer.[8] A tertiary hydroxy metabolite (*tert*-OH, Chart 1), which was isolated from rat urine after oral administration of IIPA, showed the positive optical rotation and the circular dichroism curve almost the same to those for *S*-(+)-IIPA.[8] These findings clearly demonstrated the occurrence of inversion of the *R*- to the *S*-configuration, similar to the biotransformation of ibuprofen, cicloprofen and benoxaprofen. The aim of the present investigations is to gain more insight into the mechanism of this stereospecific epimerization reaction.



IIPA: R=H
tert-OH: R=OH

Chart 1

IIPA trideuterated at the methyl group on the propionic acid residue (IIPA–CD_3) was resolved by the method of Naruto *et al.*,[9] which involved the formation of the diastereomeric amides derived from *d*-α-phenethylamine, followed by treatment of the respective amide with

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dinitrogentetroxide. The individual *S*-(+)- and *R*-(−)-IIPA–CD_3 obtained thus were administered orally to male Wistar–Imamichi rats at a dose of 50 mg/kg. The ether extracts from the pooled 24 hr urine samples were subjected to column chromatography on SiO_2 with benzene–AcOEt (7: 3) and subsequent thin-layer chromatographic purification using Kieselgel 60 F_{254} plate (Merck) with hexane–benzene–AcOEt–AcOH (15: 15: 5: 2).

tert-OH (mp 147—151°) isolated after administration of R-(−)-IIPA–CD_3 had $[\alpha]_D$ +45.1° (1% in EtOH) and the *S*-(+)-isomer content was determined to be as high as 94.5% by gas chromatographic analysis of the amide derivatives formed with *d*-α-phenethylamine. Thus, nearly complete inversion of the *R*-(−)-configuration was confirmed with the CD_3-compound. The NMR spectrum (Fig. 1) showed a singlet at δ 3.68 due to a methine proton of the propionic acid moiety (–$CH(CD_3)COOH$), similar to that of the parent *R*-(−)-IIPA-CD_3 employed. In a higher field was observed only a singlet signal at δ 1.23 of the two methyl protons. The MS spectrum (Fig. 1) exhibited a molecular ion at *m/e* 251 ($C_{15}H_{17}D_3O_3$) but no detectable peak at *m/e* 250 ($C_{15}H_{18}D_2O_3$) corresponding to the loss of a deuterium atom which can be expected to occur in the exomethylene intermediate mechanism as proposed for ibuprofen. *tert*-OH obtained after administration of *S*-(+)-IIPA–CD_3 possessed $[\alpha]_D$ +43.4°. Its NMR and MS spectra were essentially the same as those for the compound from the *R*-(−)-isomer. These combined data demonstrated clearly that inversion of the *R*-(−)-configuration of the propionic acid moiety did occur in rats with retaining the intact CD_3 group.

Racemic IIPA deuterated at the methine position of the propionic acid chain (IIPA–D_1) was then administered orally to rats at a dose of 100 mg/kg and the pooled 24 hr urine was processed for the isolation of *tert*-OH, as described above. The NMR spectrum (Fig. 2) of the isolated *tert*-OH (mp 151—154°, $[\alpha]_D$ +47.8°) revealed the composite signals due to the

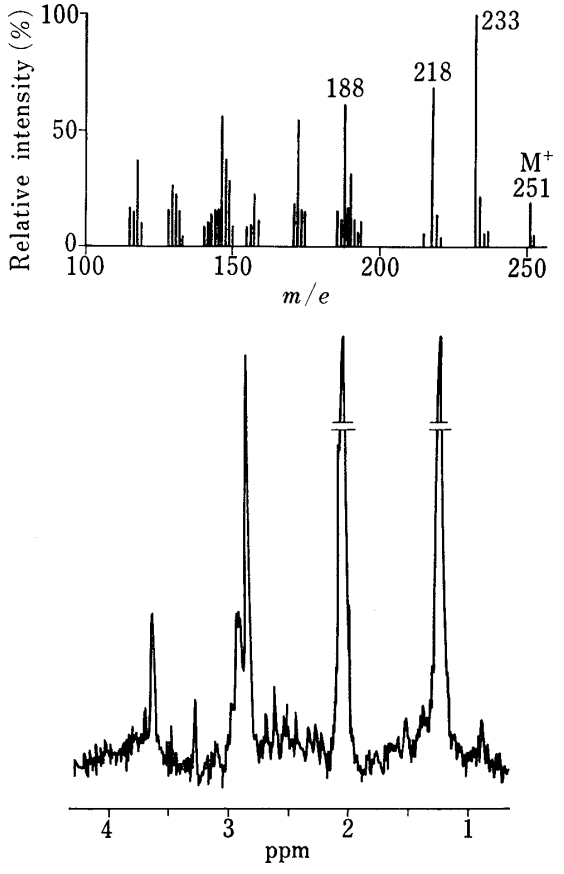

Fig. 1. MS and NMR Spectra of *tert*-OH after Oral Administration of *R*-(−)-IIPA-CD_3 to Rats

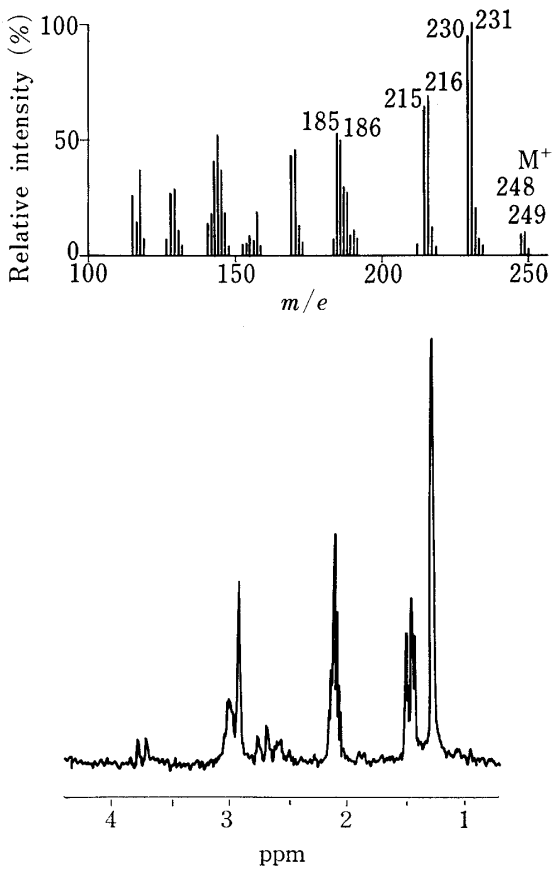

Fig. 2. MS and NMR Spectra of *tert*-OH after Oral Administration of Racemic IIPA-D_1 to Rats

methyl protons of the propionic acid moiety: a singlet at δ 1.40 as observed for the parent deuterated structure (–CD(CH_3)COOH) and a doublet at δ 1.41 (J=7 Hz) ascribable to a deuterium-lost propionic acid moiety (–CH(CH_3)COOH) with nearly the same intensity. In the MS spectrum (Fig. 2) were observed many paired ion peaks differing one mass unit with the same intensities: *m/e* 248 and 249 (M^+), 230 and 231 (M^+-H_2O), 215 and 216 ($M^+-H_2O-CH_3$), 185 and 186 (M^+-H_2O–COOH). Thus, the MS spectral data clearly indicated that *tert*-OH formed from racemic IIPA–D_1 was an isotopic mixture of an equal amount of the originally deuterated (–CD(CH_3)COOH) and deuterium-lost (–CH(CH_3)COOH) propionic acid moieties in accord with the NMR data mentioned above. This and the results from the experiments with *S*-(+)- and *R*-(−)-IIPA-CD_3 unambiguously demonstrated that only the *R*-(−)-configuration of IIPA administered to rats has been inverted almost completely to the *S*-(+)-one with simultaneous loss of the hydrogen atom on the methine group but not on the methyl group in the propionic acid moiety.

In connection with the above mentioned experiments using deuterated IIPA, an equimolar mixture of *R*-(−)-IIPA and *R*-(−)-IIPA–CD_3 was administered to rats. The MS spectrum of isolated *tert*-OH showed a pair of the molecular ion peaks at *m/e* 248 and 251 with essentially the same intensity ratio as that for the M^+ ions of the original isotopic mixture of *R*-(−)-IIPA. Therefore, it is evident that there is no observable isotope effect in this epimerization reaction between deuterated and nondeuterated *R*-(−)-IIPA.

Oral administration of the *R*-(−)-isomer of tetradeuterated ibuprofen with the side chain of –CD(CD_3)COOH to human subjects has been reported to afford the *S*-(+)-D_2-parent drug and the *S*-(+)-D_2-tertiary hydroxy metabolite with the side chain of –CH(CD_2H)COOH on a basis of the mass spectral analysis. This was taken as an evidence for the proposed reaction pathway involving the exomethylene intermediate. On the contrary, *tert*-OH isolated from rat urine after oral administration of *R*-(−)-IIPA–CD_3 did retain the intact CD_3 group. This is in sharp contrast to the results from human studies of ibuprofen and accordingly not compatible with the exomethylene-intermediate mechanism. It seems to be reasonable that this stereospecific epimerization of IIPA might proceed through the enolic form of the carboxylic acid or more likely of the activated thioacyl compound.

There remains a problem to be solved whether the disagreement in the supposed inversion mechanisms between IIPA and ibuprofen is due to the species difference or to the difference in their chemical structures. The characteristics of the enzyme system(s) concerned and the substrate specificity are interesting subjects which are now under investigations.

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