[Chem. Pharm. Bull. 33(2) 760—768 (1985)]

# Stereoselectivity in the Metabolism of the β-Adrenergic Blocking Agent, (±)-1-*tert*-Butylamino-3-(2,3-dimethylphenoxy)-2-propanol Hydrochloride (Xibenolol Hydrochloride, D-32), in Man

SEIJIRO HONMA,*[,a] TOMIHARU ITO,[b] and AKIRA KAMBEGAWA[a]

*Pharmacological Research Department, Teikoku Hormone Mfg Co.,[a] 1604, Shimosakunobe Takatsu-ku, Kawasaki 213, Japan and Hatano Research Institute Food and Drug Safety Center,[b] Ochiai, Hadano, Kanagawa 257, Japan*

(Received May 10, 1984)

After oral administration of deuterium-labeled pseudoracemic D-32 to human subjects, the enantiomeric metabolites in plasma and urine were analyzed by gas chromatography-mass spectrometry. D-32 was biotransformed to 3 major metabolites, 4-hydroxy D-32, 3-hydroxymethyl D-32 and 3-carboxy D-32. Twenty-five percent of the racemic dose was excreted into the urine as 4-hydroxy D-32, and 80% of 4-hydroxy D-32 in the urine was derived from (−)-D-32. Sixty percent of 3-carboxy D-32 in the urine was derived from (+)-D-32. About 1% of the racemic dose was excreted into the urine as unchanged material, but of this, in which (+)-D-32 amounted to 3—5 times more than (−)-D-32. The area under the plasma concentration-time curve of (−)-4-hydroxy D-32 was 2.6 times larger than that of (+)-4-hydroxy D-32. The half-lives of (−)-4-hydroxy D-32, (+)-4-hydroxy D-32 and both enantiomers of D-32 were 3.8, 2.4 and 3 h, respectively. Thus, a marked difference in the metabolism between (−)-D-32 and (+)-D-32 was found. As 4-hydroxy D-32 and 3-hydroxymethyl D-32 are the active metabolites, the pharmacological effectiveness of D-32 after oral administration is represented by the total amount of (−)-4-hydroxy D-32 and (−)-3-hydroxymethyl D-32.

**Keywords**——xibenolol HCl(D-32); β-blocker; enantiomer; stereoselective metabolism; *AUC*; mass chromatography; mass fragmentography

A new β-adrenergic blocking agent, 1-*tert*-butylamino-3-(2,3-dimethylphenoxy)-2-propanol hydrochloride (xibenolol hydrochloride, D-32) has been clinically tested in racemic form. It is generally accepted that most of the therapeutic actions of β-blocking agents, including anti-hypertensive and cardiovascular actions, are mediated mainly by the (−)-isomer.[1] It was reported from our laboratories that the pharmacological half-life of racemic D-32 was longer than the serum half life.[2] The same result was obtained for propranolol.[3,4] Moreover, the serum half-life of the (−)-isomer of propanolol is longer than that of the (+)-isomer.[4-7] These results indicate that the metabolic rate of the (−)-isomer of a β-blocking agent is different from that of the other isomer. In fact, a difference in the rate of metabolic oxidation between the optical isomers of propranolol has been described by Walle *et al.* quite recently.[8]

The objective of the present study was to investigate in detail whether there is also stereoselectivity among the three main oxidative metabolites of D-32 in man (Fig. 1). The stereochemical compositions of unchanged material and the main metabolites in urine and plasma were determined by GC-MS following oral administration of the deuterium labeled pseudoracemate of D-32. The results are described in this paper.

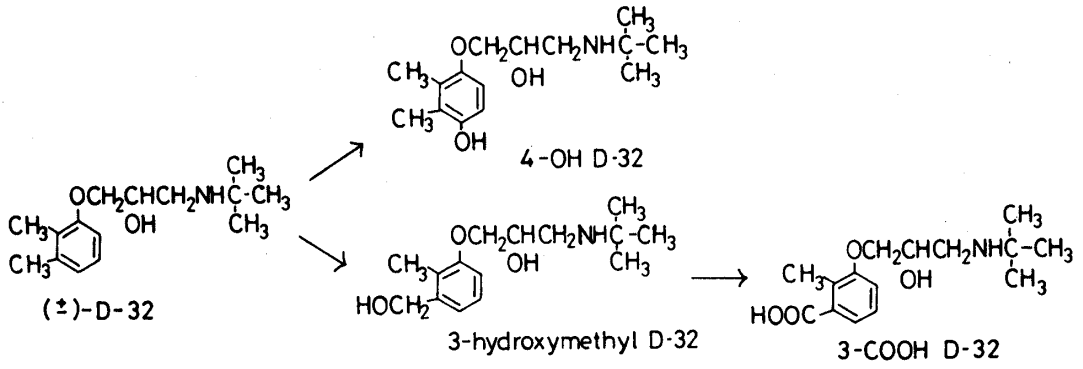


Fig. 1. The Main Metabolic Pathways of D-32 in Man

## Materials and Methods

Melting points are uncorrected. Trifluoroacetic anhydride and *N*, *O*-bis(trimethylsilyl)acetamide were purchased from Tokyo Kasei Chemical Co. β-Glucuronidase from beef liver was from Tokyo Zoki Co. Epichlorohydrin-$d_5$ (98 atom% D) and *tert*-butylamine-$d_9$ (98 atom% D) were purchased from Merck Sharp & Dohme Canada Limited.

**Syntheses of Deuterium Labeled Compounds**

**1) D-32-$d_5$ and Its Metabolites**——Over a period of 30 min, 40% NaOH in $D_2O$ (10 ml) was added to a solution of xylenol (5 g) and epichlorohydrin-$d_5$ (4 g) in MeOH (10 ml). The mixture was stirred for 18 h at room temperature. The reaction product was extracted with ether and the organic layer was washed with 4% NaOH, then dried over $Na_2SO_4$. After evaporation, the oily substance thus obtained was dissolved in MeOH (40 ml) and *tert*-butylamine (20 ml) and the mixture was refluxed for 2 h. The reaction mixture was diluted with ether and washed with $H_2O$, and the reaction product was extracted with 1 N HCl. The acidic water phase was made alkaline (pH 12) with 40% NaOH, and the free base was extracted with ether. The organic layer was dried over $Na_2SO_4$. After evaporation of the solvent, the product was recrystallized from MeOH–ether saturated with HCl gas to give D-32-$d_5$ (5.3 g), mp 142—143 °C. 3-Hydroxymethyl D-32-$d_5$ and 3-carboxy-D-32-$d_5$ were prepared using epichlorohydrin-$d_5$ and *tert*-butylamine in a similar manner.

**2) D-32-$d_9$ and Its Metabolites**——*tert*-Butylamine-$d_9$ (1.4 g) was added to a solution of 1-xylenoxypropane-2,3-oxide (2.5 g) in MeOH (1 ml), and the mixture was refluxed for 3 h. After evaporation of the solvent, the residue was recrystallized from MeOH–ether saturated with HCl gas to give D-32-$d_9$ (3.5 g) as colorless needles, mp 142—143 °C. The deuterium content measured by MS were 92 and 8% for $d_9$ and $d_8$, respectively. 3-Hydroxymethyl D-32-$d_9$ and 3-carboxy D-32-$d_9$ were prepared in a similar manner.

**3) Resolution of D-32-$d_5$ and D-32-$d_9$**——Resolution was carried out according to the method of Howe and Yost.[9,10] A solution of (±)-D-32-$d_5$ (2.5 g) and (−)-*O*, *O*′-ditoluyl-L-tartaric acid (0.772 g) in MeOH (7 ml) was allowed to stand overnight at room temperature. The resulting solid was collected by filtration and recrystallized twice from MeOH to give the desired salt, $[\alpha]_D$ −69.3°, mp 152—156 °C. The salt (2.57 g) was dissolved in $H_2O$ and the solution was adjusted to pH 13 with 40% NaOH. The free base was extracted with ether and the organic layer was washed with $H_2O$ and dried over $Na_2SO_4$. After evaporation of the solvent, the residue was recrystallized from MeOH–ether saturated with HCl gas to obtain (+)-D-32-$d_5$ (1.0 g), $[\alpha]_D$ +25.7°, mp 147—148 °C. The mother liquor of the preparation of the (+)-D-32-$d_5$ tartarate was concentrated to dryness and the residue was dissolved in $H_2O$. The solution was adjusted to pH 13 with 40% NaOH, and the free base was extracted with ether. The organic layer was dried over $Na_2SO_4$ and concentrated to dryness. The residue (0.90 g) was dissolved in MeOH (50 ml) and an equivalent quantity of (+)-*O*, *O*′-ditoluyl-D-tartaric acid was added. The tartarate of the (−)-isomer (2.0 g), $[\alpha]_D$ +66.9°, mp 152—154 °C. (−)-D-32-$d_5$, $[\alpha]_D$ −25.5°, mp 147—148 °C was obtained by the same procedure as described above.

The racemic D-32-$d_9$ was resolved by the same method as used for D-32-$d_5$ to give optically pure (−)-D-32-$d_9$, $[\alpha]_D$ −25.3° and (+)-D-32-$d_9$, $[\alpha]_D$ +25.8°.

**Metabolism**

**1) Subjects**——Six male volunteers, 36—50 years of age, were used for the experiment. All subjects were in excellent health and had taken no drugs during the preceding two weeks. Four normal men received a single 40 mg dose of pseudoracemate, (−)-D-32 and (+)-D-32-$d_5$ or (+)-D-32 and (−)-D-32-$d_5$, in gelatin capsules. Blood specimens (10 ml) were obtained with a syringe, from 0 to 8 h after drug administration. To examine the excretion of individual metabolite enantiomers, a capsule of 20, 30 or 40 mg of pseudoracemate, (−)-D-32 and (+)-D-32-$d_9$ (or $d_5$) or (+)-D-32 and (−)-D-32-$d_9$ ($d_5$), was administered to each subject. Urine was collected 2, 4, 6, 12 and 24 h after administration. To elucidate the isotopic effects, two subjects received a single dose of 40 mg of (±)-D-32 and (±)-D-

32-$d_5$ or (±)-D-32 and (±)-D-32-$d_9$ in a gelatin capsule. The urine was collected during a period of 48 h. All specimens were stored at −20 °C until analysis.

**2) Extraction from Plasma**——The following procedures were carried out on the basis of the experimental finding that the separation of D-32 and its metabolites could be satisfactorily achieved by these methods.

a) Extraction of Unchanged D-32: 1-Isopropylamino-4-(2,3-dimethylphenoxy)-3-butanol (IB, 10 ng) and 3-hydroxymethyl D-32-$d_9$ (50 ng) were added to plasma (3 ml) as internal standards. The whole was made alkaline with 0.2 M $Na_2CO_3$ (1 ml), and the mixture was shaken with *n*-heptane (20 ml). After centrifugation, the alkaline aqueous layer containing the metabolites and 3-hydroxymethyl D-32-$d_9$ was separated and kept for extraction as described in the next paragraph. From the organic layer the unchanged material and IB were extracted twice with 0.1 N HCl (2 ml), and then re-extracted with benzene (15 ml) from the acidic aqueous layer, after addition of 1 N NaOH (1 ml). The organic layer, dried over $Na_2SO_4$, was concentrated to dryness *in vacuo* at 40 °C to afford the unchanged D-32 containing IB (internal standard), which was subjected to GC-MS as described later.

b) Extraction of Metabolites: From the alkaline aqueous layer mentioned above, the metabolites and 3-hydroxymethyl D-32-$d_9$ were extracted with 55% *n*-BuOH–cyclohexane (20 ml). After centrifugation, the organic layer was diluted with *n*-heptane and the metabolites were extracted twice with 0.1 N HCl (2 ml). The aqueous layer was again made alkaline with 1 N NaOH (0.4 ml) and 0.2 M $Na_2CO_3$ (1 ml). Extraction with 55% *n*-BuOH–cyclohexane, washing with $H_2O$, drying over $Na_2SO_4$ and evaporation *in vacuo* at 50 °C gave the metabolites containing 3-hydroxymethyl D-32-$d_9$ (internal standard).

**3) Extraction from Urine**——The pH of a urine sample (30 ml) was adjusted to 4.7 with 0.1 M acetate buffer. Enzymatic hydrolysis of the sample was carried out with β-glucuronidase (50000 units) for a period of 24 h at 37 °C. Then D-32-$d_5$ (100 μg), 3-hydroxymethyl D-32-$d_5$ (1 mg) and 3-carboxy D-32-$d_5$ (1 mg) as internal standards were

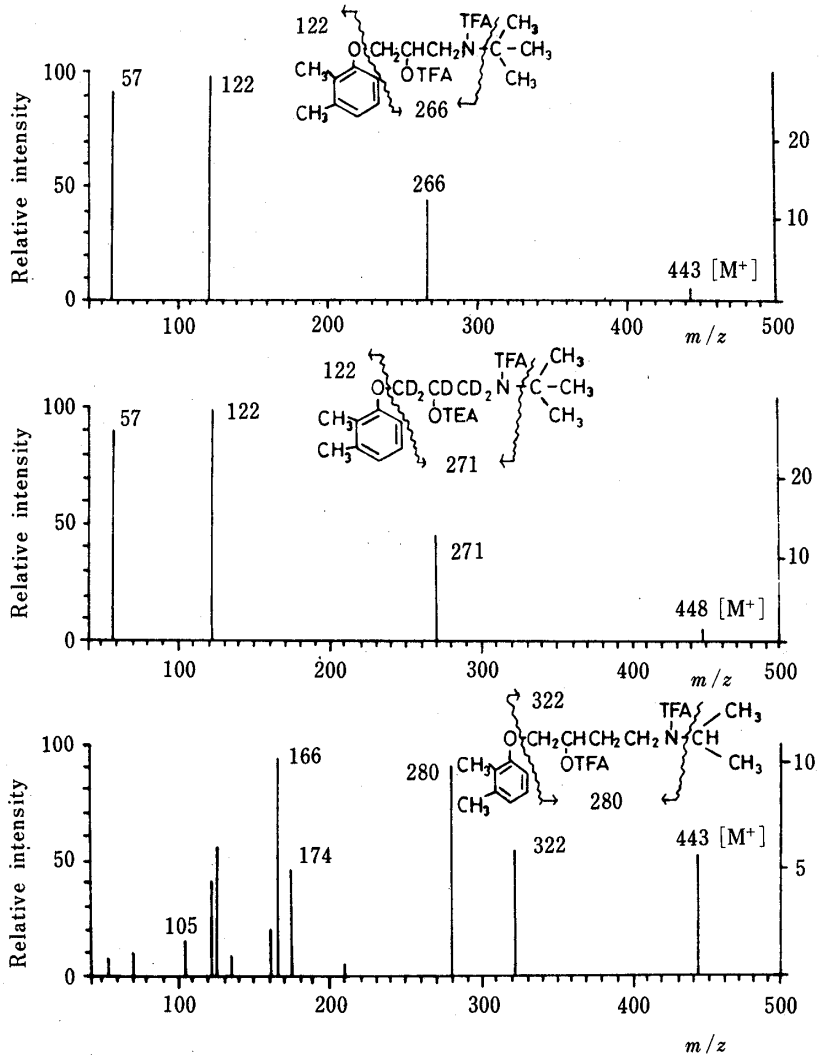


Fig. 2. MS of the Trifluoroacetic Acid Derivatives of D-32, D-32-$d_5$ and 1-Isopropylamino-4-(2,3-dimethylphenoxy)-3-butanol (IB)

added to the hydrolyzed urine. The pH was adjusted to 12 with 1 N NaOH, then extraction of D-32 and hydroxylated D-32 was performed as described in the case of plasma. A solution of the residue was adjusted to pH 4.0 with 2 N $H_2SO_4$ and percolated through a column (1 × 25 cm) packed with Amberlite XAD-2. The column was washed with $H_2O$ (30 ml), then elution with MeOH (150 ml) gave the metabolites containing internal standards. After evaporation of the solvent, the resulting gummy substance was dissolved in MeOH and treated with $CH_2N_2$–ether for 3 h. Thin layer chromatography using EtOAc–MeOH–$NH_4OH$ (12 : 3 : 0.5) as the developing solvent was carried out on the methylated material. The strip corresponding to 3-carboxy D-32 methylester was scraped off and elution with acetone and evaporation of the solvent *in vacuo* afforded a sample for analysis.

**4) Derivatization**——a) Trifluoroacetylation of D-32: A sample in a centrifuge tube was treated with 50% trifluoroacetic anhydride–EtOAc (0.5 ml) at room temperature for 1 h. After evaporation of the solvent in an $N_2$ stream, the residue was dissolved in EtOAc (30 μl) for analysis of the unchanged D-32.

b) Trifluoroacetylation of 3-Hydroxymethyl D-32 and 4-Hydroxy D-32: A sample was trifluoroacetylated as described above. The residue obtained was dissolved in 10% trifluoroacetic anhydride in EtOAc (30 μl) and the solution was directly used for GC-MS.

c) Trimethylsilylation of 3-Carboxy D-32 Methyl Ester: A methylated sample was converted to the trimethylsilyl ether (TMS) by addition of 25% *N*, *O*-bis(trimethylsilyl)acetamide in acetonitrile (0.1 ml).

**5) MS Assay**——a) Standard Curve of D-32: A mixture of D-32 (0.5—100 μg), D-32-$d_5$ (0.5—100 μg) and IB (10 μg) was trifluoroacetylated as described above and then diluted with EtOAc (1 ml). The solution was subjected to GC-MS analysis by selective ion monitoring at *m/z* 266, 271 and 280, corresponding to the side chain fragments for D-32, D-32-$d_5$ and IB (Fig. 2). The peak area ratios of D-32 and D-32-$d_5$ to IB were calculated.

b) Standard Curve of 4-Hydroxy D-32 and 3-Hydroxymethyl D-32: A mixture of 4-hydroxy D-32 (1—50 μg), 3-hydroxymethyl D-32 (1—50 μg), 3-hydroxymethyl D-32-$d_5$ (1—50 μg) and 3-hydroxymethyl D-32-$d_9$ (50 μg) was trifluoroacetylated as described above (reaction time, 30 min). MS analysis was carried out by selective ion monitoring at *m/z* 459 ($M^+$ ion of nonlabeled compound), *m/z* 464 ($M^+$ ion of 3-hydroxymethyl D-32-$d_5$) and *m/z* 468 ($M^+$ ion of 3-hydroxymethyl D-32-$d_9$).

c) Standard Curve of 3-Carboxy D-32: A mixture of 3-carboxy D-32 (1—50 μg), 3-carboxy D-32-$d_9$ (1—50 μg) and 3-carboxy D-32-$d_5$ (10 μg) was methylated and trimethylsilylated as described above. MS analysis was carried out by selective ion monitoring at *m/z* 352, *m/z* 357 and *m/z* 361 ($M^+$ ions of $d_0$, $d_5$ and $d_9$ compounds, respectively).

**6) GC-MS**——GC-MS analysis was carried out on a Shimadzu LKB-9000B GC-MS equipped with an Okitac 4300 computer. Conditions of analysis were as follows: glass column (1 m) packed with 1.5% OV-17 on Chromosorb W AW-DMCS 80/100; flash heater temperature, 200 °C; molecular separator temperature, 270 °C; ionization source temperature, 290 °C; flow rate of carrier gas (He), 30 ml/min. Fragment ions having an intensity of less than 5% of the base peak and isotope peaks were not taken into account.

**7) Calculations**——Half-lives ($T_{1/2}$) of plasma D-32 and its metabolites were calculated by least-squares linear regression analysis of the terminal exponential of the log plasma concentration–time profile. The *AUC* was calculated by means of the trapezoidal rule from the curve up to 7 h. Differences in parameters between (−)- and (+)-isomer were assessed by using the *t*-test.

## Results

To determine whether D-32-$d_5$ and D-32-$d_9$ were susceptible to isotope effects in the metabolic process, a mixture of nonlabeled and labeled D-32 was orally administered to a group of men. After hydrolysis with β-glucuronidase, the main urinary metabolites were determined by mass chromatography (Fig. 3), in which the base peak at *m/z* 266, characteristic of a compound having an alkylamino-2-propanol group, and the molecular ions were examined. Peak A corresponded to the unchanged material, while peaks B and C were identified as the trifluoroacetylated derivatives of 4-hydroxy D-32 and 3-hydroxymethyl D-32, respectively. These results are very similar to those on the urinary metabolites in rats.[11] The results indicated that D-32 substituted with five deuterium atoms in the propyl group was converted to 4-hydroxy D-32-$d_5$ and 3-hydroxymethyl D-32-$d_5$ at the same rate as nonlabeled D-32.

The mass chromatogram of 3-carboxy D-32 fraction is shown in Fig. 4. By comparison with the spectrum of authentic 3-carboxy D-32, peak D was assigned to 3-carboxy D-32 methyl ester on the basis of the base peak at *m/z* 86/88 and molecular-$CH_3$ ions. From these results, it is apparent that there were no isotope effects in the biotransformation of D-32-$d_5$ to 4-hydroxy D-32, 3-hydroxymethyl D-32 and 3-carboxy D-32.

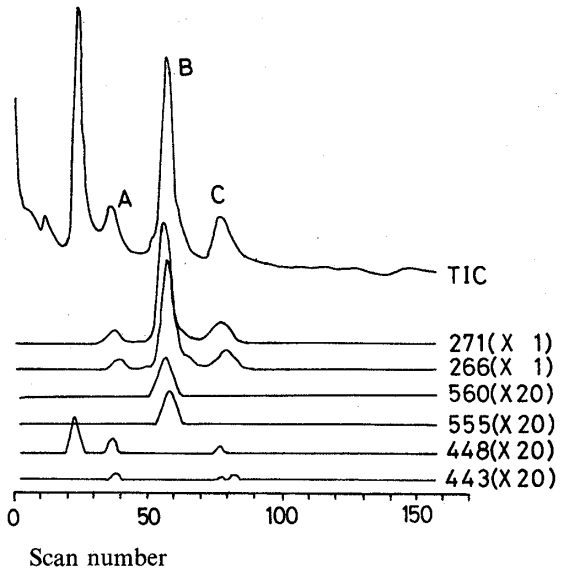


Fig. 3. Mass Chromatogram of the Basic Fraction of Urine Extract after Oral Administration of (±)-D-32 and (±)-D-32-$d_5$ ($d_0/d_5=1$) to a Human Subject

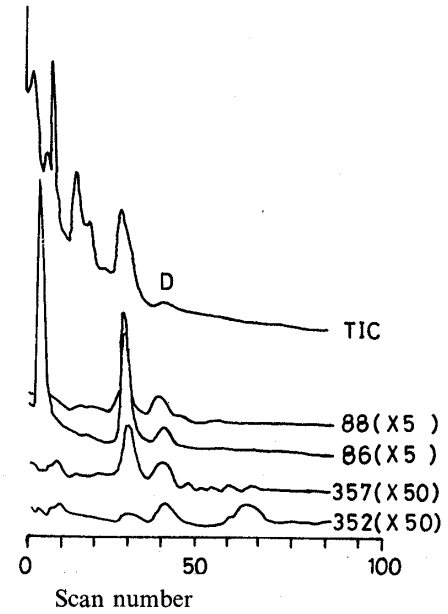


Fig. 4. Mass Chromatogram of the 3-Carboxy D-32 Fraction of Urine Extract after Oral Administration of (±)-D-32 and (±)-D-32-$d_5$ ($d_0/d_5=1$) to a Human Subject

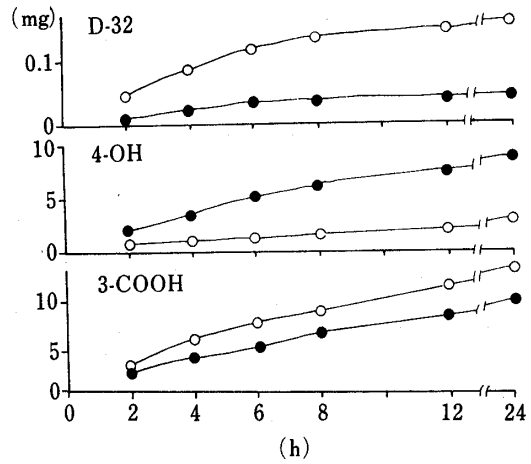


Fig. 5. Urinary Extraction of Metabolites after Oral Administration of 40 mg of Pseudoracemic D-32 to a Human Subject

●, (−) enantiomer; ○, (+) enantiomer.
Dose: (+)-D-32-$d_9$ (20 mg) and (−)-D-32 (20 mg).

After the oral administration of pseudoracemic D-32 to six subjects, the urinary metabolites were determined every 2 h during the first 12 h of a 24 h period and then at the end of the period. The ratio of the labeled metabolites and nonlabeled ones reflects the ratio of the (−) to (+) enantiomers. Eighty per cent of the total 4-hydroxy D-32 detected during the first 24 h after administration was derived from (−)-D-32. On the other hand, in the case of 3-hydroxymethyl D-32, another pharmacologically active metabolite, no difference in excretion rate was found, while 60% of 3-carboxy D-32, the main metabolite in man, was derived from (+)-D-32. The (+)-isomer of the unchanged material amounted to about 5 times more than that of the (−)-isomer. After the oral administration of another pseudoracemate, (+)-D-32-$d_9$ and (−)-D-32, to three men, the results on the urinary metabolites were similar to those given above (Table I). The time course of the urinary excretion of D-32 and its main metabolite enentiomers is shown in Fig. 5.

Figure 6 shows the serum concentration time course of the main metabolites after the oral administration of pseudoracemic D-32 containing (+)-D-32-$d_5$ and (−)-D-32. The

TABLE I. Urinary Levels of (−)- and (+)-D-32 and Metabolites after Oral Administration of Deuterium-labeled Pseudoracemic D-32 to Human (mg/24 h)

| Subject | Dose (mg) | D-32 | | 4-OH D-32 | | 3-OH D-32 | | 3-COOH D-32 | |
|---|---|---|---|---|---|---|---|---|---|
| | | (+) | (−) | (+) | (−) | (+) | (−) | (+) | (−) |
| A.K. | 30[a] | 0.062 | 0.013 | 0.394 | 1.804 | 0.258 | 0.716 | | |
| J.M. | 20[a] | 0.108 | 0.021 | 1.106 | 8.628 | 0.404 | 0.423 | | |
| M.M. | 20[a] | 0.181 | 0.030 | 0.400 | 2.030 | 0.311 | 0.291 | | |
| J.M. | 40[b] | 0.143 | 0.031 | 2.639 | 8.980 | 0.629 | 1.502 | 12.720 | 8.874 |
| M.M. | 40[b] | 0.377 | 0.072 | 2.150 | 7.525 | 0.554 | 1.104 | 15.420 | 10.795 |
| S.H. | 40[b] | 0.671 | 0.200 | 2.400 | 8.171 | 0.774 | 0.319 | 10.945 | 7.588 |

Dose: *a*) (+)-$d_0$/(−)-$d_9$ (1 : 1), *b*) (+)-$d_9$(−)-$d_0$ (1 : 1).

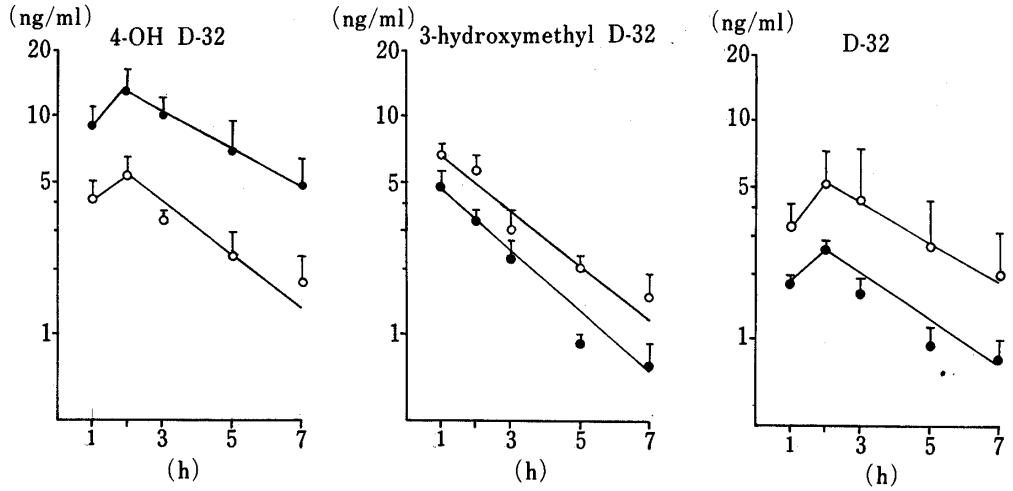


Fig. 6. Mean Plasma Concentration–Time Curves for D-32, 4-Hydroxy-D-32 and 3-Hydroxymethyl D-32 Following Single Oral Administration of (+)-D-32-$d_5$ and (−)-D-32 or (−)-D-32-$d_5$ and (+)-D-32 to Four Human Subjects

●, (−) enantiomer; ○, (+) enantiomer.

TABLE II. Plasma Pharmacokinetics of (−)- and (+)-D-32, and Metabolite Enantiomers after Oral Administration of Deuterium-labeled Pseudoracemic D-32[a] to Human

| Subject | D-32 | | | | 4-Hydroxy D-32 | | | | 3-Hydroxymethyl D-32 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *AUC* (ng·h/ml)[b] | | $T_{1/2}$ (h)[c] | | *AUC* (ng·h/ml)[b] | | $T_{1/2}$ (h)[c] | | *AUC* (ng·h/ml)[b] | | $T_{1/2}$ (h)[c] | |
| | (−) | (+) | (−) | (+) | (−) | (+) | (−) | (+) | (−) | (+) | (−) | (+) |
| S.H. | 13.41 | 49.42 | 3.8 | 3.5 | 73.56 | 25.22 | 5.8 | 3.1 | 11.40 | 20.28 | 1.9 | 2.1 |
| S.I. | 7.30 | 9.47 | 2.4 | 3.9 | 36.53 | 17.12 | 2.5 | 1.8 | 14.45 | 27.50 | 3.4 | 3.2 |
| K.K. | 8.14 | 8.54 | 2.6 | 2.6 | 36.68 | 14.87 | 3.2 | 2.9 | 18.55 | 33.12 | 1.5 | 2.1 |
| J.M. | | | | | 57.98 | 16.90 | 3.8 | 2.0 | 9.76 | 13.37 | 1.7 | 2.5 |
| X±S.E. | 9.62 ±1.91 | 22.48 ±13.47 | 3.0 ±0.4 | 3.4 ±0.4 | 51.19[d] ±9.00 | 18.53 ±2.29 | 3.8[e] ±0.7 | 2.4 ±0.3 | 13.54 ±1.93 | 23.57 ±4.30 | 2.1 ±0.4 | 2.5 ±0.3 |

*a*) D-32 pseudoracemic compound consisting of 20 mg each of (−)-D-32-$d_5$ and (+)-D-32-$d_0$.
*b*) *AUC* calculated from 0 to 7 h.
*c*) Half-life calculated by the least-squares method.
*d*) $p<0.05$. *e*) $p<0.10$.

pharmacokinetic data are shown in detail in Table II. The half lives of (−)-D-32 and (+)-D-32 did not differ significantly in the four subjects taking 40 mg of D-32. The ratio of *AUC* of (−)-4-hydroxy D-32 to that of (+)-4-hydroxy D-32 was 2.6. In the case of (−)-4-hydroxy D-32 and its isomer, the average half-lives were, however, $3.8 \pm 0.7$ and $2.4 \pm 0.3$ h, respectively ($p < 0.01$).

## Discussion

The main metabolic pathways of (±)-D-32 are shown in Fig. 1. D-32 can undergo aromatic or aliphatic hydroxylation to 4-hydroxy D-32, 3-hydroxymethyl D-32 or 3-carboxy D-32 followed by conjugation with glucuronic acid, leading to rapid excretion into the urine.[11] It was reported that the β-blocking activities of these metabolites, 4-hydroxy D-32 and 3-hydroxymethyl D-32, are from 2 to 3 times more potent than that of D-32, while the activity of 3-carboxy D-32 is much less potent than that of D-32.[12] The remarkable differences in β-blocking activity between enantiomers prompted us to clarify the metabolic fate and bioavailability of each enantiomer in man. To investigate stereoselectivity in the hydroxylation of (−)-D-32 and (+)-D-32, an equimolar mixture of (−)-D-32 and (+)-D-32, a socalled pseudoracemate, was administerd orally to men. Most of the D-32 was excreted into the urine as metabolites, 4-hydroxy D-32, 3-hydroxymethyl D-32 and 3-carboxy D-32. Both enantiomers gave the same three metabolites, but in quite different amounts, which could account for the difference in metabolic fate between the enantiomers. Eighty per cent of the total amount of 4-hydroxy D-32 found in urine was derived from (−)-D-32. In contrast, (+)-D-32 underwent oxidation of the 3-methyl group of the xylenol ring to 3-carboxyl D-32 which was excreted mainly into the urine. A small amount of 3-hydroxymethyl D-32 was also detected. This metabolite was derived in equal amounts from both enantiomers. However, the ratio of (+)-D-32 to (−)-D-32 excreted in the urine as glucuronides was four to one, which was different in the case of the dog.[13] Similar results were reported on propranolol; the amount of glucuronide of the (−)-enantiomer was 3—5 times more than that of the (+)-enantiomer in dog.[6,7]

In the case of plasma, the difference in the metabolism of the enantiomers of D-32 was also reflected in the half-lives and the bioavailabilities of pharmacologically active metabolites, 4-hydroxy D-32 and 3-hydroxymethyl D-32. Examination of the *AUC* revealed that the amount of (+)-3-hydroxymethyl D-32 was the same as that of the (−)-enantiomer, as in the case of urine. The plasma half-lives of (−)-D-32 and (−)-3-hydroxymethyl D-32 were the same as those of the (+)-enantiomers. On the other hand, the half life of (−)-4-hydroxy D-32 was obviously longer than that of the (+)-enantiomer. In addition, the plasma level of (−)-4-hydroxy D-32 was significantly higher than that of the (+) enantiomer (51 ng·h/ml and 19 ng·h/ml). The plasma concentration of D-32 was lower than that of propranolol. Nevertheless, the β-blocking activity of D-32 was more potent and long-lasting than that of propranolol, presumably would be due to the fact that the half-life of 4-hydroxy propranolol, the active metabolite, is shorter than that of 4-hydroxy D-32.

Several reports have been presented on the stereoselective kinetics and the metabolism of propranolol and oxprenolol.[4−7,13−15] The bioavailability of (+)-propranolol was 1.4 times greater than that of the (−)-enantiomer, and the amount of glucuronide of the (−)-enantiomer was 3.4 times more than that of the (+)-enantiomer in an anginal patient;[7] after intravenous administration to rats, the half life of (−)-propranolol was longer than that of the (+)-enantiomer, and (−)-propranolol was taken up into cardiac tissue; in the dog and rat, the amount of (−)-4-hydroxypropranolol in urine was 1.5—1.8 times more than that of (+)-propranolol,[13,16] as in the case of 4-hydroxylation of D-32. However in the case of man, (+)-4-hydroxypropranolol was dominant.[17] In dog, the metabolites originating from side chain

oxidation were mainly derived from (+)-propranolol.[8] All these observations support the view that the oxidation process in the metabolism of propranolol is stereoselectively controlled, as has been reported for many drugs such as amphetamine, hexobarbital, warfarin, mephenytoin and so on.[18-24] Furthermore, the regioselective and stereoselective metabolism of D-32 and propranolol might be ascribed to multiplicity of cytochrome P-450. Kaminsky *et al.*[25,26] recently reported that the different forms of cytochrome P-450 exhibit different regio- and stereoselectivities in the metabolism of warfarin. In addition, it was reported from our laboratories that (−)-D-32 was conjugated to glucuronic acid to a greater extent than (+)-D-32 in dog, both *in vivo* and *in vitro*, as in the case of propranolol.[13] The stereoselectivity in glucuronidation of the enantiomers may be explained in terms of multiplicity of glucuronyl-transferase.

In order to determine whether or not differences in elimination rates and biotransformation of deuterium-labeled enantiomers were due to isotope effects, the metabolism of two pseudoracemates, one containing (−)-D-32 and (+)-D-32-$d_9$, and the other containing (+)-D-32 and (−)-D-32-$d_9$, were examined: there was no difference in amounts of metabolites between (−)-D-32 and (−)-D-32-$d_9$, or between (+)-D-32 and (+)-D-32-$d_9$, supporting the view that metabolism of D-32-$d_9$ was not affected by isotope effects. An accurate and rapid measurement of the metabolism of both D-32 enantiomers after simultaneous administration was achieved by GC-MS analysis using deuterium labeled compounds. The sensitivities for D-32, 4-hydroxymethyl D-32 and 3-hydroxymethyl D-32 in plasma were 0.5, 5 and 5 ng/ml, respectively.

The pharmacological activities of D-32 after oral administration could be attributed to the active metabolites, (−)-4-hydroxy D-32 and 3-hydroxymethyl D-32, and unchanged (−)-D-32 in man.

**Abbreviations Used**——D-32, 1-*tert*-butylamino-3-(2,3-dimethylphenoxy)-2-propanol hydrochloride; 4-hydroxy D-32, 1-*tert*-butylamino-3-(2,3-dimethyl-4-hydroxyphenoxy)-2-propanol; 3-hydroxymethyl D-32, 1-*tert*-butylamino-3-(2-methyl-3-hydroxymethylphenoxy)-2-propanol; 3-carboxy D-32, 3-(3-*tert*-butylamino-2-hydroxypropoxy)-2-methylbenzoic acid; *AUC*, area under the plasma concentration-time curve; GC-MS, gas chromatography-mass spectrometry.

## References


1) A. S. Nies and J. G. Gerber, "Yearbook of Therapy," ed. by L. E. Hollister, Yearbook Medical Publishers, Inc., Chicago, 1980, p. 9.
2) N. Himori, S. Honma, A. Izumi, and T. Ishimori, *Nauny-Schmi. Arch. Pharmacol.*, **316**, 19 (1981).
3) A. M. Barrett and U. A. Cullum, *Br. J. Pharmacol.*, **34**, 43 (1968).
4) C. F. George, T. Fenyvesi, M. E. Conolly, and C. T. Dollery, *Eur. J. Clin. Pharmacol.*, **4**, 74 (1972).
5) K. Kawasima, A. Levy, and S. Spector, *J. Pharmacol. Exp. Ther.*, **196**, 517 (1976).
6) T. Walle and U. K. Walle, *Res. Commun. Chem. Pathol. Pharmacol.*, **23**, 453 (1979).
7) B. Silver and S. Riegelman, *J. Pharmacol. Exp. Ther.*, **215**, 643 (1980).
8) T. Walle, M. J. Wilson, U. K. Walle, and S. A. Bai, *Drug Metab. Disp.*, **11**, 544 (1983).
9) R. Howe and R. G. Shanks, *Nature* (London), **210**, 1336 (1966).
10) Y. Yost and J. L. Holtzman, *J. Pharm. Sci.*, **68**, 1181 (1979).
11) S. Honma and K. Kambegawa, *Chem. Pharm. Bull.*, **23**, 1045 (1975).
12) N. Himori, A. Izumi, and Y. Hiramatsu, *Arch. Inter. Pharm. Ther.*, **4** (1976).
13) S. Honma, Y. Kawabe, N. Koizumi, A. Kambegawa, Y. Matsuki, T. Ito, and T. Nambara, "Microsomes, Drug Oxidation, and Drug Toxicity," ed. by R. Sato and R. Kato, Japan Scientific Societies Press, Tokyo, 1982, p. 235.
14) S. A. Bai, M. J. Wilson, U. K. Walle, and T. Walle, *J. Pharmacol. Exp. Ther.*, **227**, 360 (1983).
15) T. R. Burke, W. N. Howald, and W. L. Nelson, *Res. Commun. Chem. Pathol. Pharmacol.*, **28**, 399 (1980).
16) M. L. Powell, R. R. Wagoner, C.-H. Chen, and W. L. Nelson, *Res. Commun. Chem. Pathol. Pharmacol.*, **30**, 387 (1980).
17) C. von Bahr, J. Hermansson, and M. Lind, *J. Pharmacol. Exp. Ther.*, **222**, 458 (1982).

18) A. H. Beckett and G. G. Gibson, *Xenobiotica*, **8**, 73 (1978).
19) K. Miyano, Y. Fujii, and S. Toki, *Drug. Metab. Disp.*, **8**, 104 (1980).
20) R. J. Lewis, W. F. Trager, K. K. Chan, A. Brechenridge, M. Orme, M. Roland, and W. Shary, *J. Clin. Invest.*, **53**, 1607 (1974).
21) C. Hignite, J. Vetrecht, C. Tshanz, and D. Azarnoff, *Clin. Pharmacol. Ther.*, **28**, 99 (1980).
22) L. R. Pohl, W. R. Porter, and W. F. Trager, *Biochem. Pharmacol.*, **26**, 109 (1977).
23) A. Kupfer and J. Bircher, *J. Pharmacol. Exp. Ther.*, **209**, 190 (1970).
24) A. Kupfer, R. K. Roberts, S. Schenker, and R. A. Branch, *J. Pharmacol. Exp. Ther.*, **218**, 193 (1981).
25) L. S. Kaminsky, M. J. Fasco, and F. P. Guengerich, *J. Biol. Chem.*, **254**, 9657 (1979).
26) L. S. Kaminsky, M. J. Fasco, and F. P. Guengerich, *J. Biol. Chem.*, **255**, 85 (1980).