Pharmacokinetics of the Anti-Inflammatory Tiaprofenic Acid in Humans, Mice, Rats, Rabbits, and Dogs

J. POTTIER ˣ, D. BERLIN, and J. P. RAYNAUD

Abstract □ The pharmacokinetics of tiaprofenic acid were studied in rats, dogs, mice, and rabbits using the ^{14}C-labeled compound and in humans using the radioinert compound. Its absorption was partially gastric but mainly intestinal and was total in all species. Its apparent volume of distribution was small; it was bound markedly to albumin, and its plasma clearance was high in all species. Absence of retention was confirmed in rats by the very low tissue radioactivity levels 24 hr after oral dosing. In humans, identical maximum plasma concentrations and elimination rates were recorded after the first and 40th dose during a 2-week treatment with 200 mg three times daily. During this period, constant residual levels were recorded before the first dose of the day. These results indicate that, for a dose in the therapeutic range, a steady state was reached as early as the end of the 1st day of treatment and that tiaprofenic acid did not inhibit or induce its own biotransformation. Urinary excretion accounted for one-half of the dose in rats and from 0.6 to 0.9 in humans, dogs, mice, and rabbits. Two metabolites were formed, one resulting from the reduction of the ketone group to an alcohol and the other from oxidation generating a phenol in the benzene ring in the *para*-position to the ketone group. These two metabolites were common to all species, but the latter was present in minute amounts only in dogs. Tiaprofenic acid was conjugated as an amide in dogs and as an acylglucuronide in the other species. In rats, phenobarbital induced the oxidative, but not the reductive, pathway.

Keyphrases □ Tiaprofenic acid—pharmacokinetics in humans, rats, dogs, mice, and rabbits □ Pharmacokinetics—tiaprofenic acid in humans, rats, dogs, mice, and rabbits □ Anti-inflammatory agents—tiaprofenic acid, pharmacokinetics in humans, rats, dogs, mice, and rabbits

Tiaprofenic acid[1], 5-benzoyl-α-methyl-2-thiopheneacetic acid (I) (Table I), is a highly potent anti-inflammatory and nonnarcotic analgesic agent as demonstrated both in animal (1) and in human (2–4) studies. In the present study, its animal pharmacokinetics were studied in rats and dogs—the species used to evaluate its pharmacological activity and tolerance—and, in less detail, in rabbits and mice—the species used to establish the absence of teratogenicity[2]. The absorption, distribution, excretion, and biotransformations of tiaprofenic acid were compared in these species and in humans after oral and parenteral administrations.

EXPERIMENTAL

Radiolabeled Compound—Tiaprofenic acid was labeled[3] with carbon-14 in the carbonyl group (Table I) with a specific activity of 54 mCi/mmole and a radiochemical purity of 95% as checked by TLC.

Animal and Human Studies—Male adult animals were used: Swiss mice (22 g), Sprague-Dawley rats (200 g), "Normandy" rabbits (2.4 kg), and beagle dogs (15 kg). Clinical pharmacology studies were carried out in adult subjects of both sexes. Prior to oral administration, animals and humans were fasted overnight. Unless otherwise indicated, the pharmacological dose of 5 mg/kg was administered to animals. In humans, the dosage varied from 1.5 to 3.0 mg/kg in a single administration. For the steady-state study, a daily dose of 600 mg (8.5 mg/kg), divided into three equal doses to be taken at 9:00 am, 2:00 pm, and 8:00 pm was administered for 13 days. A 40th dose of 200 mg was administered on the morning of the 14th day.

The drug was administered either as a suspension in 0.25% carboxymethylcellulose with 0.2% polysorbate 80, a solution after addition of lysine or tromethamine, capsules, or tablets. The stability of formulations containing ^{14}C-I was checked by TLC and scanning.

Blood samples were collected in dry heparin sodium in animals and with or without an anticoagulant in humans. Tissue samples were deposited on dry ice and stored at −25°. Bile was collected by catheterization of the common bile duct under prolonged anesthesia with urethan (1 g/kg ip). For intraduodenal administration, the pylorus was ligatured to avoid gastric reflux. Gastric absorption was studied in rats treated 1 hr after recovery from ligation of the pylorus under mild ether anesthesia. Pulmonary carbon dioxide was trapped in an aqueous solution of 4 *N* ethanolamine. Enzymatic induction was obtained with daily subcutaneous injections of 100 mg of phenobarbital sodium/kg for 3 days.

Hydrolysis of Conjugates—Biliary and urinary conjugates were hydrolyzed either enzymatically by leaving the material for 24 hr at 37° in a buffered acetate medium (pH 5.2) containing *Helix pomatia* juice[4] or at alkaline pH by slowly adding 0.2 *N* NaOH at 0°, away from light and under argon. In some excreta, polar products persisting after hydrolysis at alkaline pH were separated by TLC and, after elution, hydrolyzed with 1 or 6 *N* HCl for 1 hr at 100°.

Extraction and Chromatography—Plasma samples were acidified to pH ≃ 5 with 1 *M* KH_2PO_4. Urine and bile samples were acidified to pH 5 (extraction of I and II) or 2 (extraction of III) with 1 *N* HCl and then extracted twice with ethyl acetate. Extracts were chromatographed on silica gel plates[5] in various solvent systems (Table I). When ^{14}C-tiaprofenic acid was administered, the radioinert reference compounds were added before TLC to enable detection at 254 nm. Relevant areas were eluted either with methanol–acetic acid (1:1) (radiochemical assay) or with acetone or ethyl acetate–acetic acid (98:2) (spectrophotometric assay).

Radiochemical Assay—A solution of either scintillator[6] alone (0.4% w/v) in toluene or of scintillator (0.4% w/v) and naphthalene (8% w/v) in methoxyethanol–toluene (2:3) was added to the samples. Carbon dioxide formed after combustion of tissues[7] was trapped in a solution of scintillator (0.7% w/v) in toluene–phenylethylamine–methanol–water (40:33:22:5). Radioactivity was measured in a liquid scintillation spectrometer[8]. Quenching was corrected by the channel ratio method. Certain measurements were obtained by integration of the chromatographic curves with a scanner[9].

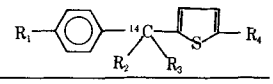
Spectrophotometric Assay—Chromatographic eluates were taken up by aqueous 1 *M* sodium acetate–ethanol (1:9). Absorbances were determined at 313 nm for I (ϵ = 15,400 M^{-1} cm^{-1}), at 248 nm for II (ϵ = 10,300 M^{-1} cm^{-1}), and at 319 nm for III (ϵ = 19,400 M^{-1} cm^{-1}). Optical interferences were eliminated by assaying control samples obtained before treatment or from untreated subjects.

For the determination of I, corrections for losses were made by the addition of labeled compound to the samples prior to analysis. Because the absorption of II is negligible above 280 nm, the determination of I is possible after chromatography in System B (Table I) even though this system does not separate I from II. This approach was checked on dog plasma, which contained relatively large quantities of II. The coefficient of correlation between radiochemical and spectrophotometric determinations of I was 0.997 (12 pairs of plasma concentrations from 3 to 100 mg/liter).

The method for the determination of I and its two metabolites in human urine after separation in System C (Table I) was checked by the analysis of control samples with compounds added *in vitro* (10 mg/liter

[1] Surgam, Laboratoires Roussel.
[2] R. Glomot, Toxicology Department, Roussel-Uclaf Research Center.
[3] Synthesized by A. Jouquey, Roussel-Uclaf Research Center.
[4] I. B. F., Gennevilliers, France.
[5] Merck, Darmstadt, 60F 254.
[6] 2-(4′-*tert*-Butylphenyl)-5-(4″-biphenyl)-1,3,4-oxadiazole, Ciba.
[7] Oxymat Intertechnique.
[8] Packard model 3385.
[9] Berthold, Wiedbad, West Germany.

Table I—Structures and R_f Values of Tiaprofenic Acid and Its Derivatives Found in Biological Media



Compound	Substituent R_1	R_2	R_3	R_4	R_f of System: Solvent System A[a]	Solvent System B[b]	Solvent System C[c]
I	H	=O		$CH(CH_3)CO_2H$	0.35	0.50	0.70
II[d]	H	H	OH	$CH(CH_3)CO_2H$	0.25	0.50	0.60
III[e]	OH	=O		$CH(CH_3)CO_2H$	0.05	0.40	0.30
IV[f]	H	=O		$COCH_3$	0.45	0.60	0.80
V[g]	H	=O		CH_2CH_3	0.70	0.80	0.85

[a] Toluene–acetic acid (88:12). [b] Benzene–dioxane–acetic acid (10:5:1). [c] Benzene–dioxane–acetic acid (9:1:1); two migrations. [d] 5-(α-Hydroxybenzyl)-α-methyl-2-thiopheneacetic acid. [e] 5-(4-Hydroxybenzoyl)-α-methyl-2-thiopheneacetic acid. [f] (2-Acetyl-5-thienyl)phenylmethanone. [g] (2-Ethyl-5-thienyl)phenylmethanone.

of each). The recovery of I [88.2 ± 2.6% (mean ± *SD*, $n = 8$)] was total when radiochemical corrections were applied (100.2 ± 2.6%). Recoveries of II and III were 94.5 ± 3.7 and 95.3 ± 1.7% ($n = 8$), respectively, and no correction was applied in these cases.

For a 5-ml sample, the limits of sensitivity were 0.1 mg/liter for I, whatever the mode of separation, 0.1 mg/liter for III, and 0.3 mg/liter for II.

Plasma Protein Binding—Binding to nondiluted plasma or to human albumin[10] in 0.1 *M* phosphate buffer (pH 7.4) was determined by ultrafiltration[11] to avoid the degradations occurring during long contact (equilibrium dialysis). Protein solutions were ultrafiltered at room temperature under a nitrogen pressure of 0.5 kg/cm^2 and magnetic stirring. In 5 min, 10% of the total volume was collected.

RESULTS

Structures of Free Compounds Found in Biological Media—The ^{14}C-label was stable; during 24 hr after intravenous administration, less than 0.01% of the radioactivity in the rat and less than 0.1% in the mouse were excreted by the lungs as ^{14}C-carbon dioxide.

Apart from tiaprofenic acid, which was found in all media studied and which was characterized by the similarity between its chromatographic properties, UV spectrum, and mass spectrum and those of a reference sample, four products were identified (II–V, Table I). Their presence was established both in humans and in the four animal species.

Compound II arises from the reduction of the ketone group of I to an alcohol. Absence of absorption in the UV spectrum around 300 nm indicated the loss of the ketone group. Its mass spectrum with a molecular peak at 262 corresponded to the introduction of two hydrogen atoms, and the fragmentation pattern was in agreement with the proposed formula.

Compound III results from an oxidation process introducing a phenol function in the benzene ring in a *para*-position to the ketone group. The maximum of absorption at 319 nm in its UV spectrum established the presence of the ketone group. Its mass spectrum revealed the introduction of an oxygen atom (molecular peak at 276) in the benzene ring (fragmentation pattern). The position of the phenol group was established by the NMR spectrum, which indicated that the proton in the *para*-position to the ketone group was absent.

Compounds II and III were identified conclusively by comparison with reference substances (1).

Compounds IV and V result from substitution of the methylacetic radical by acetyl and ethyl radicals, respectively, with the loss of one carbon atom. They were first isolated from an aqueous solution of I as degradation products; their structures were established by mass spectrometry (molecular peaks at 230 and 216, respectively, and fragmentation patterns in agreement). They were also found in small quantities *in vivo*.

In the plasma of the four animal species studied, IV and V accounted for about 5% of the total radioactivity measured after treatment with ^{14}C-I, and this proportion did not change significantly with time. Likewise, they accounted for 2–5% of the excreted radioactivity, the amount depending on the conditions of hydrolysis. Too strong alkalinization considerably increased the quantities of IV at the expense of free and conjugated I. Unlike II and III, which exist only *in vivo*, IV and V result from degradation and not from enzymatic biotransformations. Therefore, they cannot be considered as metabolites.

Animal Plasma Kinetics—Total radioactivity kinetic curves after oral and intravenous administrations of ^{14}C-I (Fig. 1) showed that absorption was rapid in the rat for the oral route and slightly slower in the dog; plasma clearance of I and its metabolites, 24 hr after administration, was almost complete in the rat but not in the dog. Tiaprofenic acid was the major constituent. Compound II was present in small quantities in rat plasma (not shown) but in appreciable quantities in dog plasma. In the plasma of both species, III was not detectable.

The kinetics of I after intravenous administration can be depicted according to a two-compartment open model. The apparent initial volumes of distribution, V_c, were calculated from biexponential plasma curves ($C = Ae^{-\alpha t} + Be^{-\beta t}$). The value of V_c is given by $V_c = \chi_0/C_0$, where χ_0 is the intravenous dose and C_0 is the plasma concentration at time $t = 0$ ($C_0 = A + B$) (5). The V_c was 17% of body weight in the rat and

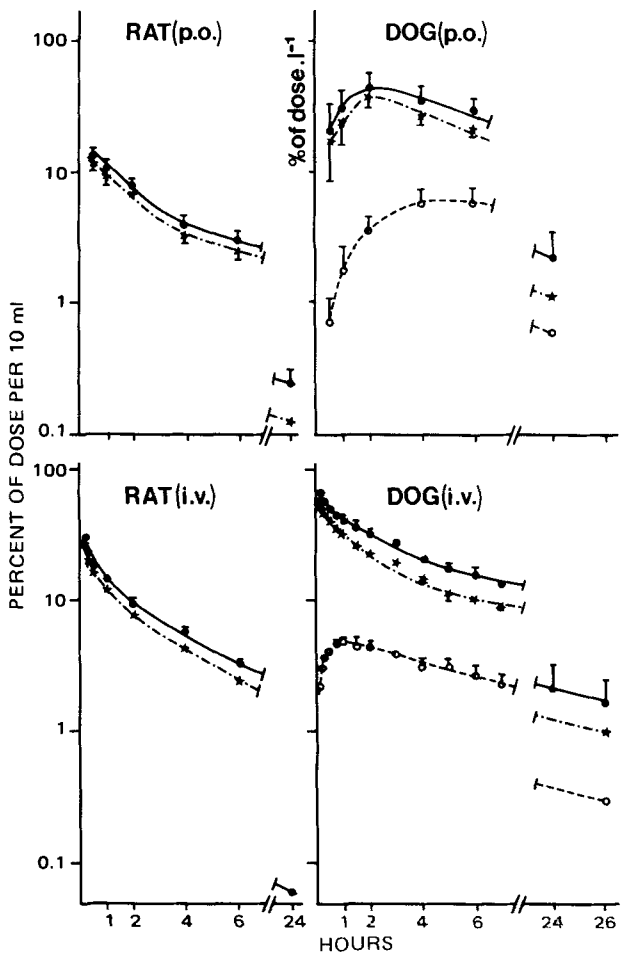


Figure 1—*Plasma kinetics in the rat and dog. Key:* ●, *total radioactivity;* ★, *tiaprofenic acid; and* ○, *II (mean* ± SE; n = *5, rat;* n = *4, dog, oral;* n = *2, dog, intravenous).*

[10] Centre National de Transfusion Sanguine, Paris, France.
[11] Pellicon Carrousel, Millipore-Diaflo Amicon P.M. 30 membranes.

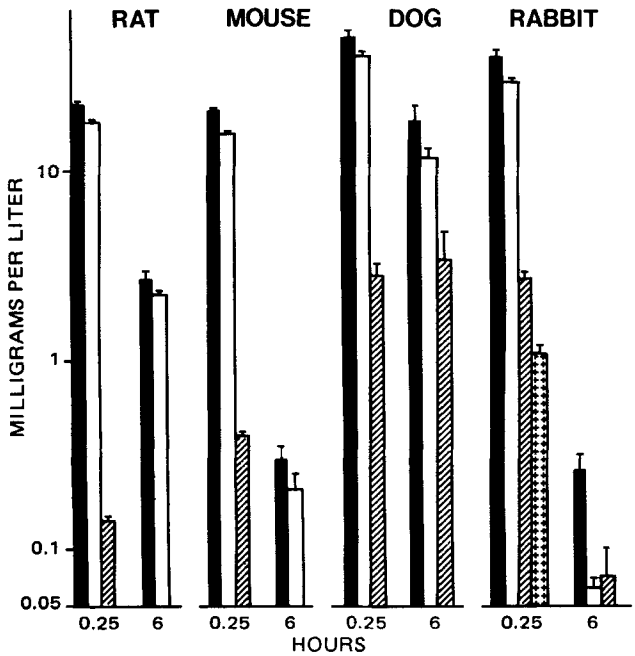


Figure 2—*Plasma levels in four animal species 0.25 and 6 hr after intravenous administration, expressed in tiaprofenic acid equivalent. Key:* ■, *total radioactivity;* □, *I;* ▨, *II; and* ⊞, *III (mean* ± SE; n = 5, *rat and rabbit;* n = 3, *dog;* n = 5, *pools of four plasmas, mouse).*

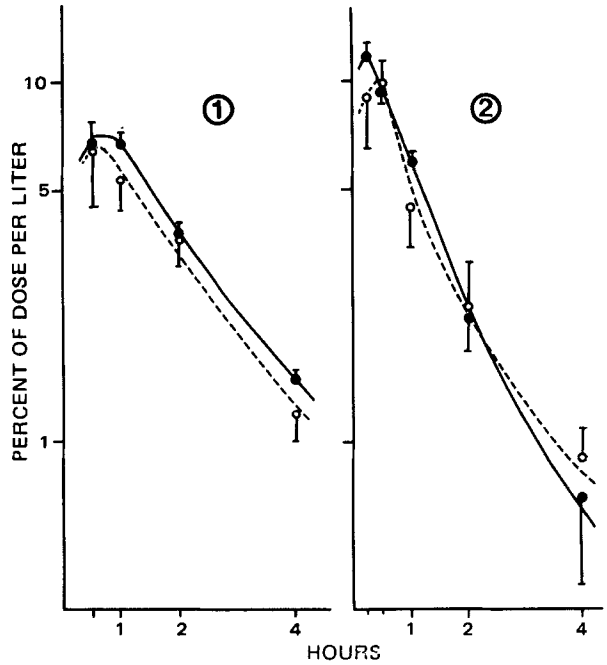


Figure 3—*Serum kinetics of tiaprofenic acid in humans after oral (1) or intramuscular (2) administration. Key:* ●, *100 mg; and* ○, *200 mg (mean* ● SE, n = *17, oral, 100 mg;* n = 5, *oral, 200 mg;* n = 5, *intramuscular, 100 mg;* n = 4, *intramuscular, 200 mg).*

8% in the dog. The extravascular diffusion space was thus smaller in the dog. The plasma clearance of I [$Cl_p = \chi_0/\int_0^\infty C\,dt = \chi_0\alpha\beta/(A\beta + B\alpha)$] per kilogram was 27 ml/hr in the dog and 96 ml/hr in the rat.

Absorption was estimated (6) from the ratio of areas under plasma curves after oral and intravenous administrations. In the rat and dog, the area ratios based on total radioactivity were 0.78 and 1.01; based on tiaprofenic acid, they were 0.87 and 1.12, indicating both a good rate of absorption and no first-pass effect.

Total plasma radioactivity and the radioactivities due to the different compounds were determined 0.25 and 6 hr after intravenous administration of ^{14}C-I in all four animal species (Fig. 2). The decrease in total radioactivity was much faster in the rabbit and mouse than in the rat and, particularly, the dog. Six hours after the injection, tiaprofenic acid represented the major fraction in all species except the rabbit. Metabolite II concentrations were very low in the rat, a little higher in the mouse, and considerably higher in the dog and rabbit. Metabolite III was detected in the rabbit only—in limited quantities and only 0.25 hr after the injection.

On the basis of a simplified one-compartment open model, $C = Ae^{-\alpha t}$, defined by the concentrations at 0.25 and 6 hr, the clearance of I ($Cl_p = \chi_0\alpha/A$) was 91 ml/hr/kg in the rat and 25 ml/hr/kg in the dog. These values are similar to those calculated in the preceding assay. Its clearance was 140 ml/hr/kg in the rabbit and 196 ml/hr/kg in the mouse. The V_c values were calculated according to this same model and enabled the classification of the animal species into two groups with V_c values equivalent to 12 and 13% (dog and rabbit, respectively) and to 24 and 26% (rat and mouse, respectively) of body weight.

Human Serum or Plasma Kinetics—The concentrations of I were assayed spectrophotometrically. Although the UV absorption of II was detected in some samples, this metabolite was present only in weak concentrations and was not measured. The presence of III could not be confirmed in serum, since a meaningful UV spectrum could not be obtained after elution.

After a single oral dose of 100 or 200 mg, peak concentrations were achieved in 0.5–1 hr. Within 4 hr of administration, the level had dropped to one-fifth of the peak value, indicating both rapid absorption and disappearance of the compound (Fig. 3). After a single intramuscular injection of the same dosages (Fig. 3), a rapid decrease in plasma levels established satisfactory diffusion from the injection site and confirmed the fast elimination rate. The V_c of I, calculated according to a one-compartment open model, was about 10% of body weight, and its clearance was 6 liters/hr.

In the steady-state study, the plasma kinetics of tiaprofenic acid after the first dose on Day 1 and after the 40th and last dose on Day 14 were similar (Fig. 4), with mean peaks of 17 mg/liter and equal elimination rate constants (0.50 and 0.46 hr^{-1}, respectively). The areas under individual curves, calculated from 0 to 5 hr by the trapezoidal rule, were not significantly different (paired t test). From Days 2 to 11, mean plasma concentrations fluctuated from 0.6 to 0.8 mg/liter. After the end of treatment (Day 14), from Days 15 to 18, concentrations were equal to or below the limit of sensitivity of the method (0.1 mg/liter).

Absorption by Stomach—The importance of gastric absorption was evaluated in rats by comparing the plasma radioactivity of a group of animals with a ligated pylorus to that of a sham group 0.5 hr after oral administration of ^{14}C-I. Plasma concentrations in control animals were close to those found previously at 0.5 hr (11.7 *versus* 13.9% of dose/10 ml). Therefore, anesthesia and laparotomy did not influence absorption. After ligature of the pylorus, the plasma concentration was only halved, 5.07 ± 0.82 (n = 10) *versus* 11.7 ± 1.08 (n = 7), indicating that the gastric mucosa absorbs tiaprofenic acid.

Tissue Distribution in Rats—Figure 5 represents plasma and tissue concentrations of total radioactivity in the rat 24 hr after ingestion of ^{14}C-I. Tissue levels were all very low and generally lower than plasma levels. Levels in central nervous system tissues were particularly low. The only tissues with concentrations higher than in the plasma were those involved in metabolism and excretion (liver and kidney) or in contact with excreta (bladder). On the basis of the tissue composition of the rat (7), the total concentrations in the whole animal, excluding the GI tract, accounted for about 2% of the dose. This very low residual fraction indicated that I and its metabolites were almost totally eliminated 24 hr after ingestion.

Excretion Routes in Animals—Renal excretion of I and its metabolites was high in all of the animal species (Table II). The total radioactivity excreted in 24 hr accounted for one-half of the dose in the rat and two-thirds of the dose in the dog after ingestion of 5 mg/kg. It accounted for three-fourths of the dose in the mouse and about nine-tenths in the rabbit after ingestion of 50 mg/kg. These high excretion levels recorded after administration of such a suprapharmacological dose are indicative of excellent absorption.

Recovery of about one-half of the dose in rat feces implies significant biliary excretion, since absorption is good in this species. This biliary excretion was measured in anesthetized rats after intravenous administration of ^{14}C-I (Fig. 6). Under conditions where bile collection prevented enterohepatic recirculation, 70% of the dose was excreted in 6 hr and 80% in 24 hr. These results confirmed the importance of biliary excretion of I and/or its metabolites.

Reabsorption of products excreted in the bile was evaluated by comparing the quantity absorbed after administration of bile of control rats

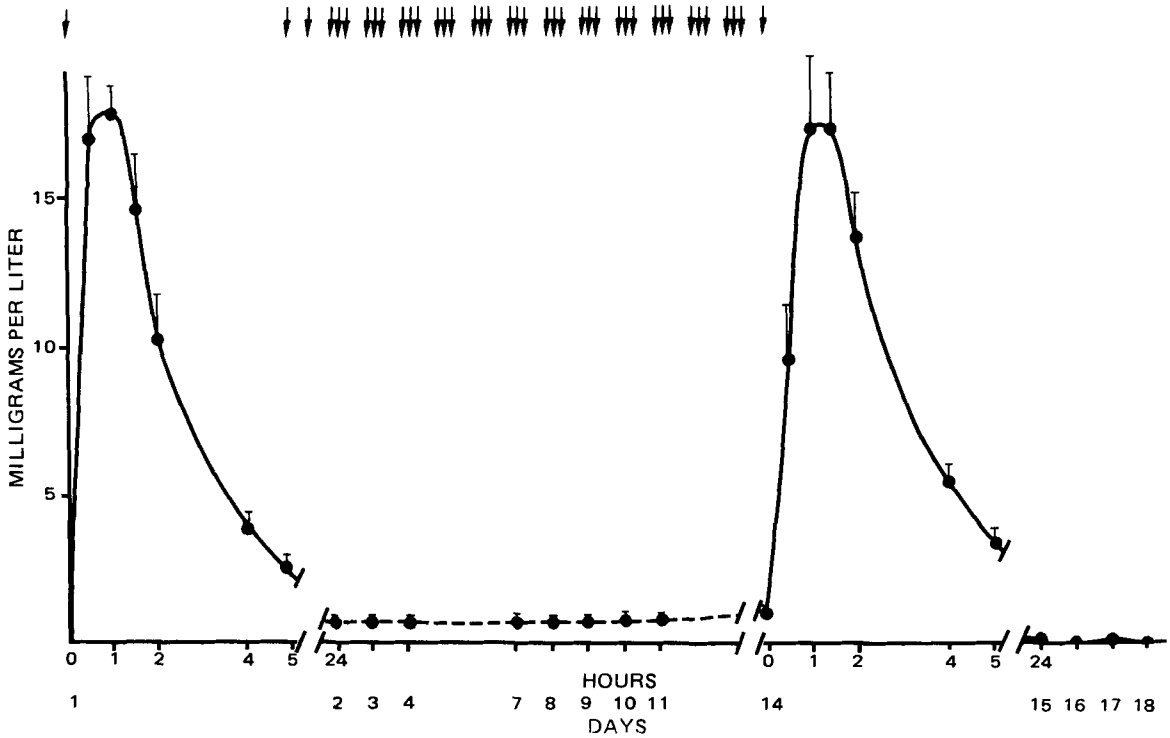


Figure 4—*Plasma concentrations of tiaprofenic acid during and after a chronic treatment with 600 mg/day. Key: ↓: dose of 200 mg (mean ± SE, n = 12).*

with ^{14}C-I added *in vitro* or of bile collected after intravenous injection of ^{14}C-I. These two types of bile samples were administered either orally to conscious rats or intraduodenally to anesthetized rats after catheterization of the common bile duct. Reabsorption, when given by the ratio of the areas under plasma radioactivity curves, was 32% (Fig. 7, top); when given by the ratio of the excreted radioactivity in the collected bile samples, it was 56% (Fig. 7, bottom). Thus, in the rat, there is a significant enterohepatic cycle.

Tiaprofenic acid was much more rapidly absorbed than its biliary metabolites, as indicated by a comparison of plasma kinetics in the first assay and of biliary excretion kinetics in the second assay.

Nature of Excreted Radioactivity in Animals—In the rat, after intravenous administration of ^{14}C-I, the percentage of radioactivity excreted within 6 hr was 29.0 ± 1.1 (n = 5) in urine (conscious animals) and 74.1 ± 2.5 (n = 4) in bile (anesthetized animals). Compounds I and II were mainly free in urine, whereas almost nine-tenths was conjugated in bile. In both fluids, the yields obtained after hydrolysis at alkaline pH were greater than those liberated by enzymatic lysis. Conjugates of I and II were consequently acylglucuronides. Their hydrolysis during collection due to their instability explains the presence of a larger free fraction in urine than in bile under the experimental conditions used.

Compound III was mainly conjugated in urine and bile. Since significant quantities of free III were liberated after enzymatic hydrolysis only, it was conjugated as an ether glucuronide or sulfate ester of the phenol group, although, in addition, an acylglucuronide with the carboxyl group could not be excluded. Tiaprofenic acid and its two metabolites represented three-fourths of the urinary radioactivity and two-thirds of the biliary radioactivity, I being the major fraction in both cases (Fig. 8). The two metabolites were excreted mainly *via* the urine. The remaining ra-

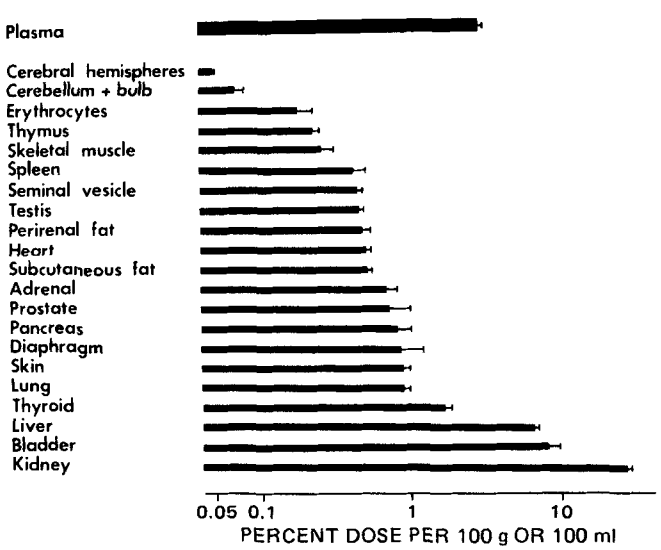


Figure 5—*Tissue distribution of total radioactivity in the rat 24 hr after oral administration (mean ± SE, n = 5).*

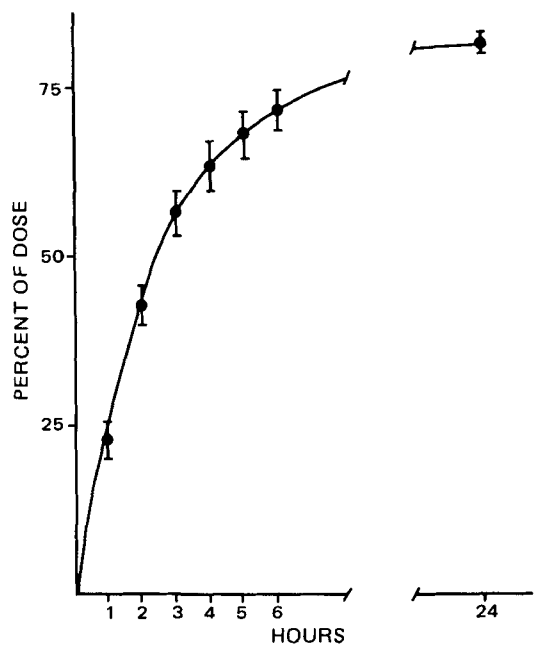


Figure 6—*Cumulative biliary excretion of total radioactivity in the rat (mean ± SE, n = 8).*

Table II—Urinary and Fecal Excretion [a] of Radioactivity in the Rat and Urinary Excretion in the Dog, Rabbit, and Mouse after Oral Administration of ^{14}C-I

	Hours	Rat (5)[b], 5 mg/kg Urine	Rat (5)[b], 5 mg/kg Feces	Dog (3), 5 mg/kg, Urine	Rabbit (3), 50 mg/kg, Urine	Mouse (2), 50 mg/kg, Urine
	0–24	46.9 ± 2.6	41.8 ± 2.9	59.3 ± 2.5	85.8 ± 4.6	73.4 ± 3.6
	24–48	1.2 ± 0.2	4.0 ± 1.3	5.2 ± 2.0	—	—
	48–72	0.3 ± —[c]	0.4 ± 0.2	—	—	—
Total	0–48			64.5 ± 0.6	—	—
Total	0–72	48.4 ± 2.7	46.2 ± 2.2	—	—	—
Grand total	0–72	94.6 ± 1.3				

[a] Expressed as means ± *SE* of percent administered radioactivity. [b] Number of animals or of groups of 10 mice. [c] *SE* < 0.05.

dioactivity was due to the noncarboxylic degradation products IV and V and primarily to polar products(s), possibly nonhydrolyzed conjugate(s). More vigorous hydrolytic methods yielded no further information because of degradation.

In the dog, the percentage of the dose excreted in the urine 6 hr after an intravenous injection of ^{14}C-I was 42.8 ± 9.4 (n = 3). Free II accounted for one-third of the total excretion (15.1 ± 6.2%, n = 3). A small quantity of free tiaprofenic acid (0.5% of dose) was unchanged after hydrolysis by enzymes or by alkaline pH. On the other hand, the residual polar fraction, after isolation by chromatography and hydrolysis by hot 6 N HCl, released a significant quantity of I (7.3 ± 1.3%, n = 3). Considering the easy degradation of I under these severe conditions, this quantity was only a minimum. The conjugate of I excreted in the urine of the dog was consequently not an acylglucuronide. Its characteristics suggest that it was an amide conjugate. Compound III was identified by its mass spectrum after hydrochloric acid hydrolysis of this same polar fraction from the urine of dogs treated orally by 100 mg of ^{14}C-I/kg. It was present in very small quantities.

In rabbit and mouse urine, collected for 24 hr after oral administration of 50 mg of ^{14}C-I/kg, tiaprofenic acid was excreted partially conjugated and accounted for most of the radioactivity, *i.e.*, 60% in rabbits and 57% in mice. Its conjugate, because it was hydrolyzed by β-glucuronidase and with a better yield at an alkaline pH, was an acylglucuronide. Compounds II and III occurred mainly in free form and accounted for 5% each of total excretion in the rabbit and 5 and 10%, respectively, in the mouse.

Urinary Excretion in Humans—Tiaprofenic acid was assayed spectrophotometrically in 24-hr urine samples. Its free form and the acylglucuronide, which was the main form, accounted for 45.2 ± 4.9 (n = 17) and 51.4 ± 3.5% (n = 5) of the dose 24 hr after oral administration of 100 and 200 mg, respectively. They accounted for 50.3 ± 3.4 (n = 5) and 39.7 ± 7.9% (n = 3) after intramuscular administration of the same doses, respectively. Metabolites II and III were also excreted partly as acylglucuronides, but the presence of an ether glucuronide or sulfate ester of the alcoholic or phenol groups cannot be excluded completely. Tiaprofenic acid and its two metabolites accounted for 60% of an oral 100-mg dose (Table III); I accounted for nine-tenths of the total excretion. The excretion of II was about double that of III.

Biotransformations of tiaprofenic acid in humans and in the four animal species studied are summarized in Scheme I.

Induction with Phenobarbital—Enzymatic induction was studied by comparing the plasma levels of I and its metabolites and their urinary excretions after intravenous injection of ^{14}C-I in rats, with or without phenobarbital pretreatment.

Compound I and its metabolites were cleared more rapidly from plasma in phenobarbital-induced rats, since plasma radioactivity was about the same 0.25 hr after the injection but was reduced by nearly 50% compared

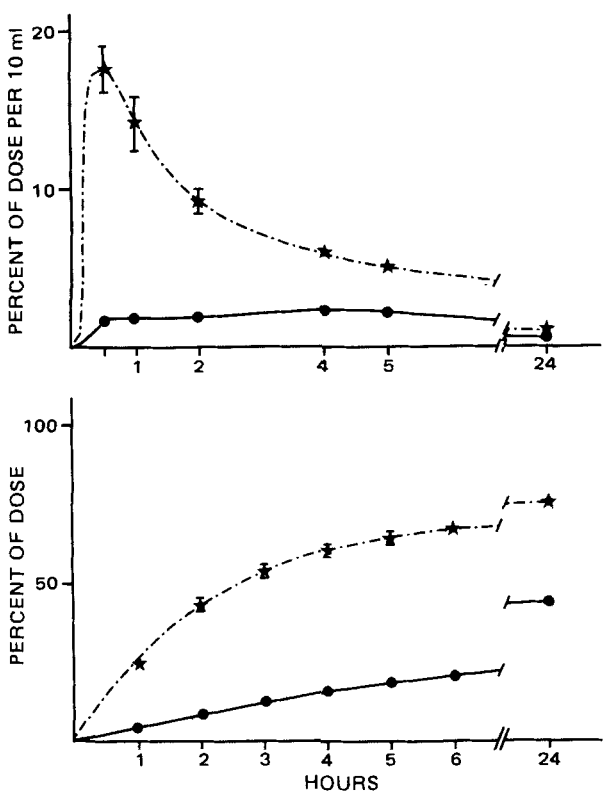
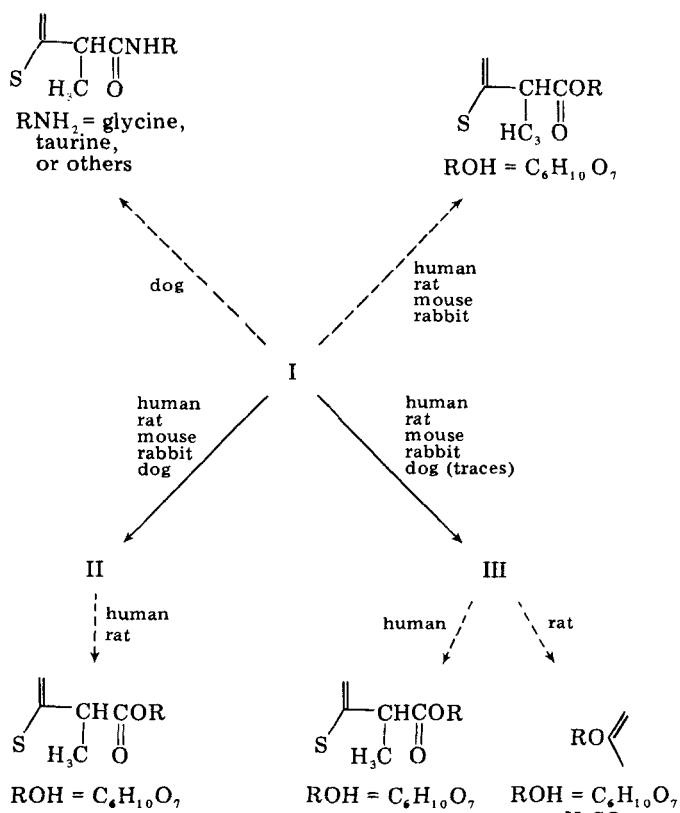


Figure 7—*Enterohepatic cycle in the rat. Top: plasma concentrations of radioactivity after oral administration of I or its biliary metabolites to conscious rats. Bottom: biliary excretion of radioactivity after intraduodenal administration of I or its biliary metabolites to anesthetized rats. Key: ★, control bile with ^{14}C-I added in vitro; and ●, bile from treated rats containing I and its metabolites. In both cases, the dose was 5 mg of I/kg or its equivalent calculated from radioactivity (mean ± SE, n = 5).*



Scheme I—Metabolic pathway of tiaprofenic acid in humans and animal species studied. Key: →, phase I biotransformations; and ⇢, phase II biotransformations (conjugations).

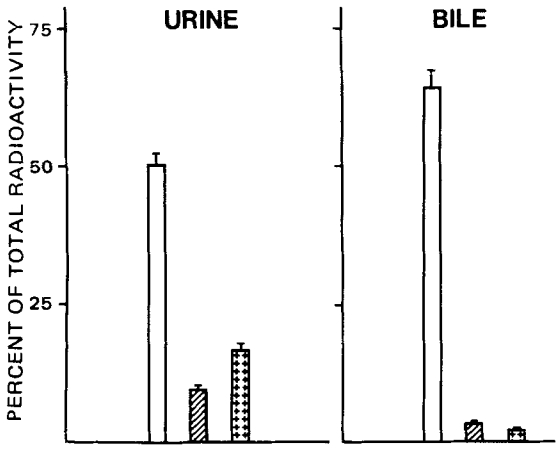


Figure 8—*Urinary and biliary levels of I and its metabolites (after deconjugation) in the rat 6 hr after intravenous administration. Key:* □, *tiaprofenic acid;* ▨, *II; and* ⊞, *III (mean* ± SE, n = 5, *urine;* n = 4, *bile).*

to controls 6 hr after the injection (Fig. 9, top). Plasma levels of I also were reduced; its plasma clearance, calculated with a one-compartment open model, was increased by 58% (139 compared to 88 ml/hr/kg in control animals). There were no significant changes in the quantities of II and III, which remained very low.

After induction by phenobarbital, the urinary excretion of radioactivity 6 hr after injection was slightly increased: 30.1 ± 1.5 *versus* 27.5 ± 2.5% (n = 5). However, the quantities of I and II were lowered, whereas the quantity of III was increased (Fig. 9, bottom). The radioactivity from other origins, mainly composed of polar products, was increased ($0.05 > p > 0.02$).

The enzymes that hydroxylate I and probably those that conjugate I and its metabolites are induced by phenobarbital. On the contrary, the enzymes that reduce I are not induced by phenobarbital, leading to a decrease in the excretion of II.

Protein Binding—The amount of radioactivity bound from a nondiluted plasma pool from rats 0.5 hr after oral administration of ^{14}C-I was 98.2 ± 0.2% (n = 5). As shown previously, 85% of the plasma radioactivity was due to I under these conditions. A similar result, 97.6 ± 0.1% (n = 3), was obtained from a plasma pool taken from control animals with identical concentrations of ^{14}C-I added *in vitro*. Similarly, 97.9 ± 0.3% (n = 3) of radioactivity was bound when I was added to a solution of human albumin (0.5 m*M*). This concentration was close to that of albumin in normal plasma.

DISCUSSION

The absorption of tiaprofenic acid was very good in all species studied, as shown by a comparison of its plasma kinetics after oral administration and after intravenous or intramuscular injection in rats, dogs, and humans, as well as by its very high urinary excretion after oral administration of a large dose in rabbits and mice.

The gastric mucosa plays a role in this absorption. In the rat, gastric absorption occurs by passive diffusion of undissociated compounds (8), and I is certainly weakly dissociated at gastric pH. Plasma radioactivity in rats with a ligated pylorus 0.5 hr after ingestion was 43% of that observed in controls, a percentage close to that obtained, under identical conditions after ingestion of ^{3}H-water, ^{14}C-ethanol, or ^{14}C-sodium acetate (50–70%). Acetic acid, like I, is only weakly dissociated at gastric pH. For

Table III—Urinary Excretion[a] of Tiaprofenic Acid (I) and Its Metabolites (II and III) in Humans for 24 hr following Oral Administration of 100 mg of I

I	II	III
43.7	1.7	0.9
81.3	2.9	0.2
50.4	4.5	2.5
51.3	2.9	1.5
43.7	2.0	3.2
54.1 ± 7.0[b]	2.8 ± 0.5	1.7 ± 0.5

[a] Expressed as percent of dose. [b] Mean ± *SE*.

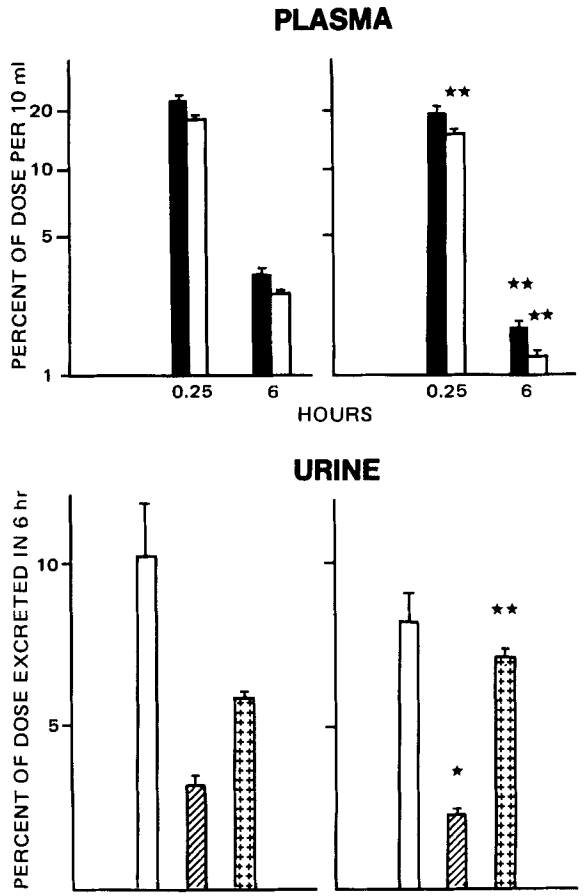


Figure 9—*Influence of phenobarbital on plasma concentrations and urine excretion in the rat. Left: control animals; right, induced animals. Key:* ■, *total radioactivity;* □, *tiaprofenic acid;* ▨, *II; and* ⊞, *III (mean* ± SE, n = 5; *significance according to Student* t *test:* ★, $0.02 > p > 0.01$; *and* ★★, $p < 0.01$).

a highly dissociated product, such as floctafenine (9), a very low (1%) percentage was recorded. Human gastric mucosa probably also absorbs I since the absorption mechanisms are qualitatively and quantitatively identical in both humans and rats (10). Absorption speed and capacity were considerable in the rat duodenum after *in situ* administration. The duodenum is certainly the main site of absorption, since, under normal conditions, residence time and the area of contact are higher in it than in the stomach.

The very high plasma concentrations recorded shortly after injection indicated that the extravascular diffusion space of tiaprofenic acid was small in all species. The V_c varied, however, according to species. In rats and mice, it was about twice that in dogs, rabbits, and humans. Like other anionic molecules such as indomethacin (11), fenoprofen (12), and tolmetin (13), tiaprofenic acid was extensively bound to the many available sites of plasma albumin.

Radioactivity was almost entirely cleared from the plasma 24 hr after administration in all species studied, including the dog where disappearance was slowest. The clearance of tiaprofenic acid in humans, 6 liters/hr, was close to that of indomethacin, 7.8 liters/hr (14). These values are relatively high, about twice those obtained for fenoprofen (15) and ketoprofen (calculated from Ref. 16) and much higher than the clearance of naproxen, 0.4 liter/hr (calculated from Ref. 17), and phenylbutazone, 0.1 liter/hr (calculated from Ref. 18). In relation to body weight, human clearance of tiaprofenic acid was of the same order as in the rat and rabbit, half that in the mouse, and four times that in the dog.

The chronic study in humans confirmed rapid elimination, since the steady state was reached as early as the end of the 1st treatment day and the concentrations were down to zero 24 hr after the last ingestion. The maximum concentrations, the areas under curves, and the elimination rate constants, which were identical after the first and 40th ingestion, showed that tiaprofenic acid cannot inhibit or induce its own biotransformations.

Renal excretion, which in rats was equal to fecal excretion, was preponderant in humans and in the other species studied. A significant en-

terohepatic cycle was demonstrated in the rat. Biliary metabolites were absorbed slower than I, since most of them were conjugated, with polarities unfavorable to intestinal absorption (19). Progressive hydrolysis of acylglucuronides by the weakly alkaline pH in the distal part of the duodenum and of all types of conjugates by bacterial enzymes (20) accounts for this difference.

Metabolites II and III were common to all species. In the dog, however, II was present in large quantities and III was present in very small quantities. They are pharmacologically less active than I (1).

In the rat, the clearance of I was greatly increased by phenobarbital, partly because of enzymatic induction of hydroxylation. This result is in agreement with the location of hydroxylase(s) in the hepatic endoplasmic reticulum, which is stimulated by phenobarbital (21). The approximately 50% increase in biliary flow induced by phenobarbital (22) increases the importance of this route of excretion and, together with enzymatic induction, accounts for the large increase in plasma clearance as opposed to the small increase in urinary radioactivity excretion. On the other hand, the enzymes that reduce the ketone group were not induced, in agreement with the location of several keto reductases in the soluble fraction from rabbit (23), rat, and human (24) liver and kidney.

No reduction of the ketone group was observed for two compounds whose structures, like I, are formed by a ring with a carboxylic radical linked to another ring by a carbonyl group: tolmetin [1-methyl-5-(4-methylbenzoyl)-2-[1*H*]-pyrroleacetic acid] (25) and ketoprofen [2-(3-benzoylphenyl)propanoic acid] (16).

For tolmetin, the only metabolite identified in rats, monkeys, and humans was the diacid formed by oxidation of the 4-methyl group. For ketoprofen, the two metabolites partially identified in rats, dogs, rabbits, and humans resulted from hydroxylation in different positions of the benzene ring not substituted by a propanoic radical.

On the other hand, Lan *et al.* (26) found that, after administration of a similar structure, 4-cyclopropylcarbonylbenzeneacetic acid[12] (VI), the alcohol derivative was excreted in low quantities in the urine of rats and monkeys but in much higher quantities in the urine of dogs. This result is parallel to the present observation of a higher urinary excretion of II in the dog than in the rat.

Regarding secondary biotransformations, a ketoprofen acylglucuronide was found in all species, including the dog (16), whereas VI was excreted in this species conjugated with taurine (26). Like VI, tiaprofenic acid was also excreted as an amide conjugate in the dog but as an acylglucuronide in the other species.

[12] S.Q. 20650.

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