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Species Differences in the Biotransformation of a New Antiarrhythmic Agent: Disopyramide Phosphate

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Abstract □ The biotransformation of disopyramide phosphate [4-diisopropylamino-2-phenyl-2-(2-pyridyl)butyramide phosphate] was studied in rats and dogs using the ^{14}C-labeled compound and in man using the unlabeled drug. Within 72 hr., 78.7 ± 1.4% of the administered radioactivity was recovered in the urine of dogs after oral administration and 44.1 ± 3.4% was recovered in the urine of rats after intraperitoneal administration. In dogs, 17.3 ± 3.4% of the urinary radioactivity was associated with the unchanged compound, 12.4 ± 1.6% with 4-isopropylamino-2-phenyl-2-(2-pyridyl)-butyramide, and 29.2 ± 2.6% with 3-phenyl-3-(2-pyridyl)-2-pyrrolidone; 18.0 ± 3.3% was present as a water-soluble conjugate which, on acid hydrolysis, gave the pyrrolidone as the major aglycone. In rats, the urinary radioactivity was predominantly (80.9 ± 2.3%) associated with the unchanged disopyramide. In this species the major metabolic pathway was aryl hydroxylation, giving two phenolic compounds, one of which was identified as 4-diisopropylamino-2-(*p*-hydroxyphenyl)-2-(2-pyridyl)butyramide. These phenolic metabolites were predominantly excreted in the bile as conjugates. In man, 56% of the administered drug was excreted unchanged in the urine while 4% was present as the secondary amine. The structural assignments of the metabolites were based on their detailed spectroscopic analysis and by comparison of their chromatographic properties with authentic samples.

Keyphrases □ Disopyramide phosphate biotransformation—species differences, rat, dog, man □ Biotransformation, disopyramide phosphate—species differences, rat, dog, man □ Urinary excretion—disopyramide phosphate biotransformation, rat, dog, man □ Pharmacokinetics, species differences—disopyramide phosphate biotransformation, rat, dog, man

Disopyramide phosphate[1] [4-diisopropylamino-2-phenyl-2-(2-pyridyl)butyramide phosphate, I, Scheme I] is an antiarrhythmic agent (1, 2) which has been shown to be useful in the clinic (3). Recent studies from these laboratories (4) showed marked species differences in the pharmacokinetic profiles of this drug in the rat, dog, and man. The purpose of the present investigation was to determine if the species differences also existed in its biotransformation. The presence of an *N,N*-diisopropyl group in disopyramide makes this compound of particular interest for metabolic studies. The isopropyl group can be expected to undergo two separate metabolic pathways: aliphatic hydroxylation to give a primary or tertiary alcohol, or *N*-dealkylation to give a primary or secondary amine (5). In this article, studies on the major route of elimination and the biotransformation of ^{14}C-disopyramide phosphate in rat and dog are presented. The urinary excretion of the unchanged compound and a metabolite from man given the unlabeled compound is also described.

MATERIALS AND METHODS

^{14}C-Labeled Disopyramide Phosphate[2]—The ^{14}C-labeled drug was prepared from phenyl(2-pyridyl)acetonitrile-1-^{14}C (50.26 μc./mg.)[3] by the method of Cusic and Sause (6). ^{14}C-Disopyramide phosphate had a specific activity of 19.5 μc./mg., and its radiochemical purity (determined by TLC) was 99.2%.

Measurement of Radioactivity—All samples were counted in a liquid scintillation spectrometer[4]. For counting aqueous solutions (urine and bile) and extracts containing polar compounds, Bray's scintillation solution (7) was used; for counting samples scraped from thin-layer chromatograms, the scintillation solution of Snyder and Smith (8) was used. Chemical quenching was corrected by the

[1] Disopyramide phosphate is also known as Norpace, SC-7031 phosphate, and SC-13957. USAN chemical name is α-[2-(diisopropylamino)-ethyl]-α-phenyl-2-pyridineacetamide.

[2] ^{14}C-Disopyramide phosphate was prepared by Mr. D. J. Zitzewitz.

[3] Obtained from Amersham/Searle Corp., Arlington Heights, IL 60005

[4] Mark I, Nuclear-Chicago Corp., Des Plaines, IL 60018

channels-ratio method, and color quenching was corrected by the internal standard method using ^{14}C-toluene.

In Vivo **Elimination Studies**—*Dogs*—Three adult female beagle dogs (8–10 kg.) were each given the drug orally (100 mg./kg. + 10 μc. of labeled compound) in gelatin capsules. Animals were housed in metabolism cages and allowed access to food and water. Separate collections of urine and feces were made for 72 hr. and stored at −20° prior to workup.

Rats—Five female rats (Charles River strain, 250–300 g.) were each given the drug intraperitoneally (100 mg./kg. + 5 μc. of labeled compound dissolved in saline). Separate collections of urine and feces were made as with dogs. To study biliary excretion of the parent drug and its metabolites, five female rats (Charles River strain, 200–250 g.) were anesthetized with sodium pentobarbital (30 mg./kg. i.p.). The common bile duct was exposed and catheterized with polyethylene tubing, and the incision was sutured. Each animal was then placed in a restraining cage for 48 hr. after intraperitoneal administration of ^{14}C-disopyramide phosphate (100 mg./kg. + 10 μc. of labeled compound).

Human Study—For quantitation and identification of urinary excretion products, the 0–24-hr. pooled urine collection of a normal human subject given 400 mg. of the unlabeled drug orally was analyzed.

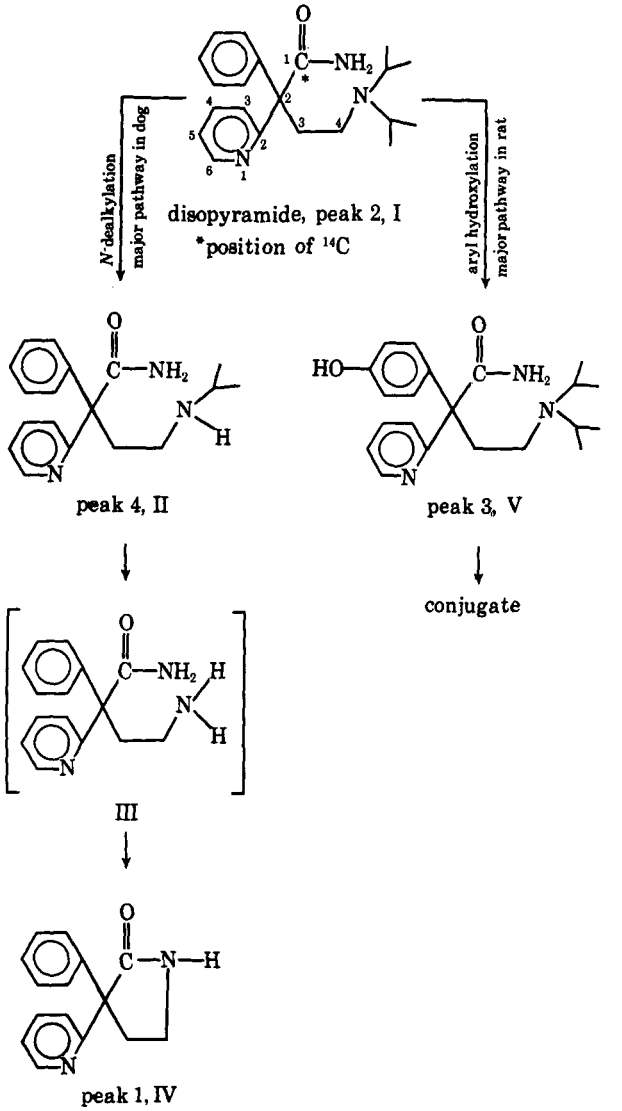
TLC—Analytical TLC was run on 5 × 20-cm. glass plates coated with 250 μ silica gel[5] HF_{254}. Thin-layer plates were activated for 1 hr. at 120° before use and developed in a solvent system of ethyl acetate–methanol–concentrated ammonium hydroxide (78:20:2). Compounds were visualized under long- or shortwave UV light or by spraying with Dragendorff or iodoplatinic acid reagents (9). Preparatory TLC (PTLC) was run on 20 × 20-cm. glass plates coated with 1 mm. of silica gel HF_{254}.

For detecting radioactive compounds on a thin-layer plate, 3-mm. bands of silica gel were scraped from the plate using an automatic zonal scraper[6], and the radioactivity in each zone was determined by liquid scintillation spectrometry. The radioactive distribution pattern of the chromatogram was then plotted by a computer-plotter[7] programmed to give the total radioactivity eluted from the plate, the number of radioactive peaks, their R_f values, and the percentage of radioactivity associated with each peak.

GLC[8]—GLC studies were made with a 1.23-m. (4-ft.) long, 4-mm. i.d., silanized spiral class column packed with 3% SE-30 ultraphase on 80–100-mesh Chromosorb W-H.P.[9]. The following operating conditions were used: column temperature, 200°; detector and injection port temperatures, 260°; and flow rate of carrier gas (helium), 65 ml./min.

Isolation of Unchanged Disopyramide and Metabolites from Urine—The isolation procedure is outlined in Scheme II; the recoveries at every stage were checked by measurement of the radioactivity. An aliquot of a pooled urine sample (200 ml.) was made alkaline (pH 10) with concentrated ammonium hydroxide and then passed through an Amberlite[10] XAD-2 column (100 g.) packed in water. The column was first eluted with water (500 ml.) followed by methanol (500 ml.). The methanol eluate B was evaporated to dryness *in vacuo*, and the residue was partitioned between chloroform (extract C) and water at pH 10. TLC analysis of extract C, in the case of dog urine, indicated the presence of four radioactive peaks with R_f values of 0.63 (peak 1), 0.44 (peak 2), 0.16 (peak 4), and 0.06 (peak 5). These compounds absorbed shortwave UV light and gave orange spots with Dragendorff reagent. Peak 1 was obtained as a crystalline compound (m.p. 172–174°), whereas peaks 2 and 4 were obtained as chromatographically homogeneous materials by PTLC of extract C. The water-soluble extract D was subjected to acidic hydrolysis by the procedure described here, and the aglycones released were isolated by PTLC.

Separation of Metabolite Peak 3 from Rat Bile—The pooled 0–48-hr. bile sample was diluted to 100 ml. with water and extracted with chloroform at pH 10. The chloroform extract contained only 15% of the biliary radioactivity. The water-soluble extract, which contained the bulk of radioactivity, was evaporated to dryness *in vacuo*, and the residue was subjected to enzyme hydrolysis. The major aglycone (peak 3) released was extracted with chloroform and separated by PTLC.



Scheme I—Proposed pathway for biotransformation of disopyramide in rat and dog. The primary amine III is postulated to be a precursor for the isolated pyrrolidone IV.

Analysis of Radioactivity in Feces of Rat and Dog—The pooled fecal samples (0–48 hr.) were homogenized with water and the suspension was freeze dried. An aliquot of the dried sample (10 g.) was then exhaustively extracted with methanol in a soxhlet extractor, and the recovery of the label in the methanol extract (100 ml.) was determined by counting 100 μl. of this solution. To investigate the chemical nature of the radioactivity, the methanol extract was subjected to thin-layer radiochromatographic analysis.

Hydrolysis of Water-Soluble Conjugated Fractions—For enzyme hydrolysis, aliquots of the conjugated extract (1 × 10^6 d.p.m.) in acetate buffer (25 ml.) at pH 5.1 were heated on a steam bath for 20 min. and then incubated at 37° for 48 hr. with either 1 ml. of β-glucuronidase[11] or 0.1 ml. of a β-glucuronidase–sulfatase mixture[12]. In a control experiment, this procedure was repeated without the addition of the enzyme. Acidic hydrolysis was done by refluxing an aliquot of the conjugated extract (4 × 10^6 d.p.m.) in 2 *N* hydro-

[5] E. Merck, Darmstadt, Germany.
[6] Analab Inc., North Haven, Conn.
[7] Cal Comp, California Computer Products, Inc., Anaheim, Calif.
[8] Hewlett-Packard model 5750, with a flame-ionization detector system.
[9] Johns-Manville, Trenton, N. J.
[10] Rohm & Haas Co., Philadelphia, Pa.

[11] β-Glucuronidase (Ketodase, Warner-Chilcott, Morris Plains, N.J.) was derived from beef liver and contained 500 Fishman units/ml.
[12] β-Glucuronidase–sulfatase mixture (Calbiochem, Los Angeles, Calif.) was derived from *Helix pomatia* and contained 111,000 Fishman units/ml. β-glucuronidase and 58,000 Fishman units/ml. aryl sulfatase.

pooled urine (100% of ^{14}C)

Amberlite XAD-2 resin column chromatography

aqueous eluate, A

methanol eluate, B
rat: 95.3 ± 2.6%
dog: 97.8 ± 2.4%

solvent partition at pH 10

chloroform layer, C
rat: 94.8 ± 3.1%
dog: 68.5 ± 4.2%

aqueous layer, D
rat: 0.5 ± 0.1%
dog: 18.0 ± 3.3%

TLC

	rat	dog
Peak 1	7.2 ± 1.2%	29.2 ± 2.6%
Peak 2	80.9 ± 2.3%	17.3 ± 3.4%
Peak 3	4.1 ± 0.5%	-
Peak 4	6.3 ± 0.7%	12.4 ± 1.6%
Peak 5	-	6.9 ± 1.9%

Scheme II—Separation of unchanged disopyramide and its metabolites from pooled urine (0–72 hr.) of rat and dog

chloric acid (20 ml.) for 1 hr. The released aglycones obtained by the hydrolytic procedures were extracted with chloroform at pH 9 and analyzed by TLC.

Derivative Formation—Methylation of the phenolic metabolite (peak 3) was carried out in methanol containing an excess of an ethereal diazomethane. The reaction flask was stoppered and allowed to stand at room temperature for 12 hr. The reaction solution was evaporated to dryness *in vacuo*, and the product was subjected to TLC and GLC analyses using the conditions described.

Spectroscopic Analysis—IR spectra were measured in chloroform using an ultramicro cell[13]. UV absorption spectra were determined in methanol, on a ratio recording spectrophotometer[14]. Mass spectra were obtained by the direct-insertion probe technique at 70 ev. and 200° on a mass spectrometer[15]. NMR spectra were determined on a spectrometer[16] in deuterochloroform ($CDCl_3$) solution.

Synthesis of Reference Compounds—*4-Isopropylamino-2-phenyl-2-(2-pyridyl)butyramide*[17] *(II)*—*m*-Chloroperbenzoic acid (≃85%, 4.1 g.) was added portionwise during 8 min. to a stirred solution of disopyramide (I, 6.8 g.) in methylene chloride (150 ml.) at 4–6°. After standing at room temperature for 35 min., the reaction mixture was washed with a solution containing sodium sulfate (6.0 g.) and concentrated ammonium hydroxide (10 ml.) in water (60 ml.). The aqueous washing was extracted with methylene chloride, and the combined methylene chloride extract was dried (sodium sulfate) and evaporated to dryness. The residue was dissolved in benzene (50 ml.) and boiled on a steam bath for 7 min., and the solution was then evaporated to dryness. The residual oil was crystallized from ether to yield microcrystals of 4-(*N*-isopropyl-*N*-hydroxylamino)-2-phenyl-2-(2-pyridyl)butyramide (VI, 3.4 g.), m.p. 132–133°; IR $\nu_{max.}$: 3590, 3450, 1675, 1590, and 1570 $cm.^{-1}$; NMR τ: 1.45 (1H, bd. d, 6-pyridyl *H*), 2.2–2.9 (8H, m, aryl *H*), and 9.05 (6H, d, J = 6.5 Hz., isopropyl CH_3).

Anal.—Calc. for $C_{18}H_{23}N_3O_2$: C, 68.98; H, 7.40; N, 13.41. Found: C, 68.89; H, 7.45; N, 13.58.

Concentrated hydrochloric acid (34 ml.) was added slowly to the stirred solution of the hydroxylamine VI (9.0 g.) in a water–ethanol mixture (1:20, 200 ml.). Zinc dust (10 g.) was then added portionwise with stirring during an 18-min. period. After stirring the reaction mixture for 15 min. at room temperature, granular potassium

[13] Beckman IR-12.
[14] Beckman DK-2A.
[15] Varian M-66.
[16] Varian Associates HA-100 MHz.
[17] II was synthesized by Mr. C. H. Yen, Department of Chemical Research, G. D. Searle & Co.

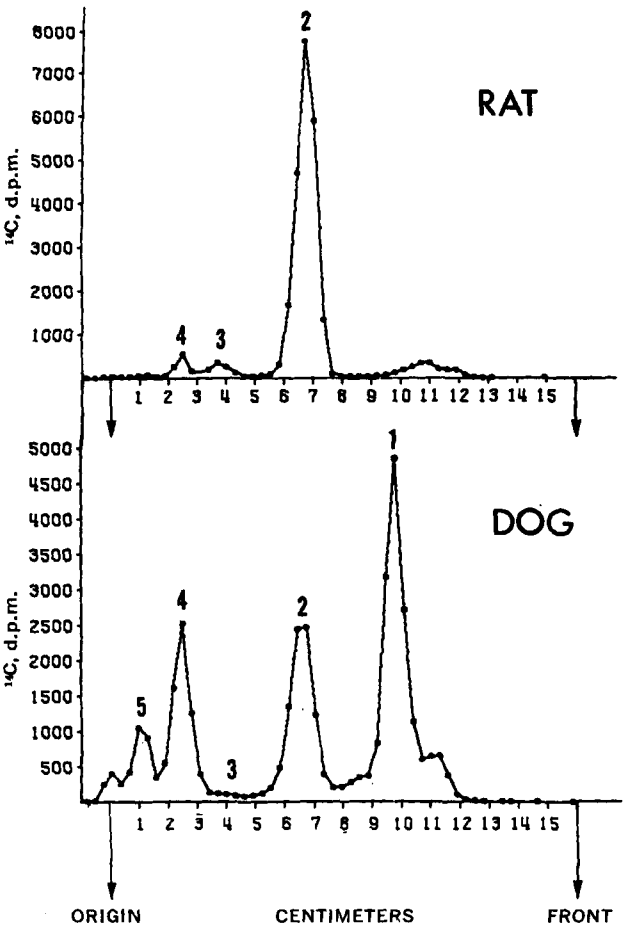


Figure 1—*Thin-layer radiochromatograms of urinary radioactivity (extract C of Scheme II) after intraperitoneal and oral administration of ^{14}C-disopyramide phosphate to the rat and dog, respectively. Peak 2 is due to unchanged disopyramide. For identities of metabolite peaks, see Scheme I.*

carbonate (15.2 g.) was added and stirring was continued for 30 min. The supernate containing a fine suspension was decanted, and the residue was washed several times with ethanol. The combined alcoholic solution was neutralized with 4% potassium carbonate solution, evaporated to 60 ml., made basic with aqueous sodium hydroxide solution, and then extracted with methylene chloride. Evaporation of the dried (sodium sulfate) methylene chloride solution yielded an oil which was crystallized from an ether–*n*-pentane mixture (1:1), giving II (6.6 g.), m.p. 92–94°. See Table I for spectral data.

Anal.—Calc. for $C_{18}H_{23}N_3O$: C, 72.69; H, 7.80; N, 14.13. Found: C, 72.74; H, 7.90; N, 14.12.

4-Diisopropylamino-2-(*p*-methoxyphenyl)-2-(2-pyridyl)butyramide (X)—To the stirred solution of *p*-methoxyphenylacetonitrile[18] (VII, 14.7 g.) and 2-bromopyridine (15.8 g.) in dry toluene (60 ml.) was added sodamide (18.0 g.) over 45 min. The reaction mixture was stirred at 100° for 2 hr. and cooled to room temperature, and then crushed ice was added portionwise with stirring until the reaction subsided. The toluene layer was washed with water and then extracted with 6 *N* hydrochloric acid (3 × 100 ml.). The acidic layer was then made basic with 10% sodium hydroxide solution and extracted with toluene (2 × 100 ml.), which was dried (sodium sulfate) and evaporated to dryness. The residue was crystallized from a ether–hexane (1:1) mixture to yield 2-(*p*-methoxyphenyl)-2-(2-pyridyl)acetonitrile (VIII, 11.0 g.), m.p. 89–90°.

Anal.—Calc. for $C_{14}H_{12}N_2O$: C, 74.99; H, 5.38; N, 12.49. Found: C, 74.77; H, 5.64; N, 12.77.

A stirred solution of VIII (11.0 g.) and diisopropylaminoethyl chloride hydrochloride (12.1 g.) in freshly distilled methyl ethyl

[18] Eastman Kodak Co., Rochester, N. Y.

Table I—Spectral Properties of Unchanged Disopyramide and Its Metabolites Isolated from Urine

	IR, $\nu_{max.}^{CHCl_3}$ cm.$^{-1}$	Mass Spectra (Prominent Peaks), *m/e*	NMR[a] (τ Values) —CON*H*—	H_3C—C	Aryl-*H*	6-Pyridyl-*H*
Disopyramide (Peak 2)	3460, 1685, 1590, 1570, 1500, 1475, 1450, 1432	239, 212, 195, 194, 182, 180, 167	4.1 brs	9.10 (12H) d, J = 6.5	2.2–3.1 (8H) m	1.45 bd.d
Peak 4	3470, 1685, 1592, 1575, 1500, 1475, 1450, 1435	280, 239, 212, 195, 194, 182, 180, 167	4.0 brs	8.90 (6H) d, J = 6.5	2.2–3.1 (8H) m	1.43 brs
Peak 1	3440, 1710, 1592, 1575, 1498, 1475, 1455, 1438	238, 212, 195, 194, 182, 180, 167	3.6 brs		2.2–3.1 (8H) m	1.42 brs

[a] Chemical shifts were recorded in τ units (p.p.m.) relative to tetramethylsilane as the internal standard. Multiplicity of signals is designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; brs, broad signal; and bd.d, broad doublet. Coupling constants J are given in hertz.

ketone (250 ml.) was heated to reflux under nitrogen. To this, 85% granular potassium hydroxide (16.9 g.) was added gradually over 1.5 hr. The reaction mixture was cooled to room temperature and then water (300 ml.) and redistilled ether (300 ml.) were added. The ethereal layer was extracted with 6 *N* hydrochloric acid (3 × 100 ml.), and the combined aqueous extracts were made basic with 45% potassium hydroxide solution and reextracted with ether (3 × 100 ml.). Evaporation of the dried (sodium sulfate) ether extract gave 4-diisopropylamino-2-(*p*-methoxyphenyl)-2-(2-pyridyl)butyronitrile (IX, 18.8 g.) as an oil, IR $\nu_{max.}$: 2250, 1650, and 1590 cm.$^{-1}$; NMR τ: 1.40 (1H, bd. d, 6-pyridyl *H*), 2.57 and 3.15 (2H, d, J = 9 Hz., *m*- and *o*-phenyl *H*), 2.2–2.9 (3H, m, 3-, 4-, and 5-pyridyl *H*), 6.25 (3H, s, —OCH_3), and 9.06 (12H, d, J = 6.5 Hz., 2 isopropyl CH_3).

To a solution of IX (1.38 g.) in ethanol (10 ml.) were added potassium hydroxide pellets (3.2 g.) and water (0.4 ml.). The mixture was refluxed for 22 hr., after which it was diluted with water (50 ml.) and extracted with chloroform (3 × 25 ml.). The chloroform extract was reextracted with 2 *N* hydrochloric acid (3 × 25 ml.); then the combined acidic layers were made basic and reextracted with chloroform. Evaporation of the dried (sodium sulfate) chloroform extract gave X (700 mg.) as a colorless gum. This compound resisted crystallization; however, it was found to be homogeneous on TLC and GLC analyses (Table II); IR $\nu_{max.}$: 3460, 1680, 1610, and 1772 cm.$^{-1}$; NMR τ: 1.40 (1H, bd. d, 6-pyridyl *H*), 2.57 and 3.15 (2H, d, J = 9 Hz., *m*- and *o*-phenyl *H*), 6.25 (3H, s, —OCH_3), and 9.36 (12H, d, J = 6.5 Hz., 2 isopropyl CH_3).

RESULTS

Excretion of Label in Urine of Rat and Dog—The excretion of the total label measured at various times after intraperitoneal administration of the labeled drug to rats and after oral administration to dogs is shown in Table III. Within 72 hr., 78.7 ± 1.4% of the administered label was excreted in the urine of dogs, 75.2 ± 1.1% of the ^{14}C being excreted in the first 24 hr. The urinary excretion of ^{14}C in rats during a 72-hr. period was 44.1 ± 3.4%, with 42.7 ± 3.4% of the label being excreted in the first 24 hr. The radioactivity in the pooled urine sample was fractionated according to the method outlined in Scheme II. Amberlite XAD-2 column chromatography of the urine removed large amounts of nonradioactive materials in the aqueous eluate. The methanol eluate B contained 95–98% of the radioactivity applied to the column. This eluate was evaporated to dryness *in vacuo*, and the residue was extracted with chloroform at pH 10 to give the chloroform-soluble extract C and the water-soluble extract D. In the rat, 94.8 ± 3.1% of the urinary ^{14}C was recovered in the chloroform extract C; in the dog, this extract contained 68.5 ± 4.2% of the urinary label. The thin-layer radiochromatograms of extract C from rat and dog urine are shown in Fig. 1, and the percentages of the urinary label associated with individual compounds are listed in Scheme II. In the rat the major excretory product was unchanged disopyramide (peak 2), which accounted for 80.9 ± 2.3% of the label in the urine. Three minor metabolites, peaks 1, 3, and 4, accounted for 7.2 ± 1.2, 4.1 ± 0.5, and 6.3 ± 0.7%, respectively, of the urinary ^{14}C. In the dog, the unchanged compound accounted for only 17.3 ± 3.4% of the urinary label. In this species, the major portion of the ^{14}C was associated with three metabolites, peaks 1, 4, and 5, which accounted for 29.2 ± 2.6, 12.4 ± 1.6, and 6.9 ± 1.9%, respectively, of the urinary radioactivity. To check the recovery of unchanged disopyramide from urine in isolation Scheme II, a known amount of the label was added to the control

Table II—TLC and GLC Characteristics[a] of Disopyramide, Its Metabolites, and Related Compounds

Compound	TLC, R_f	GLC, Retention Time, min.
4-Diisopropylamino-2-phenyl-2-(2-pyridyl)butyramide, I (Peak 2, disopyramide)	0.44	13.8
3-Phenyl-3-(2-pyridyl)-2-pyrrolidone, IV (Peak 1)	0.63	4.7
4-Isopropylamino-2-phenyl-2-(2-pyridyl)butyramide, II (Peak 4)	0.16	27.7[b]
4-Diisopropylamino-2-(*p*-methoxyphenyl)-2-(2-pyridyl)butyramide, X (Peak 3, methyl ether)	0.44	30.6

[a] See under *Materials and Methods* for experimental details. [b] Retention time of acetylated derivative.

Table III—Time Course Recovery of Radioactivity in Urine and Bile after ^{14}C-Disopyramide Phosphate Administration to the Rat and Dog

	Percent Administered ^{14}C 0–6 hr.	6–24 hr.	24–48 hr.	48–72 hr.	Total
Rat urine after intraperitoneal dose					
1	32.5	13.2	1.2	0.3	47.2
2	44.1	9.1	0.8	0.2	54.2
3	29.4	8.5	1.3	0.4	39.6
4	36.4	6.9	1.3	0.6	45.2
5	28.9	4.3	0.8	0.5	34.5
Mean	34.3	8.4	1.1	0.4	44.1
± *SEM*	2.80	1.46	0.12	0.07	3.35
Rat bile after intraperitoneal dose					
6	26.0	3.7	0.1		29.8
7	17.2	3.2	0.2		20.6
8	33.9	3.0	0.1		37.0
9	34.8	1.6	0.2		36.6
10	23.4	6.3	0.2		29.9
Mean	27.1	3.6	0.2		30.8
± *SEM*	3.30	0.77	0.02		2.98
Dog urine after oral dose					
1	13.9	63.4	3.5	0.7	81.5
2	48.7	24.9	2.8	0.3	76.7
3	32.7	42.0	2.9	0.3	77.9
Mean	31.8	43.4	3.1	0.4	78.7
± *SEM*	10.06	11.14	0.22	0.13	1.4

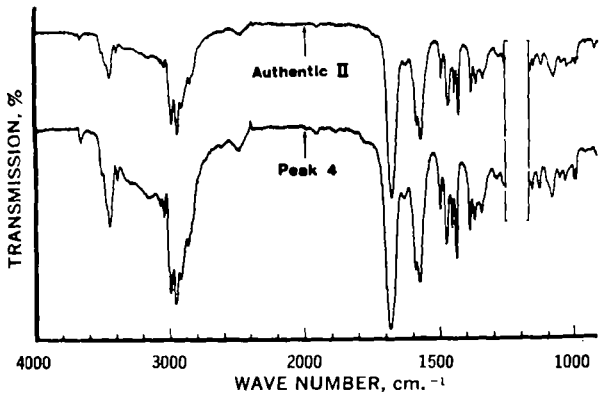


Figure 2—*IR spectral ($CHCl_3$) comparison of metabolite peak 4 isolated from human urine with an authentic sample of II. The artifact peaks in the chloroform opaque region (1180–1260 cm.$^{-1}$) were deleted.*

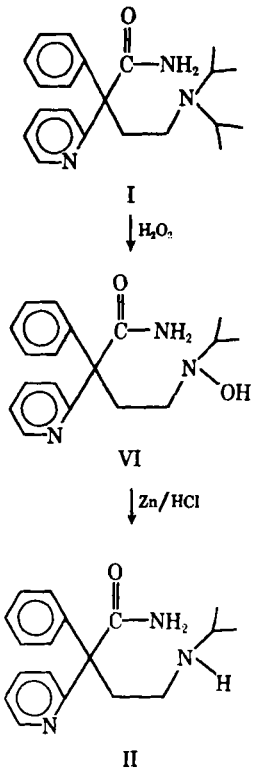
urine sample and this scheme was repeated; 98–100% of the added disopyramide was recovered in the chloroform extract C.

In rat urine, only 0.5 ± 0.1% of the urinary label was present in the water-soluble extract D. However, in the dog the corresponding figure for this extract was much higher (18.0 ± 3.3%). To investigate the nature of this water-soluble material, extract D from dog urine was subjected to acidic hydrolysis. The major aglycone released (75.3 ± 3.8%) had an R_f value identical to peak 1, the major compound in the free fraction of urine.

Analysis of Radioactivity in Feces of Rat and Dog—The mean recovery of the label in the 0–48-hr. fecal collection was 20% (oral dose) in the dog and 40% (intraperitoneal administration) in the rat. To investigate the nature of the fecal radioactivity, the methanol extract of the freeze-dried fecal sample was subjected to thin-layer radiochromatographic analysis. The unchanged drug accounted for 17.8% of the fecal radioactivity in the rat and 47.5% in the dog. The major portion (47.5%) of the radioactivity in the feces of the rat was associated with metabolite peak 3. This metabolite in dog feces accounted for only 5.5% of the fecal radioactivity. In this species, the major fecal radioactivity was associated with peak 1, which was also the major metabolite in the urine.

Biliary Excretion of Label in the Rat—Since the recovery of the label in the urine of the rat was not as high as that in the dog, the importance of biliary excretion of disopyramide and its metabolites in this species was investigated in bile duct-cannulated animals. The excretion of the label in the bile of these animals after intraperitoneal administration of ^{14}C-disopyramide phosphate is illustrated in Table III. Within 6 hr., 27.1 ± 3.3% of the administered ^{14}C was excreted in the bile. Extraction of the pooled bile sample (0–48 hr.) with chloroform at pH 10 gave only 15 ± 1.1% of the radioactivity in the organic phase. The major excretory products in the bile were water-soluble conjugates. To investigate the nature of these conjugates, aliquots of the extract were subjected to either acid or enzyme hydrolysis. On acidic hydrolysis, 40–50% of the radioactivity was released as a free fraction. Hydrolysis with β-glucuronidase alone released 13.4 ± 1.8%; while with a mixture of β-glucuronidase plus aryl sulfatase, 82.9 ± 3.0% was released. In a control experiment, in which no enzyme was added, there was a release of 5.2 ± 0.4%. These hydrolytic experiments suggested the presence of a sulfate conjugate. TLC analysis of the free fraction released by these procedures gave peak 3 as the major aglycone in all cases.

Urinary Metabolic Composition in Man—For isolation of unchanged disopyramide and its metabolite from human urine, the procedure used for the urine of the rat and dog (Scheme II) was employed. The 24-hr. urine sample of the human subject, who had taken 400 mg. of disopyramide phosphate orally, was passed through an Amberlite XAD-2 resin column. The column was first eluted with water followed by methanol. The methanol eluate was evaporated to dryness *in vacuo*, and the residue was fractionated between chloroform (extract C) and water (extract D) at pH 10. TLC analysis of the chloroform extract C revealed the presence of unchanged disopyramide (R_f 0.44) and a polar metabolite with an R_f value (0.16) identical to peak 4.



Scheme III—Synthesis of reference monoalkylated metabolite (peak 4) of disopyramide

An estimation of the concentration of each compound was obtained as follows. Five milligrams of extract C was chromatographed on a PTLC plate; the UV absorbing bands corresponding to the unchanged drug and metabolite peak 4 were scraped from the plate, powdered, and shaken with 10 ml. of methanol for 30 min. The extinction of the solution was measured at 262 nm. For the background value, an equal amount of silica gel was scraped from a blank area of the plate and its UV absorption was determined. The concentrations of disopyramide and metabolite (peak 4) were determined from their respective standard curves. Unchanged disopyramide in a 0–24-hr. urine sample accounted for 56% of the administered dose, while the metabolite peak 4 was equivalent to 4% of the administered drug. After acid hydrolysis of the water-soluble extract D, no Dragendorff positive aglycones related to disopyramide were detected.

Identification of Unchanged Disopyramide and Its Metabolites—The identity of peak 2 as unchanged disopyramide in the urine of the rat, dog, and man was established by comparing the IR spectra as well as TLC and GLC properties (Table II) of the isolated material with an authentic sample.

Peak 4—This metabolite was present in the urine of the rat, dog, and man. Its IR and NMR spectra and chromatographic (TLC and GLC) characteristics were identical to those of an authentic sample of II. A typical IR spectral comparison of this metabolite isolated from human urine with that of an authentic sample is illustrated in Fig. 2. A synthetic procedure for preparing this metabolite is outlined in Scheme III, and Table I lists its spectral characteristics. The IR spectrum of II showed close similarities to disopyramide. However, in the NMR spectrum, disopyramide gave signals due to two *N*-isopropyl groups at 9.10τ (12H, d, J = 6.5 Hz.); in the spectrum of peak 4, signals due to only one such group at 8.90τ (6H, d, J = 6.5 Hz.) were apparent. The mass spectra of the two compounds showed close similarities. Neither compound gave the molecular ion peak, but both possessed common high intensity peaks at *m/e* 167, 195, and 212. The high intensity peak at *m/e* 239 in the spectrum of disopyramide was of weak intensity in the monoalkylated compound, but there was a high intensity peak at *m/e* 280.

Peak 1—This compound was isolated as a crystalline material (m.p. 172–174°) from the urine of the dog. Its IR spectrum is shown in Fig. 3. The characteristic change in the IR spectrum of peak 1 was

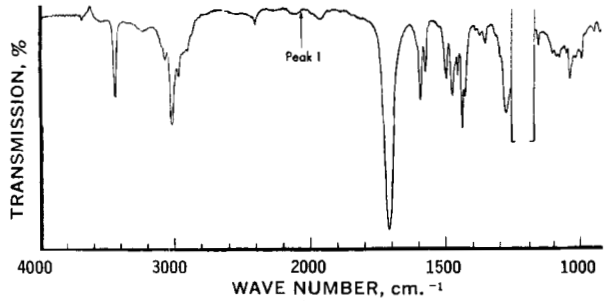


Figure 3—*IR spectrum ($CHCl_3$) of metabolite peak 1 isolated from dog urine. The artifact peaks in the chloroform opaque region (1180–1260 cm.$^{-1}$) were deleted.*

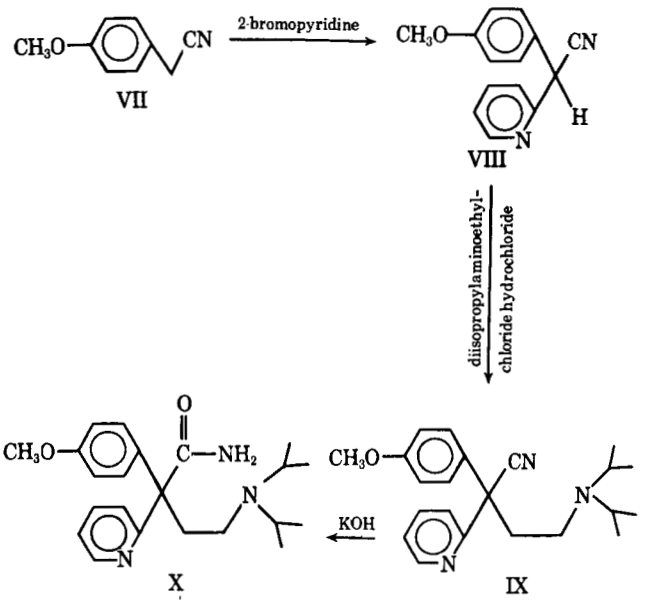
the strong carbonyl absorption band at 1710 cm.$^{-1}$. This shift in the carbonyl absorption band from 1685 cm.$^{-1}$, as found in the spectra of disopyramide and of peak 4 (Fig. 2), was considered to be due to the conversion of the primary amide group to a pyrrolidone. The sharp absorption band at 3440 cm.$^{-1}$ in the spectrum of peak 1 was assigned to the >N—H group. The UV spectrum of peak 1 was similar to that of both disopyramide and peak 4; and in the NMR spectrum, no signals due to the isopropyl group were seen. In the mass spectrum, unlike disopyramide and peak 4 (Table I), this compound gave a strong molecular ion peak at *m/e* 238 ($C_{15}H_{14}N_2O$). These spectral data were consistent with Structure IV [3-phenyl-3-(2-pyridyl)-2-pyrrolidone (11)] shown in Scheme I.

Peak 5—This polar metabolite, found in dog urine, gave a deep purple color with ninhydrin reagent, suggesting the presence of a primary amine. Peak 5 was, however, very unstable, and attempted isolation by PTLC resulted in conversion to the pyrrolidone (IV). Based on these observations, the structure 4-amino-2-phenyl-2-(2-pyridyl)butyramide (III) was proposed for this metabolite.

Peak 3—This peak was the major aglycone obtained after enzyme hydrolysis of the conjugated fraction in the bile of rat. The UV spectrum of this material showed a bathochromic shift (245 nm. → 272 nm.) on addition of alkali; on reacidification of the alkaline solution, the original spectrum was obtained. On methylation with diazomethane, peak 3 gave less polar methylated products which, upon GLC analysis, showed a 60:40 mixture of two compounds with retention times of 30.6 and 43.7 min., respectively. The retention time and the TLC R_f value of the less polar product were identical to those of the authentic sample of X, prepared by the synthetic procedure shown in Scheme IV.

DISCUSSION

The results of the present investigation show that the main pathway of disopyramide metabolism involves *N*-dealkylation of the isopropyl groups and aryl hydroxylation. A marked species difference in the biotransformation of this drug was observed. In the dog, the major portion of the label after an oral dose of ^{14}C-disopyramide phosphate was recovered in the urine, and the biotransformation products were predominantly the *N*-dealkylated compounds: the pyrrolidone IV and the secondary amine II. The pyrrolidone IV was also obtained as the major aglycone after acid hydrolysis of the water-soluble conjugates in the urine. It was also the major metabolite in the feces. These *N*-dealkylated compounds together accounted for 50% of the administered label in the dog. In the rat, a much smaller portion of the administered label was excreted in the urine, but the urinary radioactivity was predominantly due to the unchanged compound. In this species, the major metabolic pathway was aryl hydroxylation to furnish two phenolic metabolites. One was identified as 4-diisopropylamino-2-(*p*-hydroxyphenyl)-2-(2-pyridyl)butyramide (V) by comparing the TLC and GLC characteristics of the methyl ether derivative (X) of this compound with the methylated derivative of peak 3. In the bile of the rat, the two phenolic compounds were present predominantly as sulfate conjugates, accounting for 30% of the administered ^{14}C. In man, disopyramide was excreted predominantly unchanged in the urine. Only the secondary amine II was identified as a minor metabolite in the urine. This amine was present in the urine of all three species studied. Species differences in drug metabolism are important considerations in evaluating toxicity and biological activity of drugs. Hucker (12) reviewed a wide variety of drugs that exhibit species differences in their metabolism; disopyramide constitutes another example of such drugs.



Scheme IV—Synthesis of the reference methyl ether derivative of hydroxylated metabolite (peak 3) of disopyramide

The logical precursor of the pyrrolidone IV, the major urinary metabolite of the dog, is the primary amine III (Scheme I). This compound can be derived by metabolic *N*-dealkylation of the two isopropyl groups of disopyramide. Such an amine would be expected to be unstable and readily cyclized to IV. The presence of III was suggested in peak 5, a minor metabolite present in dog urine. This polar material was very unstable, and any chromatographic attempts to isolate this compound resulted in its cyclization to IV. During the chromatographic separation, the secondary amine II did not cyclize to the corresponding *N*-isopropylpyrrolidone. This stability is presumably due to the steric hindrance afforded by the bulky isopropyl group.

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