to the starting ester in DMSO, a slurry of $NaOCH_3$ in DMSO was added with stirring over a 1-hr period while maintaining the temp below 30°. After complete addn and 15 min of addl stirring, acidification (excess 3 *N* HCl), filtration, and recrystn (IPO) gave a 59% yield of the desired ester Ic.

2-Methyl-4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylic Acid Methyl Ester 1,1-Dioxide (VI).—A yellow soln resulted from a combination of 2.95 g (0.012 mole) of 4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylic acid methyl ester 1,1-dioxide, 2.4 ml of MeI, 11 ml of H_2O, 40 ml of EtOH, and 12 ml of 1 *N* NaOH. After standing at room temp for 18 hr the resulting heavy yellow ppt was filtered, washed with H_2O, and dried to give 2.4 g (78%) of VI, mp 162–165°. A sample in MeOH soln gave a wine-red color with 5% $FeCl_3$. Titration in 2:1 dioxane–H_2O indicated neut equiv 266 (calcd 269) and pK_a = 8.4; nmr ($DCCl_3$) τ −2.04 (s, 1, exchanges with D_2O, enol OH), 1.8–2.4 (m, 4, arom protons), 6.05 (s, 3, OCH_3), 7.06 (s, 3, NCH_3). *Anal.* ($C_{11}H_{11}NO_5S$) C, H, N.

By an analogous alkylating procedure using either EtI, PrI, allyl bromide, or benzyl bromide the corresponding 2-alkyl-4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylic acid methyl ester 1,1-dioxides were made (see Table IV).

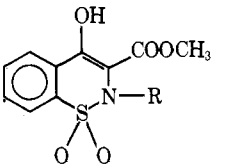
2-Benzyl- and 2-Allyl-2*H*-1,2-benzothiazin-4(3*H*)-one 1,1-Dioxides.—The 2-step procedure employed for prepg these compds is essentially the same as that reported by Zinnes,[8] *et al.*, for the 2-Me analog. Alkylation of 2*H*-1,2-benzothiazin-4(3*H*)-one 1,1-dioxide ethylene ketal[8] (II) with benzyl bromide gave a 75% yield of **2-benzyl-2*H*-1,2-benzothiazin-4(3*H*)-one 1,1-dioxide ethylene ketal**, mp 83–86°. *Anal.* ($C_{17}H_{17}NO_4S$) C, H, N. Hydrolysis[8] of this ketal gave 88% yield of **2-benzyl-2*H*-1,2-benzothiazine-4(3*H*)-one 1,1-dioxide**, mp 123–126°. *Anal.* ($C_{15}H_{13}NO_3S$) C, H, N.

Alkylation of 2*H*-1,2-benzothiazin-4(3*H*)-one 1,1-dioxide ethylene ketal[8] (II) with allyl bromide gave a 88% yield of **2-allyl-2*H*-1,2-benzothiazin-4(3*H*)-one 1,1-dioxide ethyl eneketal**, mp 75–77°. *Anal.* ($C_{13}H_{15}NO_4S$) C, H, N. Hydrolysis[8] of this ketal gave a 78% yield of **2-allyl-2*H*-1,2-benzothiazin-4(3*H*)-one 1,1-dioxide** as a viscous liquid which was purified by column chromatog (silica gel G, E. Merck, A. G., eluted with $CHCl_3$). A sample was vacuum distd for analysis. *Anal.* ($C_{11}H_{11}NO_3S$) C, H, N.

2,*N*-Dimethyl-4-hydroxy-2*H*-1,2-benzothiazin-3-carboxanilide 1,1-Dioxide (**45**).—A yellow soln of 4.0 g (0.015 mole) of VI and 1.8 g (0.017 mole) of *N*-methylaniline in 300 ml of xylene was placed under N_2 and refluxed for 22 hr. Distn of solvent to a final vol of 30 ml and cooling gave a yellow solid which, after recrystn from EtOH yielded 2.1 g (40%) of **45**: mp 162–165°; a $FeCl_3$ test was positive (red); ir (enol form) 6.0, 6.20, 6.26 μ; titration in 2:1 dioxane–H_2O indicated neut equiv 354 (calcd 344) and pK_a = 9.8; nmr (DMSO-d_6) τ 1.92–2.31 (m, 4, aromatic protons), 2.64 (s, 5, C_6H_5), 6.65 [s, 3 $CH_3(C_6H_5)N$], 7.60 (s, 3, the 2-CH_3).

TABLE IV

2-ALKYL-4-HYDROXY-2*H*-1,2-BENZOTHIAZINE-3-CARBOXYLIC ACID METHYL ESTER 1,1-DIOXIDES



R	Yield, %	Mp, °C	Crystn solvent[a]	Formula[b]
Et	35	97–100	E–W	$C_{12}H_{13}NO_5S \cdot 0.5H_2O$
Pr	21	125–127	E–W	$C_{13}H_{15}NO_5S \cdot 0.5H_2O$
$CH_2CH{=}CH_2$	62	97–100	E–W	$C_{13}H_{13}NO_5S$
$CH_2C_6H_5$	64	157–159	E	$C_{17}H_{15}NO_5S$

[a] See footnote *c* of Table I. [b] Satisfactory analyses for C, H, N were obtained for all of these compds.

Acknowledgment.—The authors are grateful to Miss Josephine Chiaini and Messrs. Nelson Treadway, Jr., Paul Kelbaugh, and Joseph Sadosky for their valuable technical assistance. We thank the Preparations Laboratory of Pfizer, Inc., especially Mr. Ernest J. Bianco and Dr. Susumu Nakanishi, for developing the conditions for large scale preparation of intermediate compounds.

Excretion and Metabolism of a Nonsteroidal Antiinflammatory Agent, 4-Hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxanilide 1,1-Dioxide, in Rat, Dog, Monkey, and Man

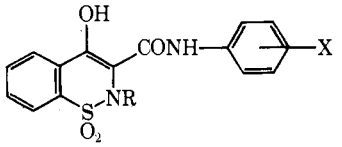
JOSEPHINE CHIAINI, EDWARD H. WISEMAN,* AND JOSEPH G. LOMBARDINO

Medical Research Laboratories, Pfizer Inc., Groton, Connecticut 06340

Received May 29, 1971

The nonsteroidal antiinflammatory agent, 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxanilide 1,1-dioxide has a plasma half-life of 6 hr in the rat, 30 hr in the dog, 4.5 hr in the monkey, and 21 hr in man. The principle metabolite in man, monkey, and rat, formed by hydroxylation of the carboxanilide moiety, is excreted in the urine as an acid-labile conjugate. The dog eliminates the drug in the urine mainly as a water-soluble conjugate of the parent drug.

4-Hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxanilide 1,1-dioxide (I) is a member of a series of potent antiinflammatory 1,2-benzothiazine-3-carboxanilides.[1] This paper is an account of pharmacokinetic and metabolic studies with I in man, monkey, dog, and rat.



I, R = CH_3; X = H
II, R = CH_3; X = OH
III, R = [^{14}C]CH_3; X = OH
IV, R = H; X = H

Experimental Section

Pharmacokinetic and metabolism studies were carried out in male albino rats (Charles River), mongrel dogs, and rhesus monkeys, maintained in metabolism cages with free access to

(1) J. G. Lombardino, E. H. Wiseman, and W. M. McLamore, *J. Med. Chem.*, **14**, 1171 (1971).

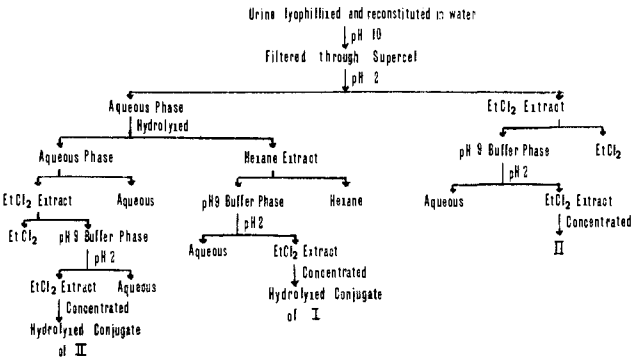


Figure 1.—Isolation of metabolites from urine of subjects receiving 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxanilide 1,1-dioxide (I).

food and H_2O.[2] Blood samples for anal. of drug concns were drawn from the abdominal aorta of rats maintained under pentobarbital anesthesia, or from the jugular vein of dogs and monkeys. Plasma and urine samples were stored at 4° until assayed. Drug plasma half-life in lab animals was detd after iv administration of drug soln (10 mg/kg). In the rat, the drug was injected into the tail vein; groups of 3 animals were sacrificed at each time interval. The drug was injected into the cephalic vein of dogs and monkeys, and serial blood samples were withdrawn at intervals. Studies in man were carried out in healthy male volunteers during and subsequent to termination of chronic drug administration. Drug was administered orally and blood samples were drawn by venipuncture into heparinized tubes. Drug plasma half-life was determined by analyzing the decay of plasma drug concns after drug withdrawal following chronic administration.

Spectrophotometric assays were developed for I and II in biological fluids. All assays were calibrated by carrying appropriate samples of known concn (1–80 μg/ml) through the entire procedure. The assay for I was as follows: samples (2 ml) were acidified with 1 *N* HCl (0.5 ml) and extd by shaking with heptane contg 1.5% *i*-AmOH (5 ml). The layers were sepd by centrifugation, and an aliquot (4 ml) of the org layer was extd with pH 9 carbonate buffer (5 ml where drug concns were anticipated to be above 5 μg/ml; 1 ml where drug concns were anticipated to be in the range 0.5–5 μg/ml). The optical density of the aq layer was detd at 255 and 350 mμ using a Beckman Model DU spectrophotometer. The ratio of the absorbances at 255 and 350 mμ is essentially const (1.0 ± 0.1), providing authentication of the material present in the sample. II was not extd from an acid soln by heptane–1.5% *i*-AmOH and concns of up to 100 μg/ml did not interfere in the assay for I.

Plasma and urine samples were assayed for II as follows: the sample (2 ml) was acidified with 1 *N* HCl (0.5 ml) and extd by shaking with $(ClCH_2)_2$ (10 ml). After centrifugation to sep the layers, an aliquot of the org phase (8 ml) was extd with pH 9.0 carbonate buffer (5 ml). The opt density of the aq ext was detd at 250 and 360 mμ in a Beckman Model DU spectrophotometer. The ratio of the absorbances, 250:360, is essentially const (1.1 ± 0.1), providing authentication of the material present in the sample. I was not extd from $(ClCH_2)_2$ by pH 9 buffer ($(ClCH_2)_2$–pH 9 buffer partition coefficient = 19.6) and did not interfere with the assay for II.

Urine samples were subjected to acid hydrolysis under the following condns: the urine sample (2 ml) was acidified with concd HCl (0.5 ml) and extd with $(ClCH_2)_2$ (10 ml) to remove unconjugated material. After centrifugation, aliquots of the urine layer (0.5 ml) were dild to 2.5 ml with H_2O (2 ml) and heated on a steam bath for 30 min. After cooling to room temp, the samples were assayed for I or II by adding the appropriate solvent as described above.

Samples of biological fluids from animals that had received labeled drug (III)[3] were assayed in duplicate by liquid scintillation counting techniques in a Nuclear-Chicago liquid scintillation spectrometer (Model 6860). Samples were corrected for counting efficiency by internal standardization. The scintillator soln was composed of 30% EtOH and 70% PhMe, contg 0.33% Omnifluor (New England Nuclear). Urine samples were assayed directly by dissolving 0.2 ml of the specimen in 15 ml of scintillator soln. Plasma samples (0.2 ml) were dissolved in Hyamine (1 ml) soln to which was added scintillator soln (15 ml). Fecal samples were homogenized in dil NaOH and freeze-dried. A portion (3–8 mg) was dissolved in 1 ml of Hyamine soln to which was added scintillator soln (15 ml).

Compds I, II, and IV, in aq media had different chromatographic mobilities (Table I). Drug-related fragments were isolated from urine by the scheme shown in Figure 1. Material isolated from urine was identified by comparison of the mass spectra with that of authentic samples,[1] using a Hitachi Perkin-Elmer Model RMU-6E mass spectrometer.

TABLE I

CHROMATOGRAPHIC BEHAVIOR OF 1,2-BENZOTHIAZINE-3-CARBOXANILIDES

	Paper chromatographic system			Detection	
Compd	1[a]	2[b]	3[c]	Uv light	Pauli's reagent[d]
I	0.84	0.91	Off paper	Fluorescence	Yellow
II	0.76	0.10	0.36	Absorbance	Rusty orange
IV	0.68	Origin	0.10	Absorbance	Bleach

[a] The mobile phase was BuOH–dioxane–2 *N* NH_4OH (5:1:4) (7 hr pass). [b] Papers, impregnated with Me_2CO–$HCONH_2$–AcOH (65:35:2) were run using PhH satd with $HCONH_2$ as the mobile phase (1 hr pass). [c] The same conditions as 2 except that the papers were run overnight (15 hr pass). [d] Spray with 1:1 soln of 5% $NaNO_2$ and 0.1% sulfanilic acid in 1.5% HCl; air dry and spray with 5% Na_2CO_3.

Results and Discussion

Striking differences exist between rat, dog, monkey, and man in the metabolism of I (Figure 2). Although all 4 species hydroxylate the 3-carboxanilide moiety, metabolites related to II account for about 70% of a single dose in man, about 50% in the rat and monkey, and about 10% in the dog. In the latter species, about 70% of administered drug is excreted in the urine as a H_2O sol conjugate of the 4′-OH function of the parent drug; a further 10% is excreted unchanged. Rat, monkey, and man all extensively metabolize I; in man, solely to II and its conjugate, and in rat and monkey to those metabolites and also to an unidentified polar metabolite. Studies with radio-labeled I (III) indicate that all drug fragments are cleared in the urine of the dog and monkey, but that the unknown polar metabolite is also excreted in the feces of the rat.

Differences in the pharmacokinetics of I and its major metabolite II in the 4 species studied reflect the metabolic dispositions described above. In the dog, the plasma half-life of I is twice as long as that of II (Table II). The average plasma concn of I, 24 hr after the 96th daily dose (10 mg/kg, oral) was 25 μg/ml; the average plasma concn of II was 11 μg/ml. Thus the difference in plasma half-life between I and II in the dog was reflected in the plateau plasma concns achieved during multiple dosing. During human toleration studies,[4] after 30 days of repeated administration (300 mg, daily) concns of I averaged 34 μg/ml and those of II averaged 40 μg/ml. These data were consistent with

(2) The research described in this report involved animals maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

(3) Prepared by using $[^{14}C]CH_3I$ and methods described in ref 1; specific activity, 3.8 μCi/mmole.

(4) Carried out in subjects under the care of Dr. J. R. Migliardi, of these laboratories.

TABLE II

PLASMA HALF-LIFE OF 1,2-BENZOTHIAZINE-3-CARBOXANILIDES IN VARIOUS SPECIES

Species	I, hr	II, hr
Rat	6[a]	7[a]
Dog	30[a]	14[a]
Monkey	4.5[b]	11[c]
Man	21[d]	37[d]

[a] Detd following iv administration (10 mg/kg). [b] Detd following iv administration (34 mg/kg). [c] Detd following single, iv dose of III and assuming that the plasma radioactivity after 40 hr was due solely to II. [d] Detd following withdrawal from oral, multiple dose regimen of I (300 mg daily).

the longer half-life in man of the metabolite II compared with that of the parent drug I. Although comparable chronic data were not sought in the monkey, it might be postulated from the plasma half-lives of I and II that the plateau concns of II would be higher than that of the administered drug I.

Almost without exception, drug metabolites have lower oil–H_2O partition coefficients than the parent drug. It is generally accepted that this increased hydrophilicity aids the process of excretion by the kidney (urine) and/or liver (bile).[5] It is therefore of interest that the metabolite II of the carboxanilide I, has a longer plasma half-life (37 hr) in man than its parent drug (21 hr). A similar phenomenon was noted[6] in a related series of antiinflammatory isoquinolinedione carboxanilides. In man, the parent drug (V) had a plasma half-life of 8.5 hr, whereas the major metabolite (VI) had a plasma half-life of 15 hr. Recalculation of the data presented by Burns, *et al.*,[7] indicates that, in man, the OH metabolite of Tromexan has a plasma half-life (4 hr) in excess of that of the parent drug (1 hr). Although metabolism does not increase the half-life, in man the OH metabolite of phenylbutazone has the same extended half-life as that of its parent (72 hr).[8] Thus hydroxylation of a Ph ring in certain acidic drugs appears to have the effect of rendering elimination more difficult.

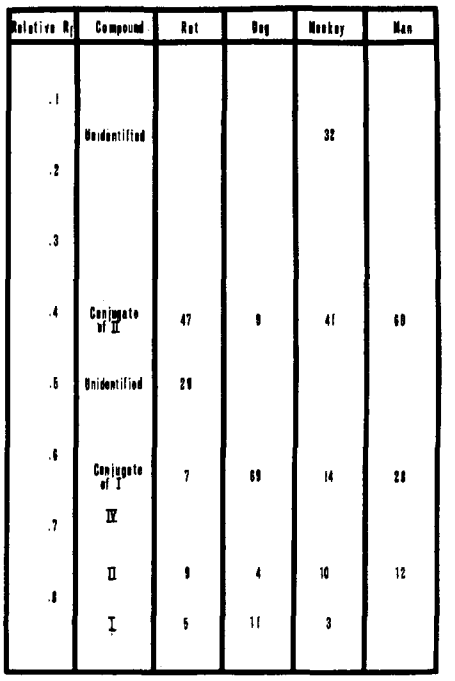
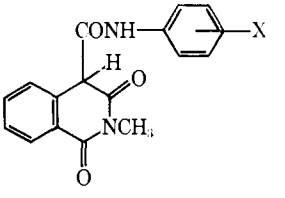


Figure 2.—Relative percentages of urinary metabolites from animals receiving 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxanilide 1,1-dioxide (I).



V, X = H
VI, X = OH

(5) R. T. Williams, "Detoxication Mechanisms," Wiley, New York, N. Y., 1959.

(6) E. H. Wiseman, E. J. Gralla, J. Chiaini, J. R. Migliardi, and Y.-H. Chang, *J. Pharm. Exp. Ther.*, **172**, 138 (1970).

(7) J. J. Burns, M. Weiner, G. Simson, and B. B. Brodie, *ibid.*, **108**, 33 (1953).

(8) J. J. Burns, R. K. Rose, S. Goodwin, J. Reichenthal, E. C. Horning, and B. B. Brodie, *ibid.*, **113**, 481 (1955).