acid was dissolved in Et_2O (80 ml); 7-ACA *tert*-butyl ester (3.3 g, 0.01 mol) was added, followed by DCCI (2.1 g, 0.01 mol). The mixture was stirred at room temperature for 5 hr. The precipitated DCU was removed and the filtrate was evaporated *in vacuo*. The semisolid residue was dissolved in Et_2O and precipitated with petroleum ether; the resulting solid was dried *in vacuo* to give the 7-mandelamidocephalosporanic acid protected as the *tert*-butyl and dichloracetyl diester (75-95% crude yield). The diester (0.005 mol) was dissolved in cold TFA (25 ml) and the solution was stirred at room temperature for 45 min. The TFA evaporated *in vacuo* and the residue was treated with 5% aqueous Na_2CO_3 (pH 9.5) for 1 hr and worked up as described under procedure A.

Procedure D. Preparation of 7-(Aminomandeloyl)cephalosporanic Acids (Compounds 26-28). The appropriate 7-(dichloroacetylnitromandeloyl)cephalosporanic acid *tert*-butyl ester (0.01 mol) prepared by procedure C was dissolved in MeOH (300 ml) containing 10 g of prereduced 5% Pd/C and hydrogenated on a Parr apparatus at 3.5 kg/cm^2 for 3 hr. The mixture was filtered and evaporated *in vacuo* and the residue was triturated with Et_2O. The resulting solid was dissolved in 20 ml of TFA for 30 min at room temperature. The excess TFA was evaporated and the residual TFA salt of the product was triturated with Et_2O. It was converted to the sodium salt with NaOMe as described in procedure A.

7-[(*R*)-(−)-Mandelamido]-3-(*N*-pyridiniummethyl)-3-cephem-4-carboxylate (52). This compound was prepared by displacement of the acetoxy group of 1 with pyridine using the method described by Arkley, *et al.*[10] *Anal.* ($C_{21}H_{19}N_3O_5S\cdot 2.5H_2O$) C, H, N.

Sodium 7-[(*R*)-(−)-Mandelamido]-3-azidomethyl-3-cephem-4-carboxylate (53). This compound was prepared in 35% yield by procedure A substituting 7-amino-3-azidomethyl-3-cephem-4-carboxylic acid[4] for 7-ACA. *Anal.* ($C_{16}H_{14}N_5NaO_5S\cdot 1.25H_2O$) C, H; N: calcd, 16.14; found, 15.66.

Sodium 7-[(*R*)-(−)-Mandelamido]-3-(5-methyl-1,3,4-thiadiazol-2-ylthiomethyl)-3-cephem-4-carboxylate (54). A mixture of 1 (4.55 g, 0.01 mol) and 5-methyl-1,3,4-thiadiazole-2-thiol (1.32 g, 0.01 mol) in 100 ml of pH 6.4 phosphate buffer was heated at 60° with stirring for 21 hr. The cooled (25°) mixture was layered with EtOAc and acidified to pH 2 with dilute HCl. The aqueous phase was extracted twice more with EtOAc and the combined dried extracts were evaporated to give 4.9 g solid. Chromatography on silica gel using a $CHCl_3$-MeOH gradient gave 2.91 g of the cephalosporin free acid. Treatment of an acetone solution of this acid with a slight excess of 30% sodium 2-ethylhexanoate in isopropyl alcohol gave 2.3 g (44%) of the sodium salt. *Anal.* ($C_{19}H_{18}N_4NaO_5S_3\cdot H_2O$) C, H, N.

Acknowledgment. We are grateful to Dr. Alfred Burger and Mr. R. J. Ferlauto for their encouragement and valuable discussions of the subject matter. We wish to thank Mr. John Zarembo and his staff in the Analytical & Physical Chemistry Department of Smith Kline and French Laboratories for the elemental analyses, Dr. Roger C. Parish for his contributions to the design and preparation of the compounds described, and Dr. A. Magnani, Dr. A. W. Chow, Mr. G. Gallagher, Jr., and Mr. S. G. Weiss for their synthetic contributions. Eleven of the (*R*)-mandelic acids used in the syntheses were prepared and resolved by Dr. J. J. Lafferty and Miss I. Uzinskas of our laboratories. We are especially grateful for their valuable contribution to this work.

References

(1) J. R. E. Hoover, U. S. Patent 3,167,549 (1965).
(2) H. Vanderhaeghe, P. van Dijck, M. Claesen, and P. DeSomer, *Antimicrob. Ag. Chemother.*, 581 (1961).
(3) R. J. Stedman, *J. Med. Chem.*, **9**, 444 (1966).
(4) L. A. Wetherill, W. Graham, and M. J. Covil, British Patent 1,104,938 (1968).
(5) W. E. Wick and W. S. Boniece, *Appl. Microbiol.*, **13**, 248 (1965).
(6) J. P. Currie, *Postgrad. Med. J., Suppl.*, **43**, 22 (1967).
(7) J. L. Spencer, E. H. Flynn, R. W. Roeske, F. Y. Siu, and R. R. Chauvette, *J. Med. Chem.*, **9**, 746 (1966).
(8) W. E. Wick and D. A. Preston, *Antimicrob. Ag. Chemother.*, **3**, 221 (1972).
(9) B. B. Corson, R. A. Dodge, S. A. Harris, and J. S. Yeaw, "Organic Syntheses," Collect. Vol. I, H. Gilman, Ed., Wiley, New York, N. Y., 1941, p 336.
(10) V. Arkley, S. Eardley, and A. G. Long, British Patent 1,028,563 (1966).

Differential Effects of Benzodioxane, Chroman, and Dihydrobenzofuran Analogs of Clofibrate on Various Parameters of Hepatic Drug Metabolism†,‡

Neil J. Lewis, Dennis R. Feller, Guiragos K. Poochikian, and Donald T. Witiak*

Divisions of Medicinal Chemistry and Pharmacology, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210.
Received June 20, 1973

The influence of pretreatment with clofibrate (1) and related analogs on various parameters of hepatic drug metabolism in rats was investigated. Of the analogs tested, only ethyl 5-chloro-2,3-dihydrobenzofuran-2-carboxylate (4) and ethyl 6-chlorochroman-2-carboxylate (6) possessed inductive properties similar to 1. Increases were noted in the activities of ethylmorphine *N*-demethylase and/or aniline hydroxylase and cytochrome P-450 in liver microsomes obtained from animals pretreated with **1**, **4**, and **6**. Deschloro analogs (**5** and **7**) of **4** and **6** were ineffective as hepatic enzyme inducers. Presence of a chloro substituent was favorably correlated to the inductive properties noted within this series of compounds.

We recently described a comparative analysis of the hypocholesterolemic and hypotriglyceridemic activities of a number of cyclic ethyl ester analogs of clofibrate [ethyl 2-methyl-2-(4-chlorophenoxy)propionate, **1**].[1] A rat model was utilized in which hyperlipemia was induced by intraperitoneal injection of Triton WR-1339. Under these conditions, clofibrate (**1**), ethyl 1,4-benzodioxane-2-carboxylate (**2**), ethyl 5-chloro-2,3-dihydrobenzofuran-2-carboxylate (**4**), and ethyl 6-chlorochroman-2-carboxylate (**6**) significantly reduced serum cholesterol levels. The deschloro analogs **5** and **7** were inactive. In these same hyperlipemic rats, esters **1**, **2**, and **6** significantly reduced plasma triglyceride levels. Deschloro analogs **5** and **7** were inactive and the apparent hypotriglyceridemic effect of chloro analog **4** was not statistically significant. Chlorochroman ana-

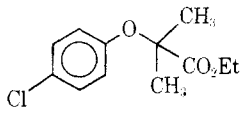
†The authors gratefully acknowledge support of this work through Grant HL 12740 from the U. S. Public Health Service.
‡This article is dedicated to Alfred Burger in recognition of his many significant contributions in the field of medicinal chemistry and because of his stimulating influence on the author's own research.

Table I. Effect of Pretreatment with Phenobarbital and Analogs **1–7** on Liver Weight, Liver/Body weight, and Microsomal Protein in Male Rats

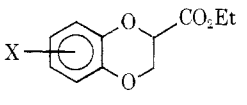
Compd	Dose,[a] mmol/kg	Liver weight, g[b]		Liver/body weight, %[b]		Microsomal protein, mg/g of liver[c]	
		Control	Test	Control	Test	Control	Test
Phenobarbital	0.16	7.70 ± 0.37	9.80 ± 0.40[d]	4.39 ± 0.13	5.26 ± 0.27[d]	17.2 ± 2.1	23.2 ± 1.7[d]
1	0.40	5.53 ± 0.50	5.90 ± 0.27	4.82 ± 0.13	4.94 ± 0.10	21.2 ± 0.8	26.6 ± 0.4[d]
	0.80	7.72 ± 0.27	7.08 ± 0.34	4.96 ± 0.18	4.94 ± 0.10	25.3 ± 0.6	32.3 ± 1.8[d]
2	0.40	5.78 ± 1.10	6.52 ± 0.49	4.39 ± 0.69	3.97 ± 0.47	26.9 ± 1.6	24.2 ± 1.6
	0.80	4.72 ± 1.10	6.68 ± 1.10	4.87 ± 0.58	4.88 ± 0.57	33.2 ± 0.4	34.0 ± 1.0
3	0.40	4.57 ± 0.26	3.95 ± 0.26	4.26 ± 0.12	3.77 ± 0.19	20.0 ± 1.0	27.0 ± 1.2[d]
	0.80	4.60 ± 0.29	4.34 ± 0.24	4.14 ± 0.23	4.01 ± 0.17	22.8 ± 1.2	26.4 ± 2.4
4	0.40	4.85 ± 0.52	4.18 ± 0.69	4.57 ± 0.25	4.20 ± 0.42	16.6 ± 0.0	19.5 ± 0.5[d]
	0.80	5.42 ± 0.92	5.96 ± 0.96	4.24 ± 0.28	4.63 ± 0.54	24.3 ± 0.6	24.7 ± 1.5
5	0.40	3.91 ± 0.39	3.73 ± 0.57	4.06 ± 0.29	3.89 ± 0.28	21.6 ± 3.3	24.2 ± 2.0
	0.80	5.14 ± 0.37	5.27 ± 1.03	4.09 ± 0.28	3.92 ± 0.21	26.8 ± 1.8	27.8 ± 2.2
6	0.40	4.88 ± 0.44	4.48 ± 0.13	4.37 ± 0.43	4.37 ± 0.18	22.2 ± 1.4	21.3 ± 0.9
	0.80	5.87 ± 0.28	5.12 ± 0.24[d]	4.26 ± 0.06	4.08 ± 0.17	28.8 ± 1.0	26.4 ± 0.4
7	0.40	4.71 ± 0.45	5.20 ± 0.34	4.11 ± 0.43	3.86 ± 0.20	21.6 ± 3.2	24.2 ± 2.1
	0.80	6.09 ± 0.98	6.91 ± 1.42	4.16 ± 0.34	4.27 ± 0.35	23.4 ± 2.8	27.0 ± 0.8

[a]Compounds were given orally in coconut oil once daily for 7 days. [b]Values represent the mean ± S.E. of $n = 6$. Control refers to coconut oil pretreatment and Test refers to drug pretreated animals. [c]Values represent the mean ± S.E. of $n = 3$. [d]Significantly different from control ($p < 0.05$).

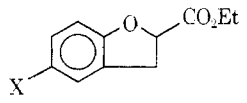
log **6** compared most favorably with clofibrate (**1**). However, none of the experimental drugs were as effective as **1** when assessed for their ability to return lipoprotein patterns to normal in hyperlipemic rats in conjunction with their hypocholesterolemic and hypotriglyceridemic activities.



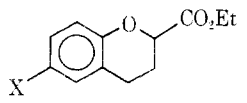
1



2, X = H
3, X = Cl[a]



4, X = Cl
5, X = H



6, X = Cl
7, X = H

[a]A mixture of position isomers in which the halogen is found 50% at position 6 and 50% at position 7.

As part of an extensive investigation designed to probe the nature of biochemical sites which are either blocked or stimulated *in vivo* by administration of the parent esters[1,2] or *in vitro* by utilization of the corresponding carboxylic acid hydrolysis products,[3-6] we initiated a comparative study to determine the effect of chronic analog administration on various parameters of hepatic drug metabolism. Several investigators[7-9] have demonstrated an increase in liver weight and microsomal protein content after clofibrate administration to rats. We recently established that increases in microsomal protein and liver weight after clofibrate (**1**) administration are dependent upon the age of the animal.[10] Pretreatment with **1** significantly reduced pentobarbital and zoxazolamine sleeping times and increased the rates of microsomal metabolism of pentobarbital, ethylmorphine, and aminopyrine.[10] However, specific activities of microsomal enzymes involved with the hydroxylation of zoxazolamine or aniline remained unaffected after chronic clofibrate administration. Changes in various microsomal enzymes induced by administration of **1** also were accompanied by an elevation in cytochrome P-450, a component of the electron transport system usually associated with alterations in drug oxidations.[11] Since clofibrate (**1**) interacts with systems necessary for lipid metabolism[12-14] and with cytochrome P-450, which is involved in cholesterol metabolism and biosynthesis *in vitro*,[15-17] the present study was undertaken to ascertain the effect of chronic administration of clofibrate analogs on hepatic drug metabolism in rats. The structure–activity data thus obtained can be utilized for the future design of antilipemic drugs.

Experimental Section

The synthesis for experimental hypolipemic drugs, excluding analog **3**, has been described previously.[2-5] Clofibrate (**1**) was obtained from Ayerst Laboratories, New York, N. Y. For these preliminary studies **ethyl 6- and 7-chlorobenzodioxane-2-carboxylate (3)** was synthesized from 4-chlorocatechol by the method described for the preparation of **2** from catechol[4] and was tested as a 50/50 mixture of the two position isomers as determined by glpc.

Male Harlan Wistar rats (80–130 g) were given each drug dissolved in coconut oil orally at either of two dose levels (0.40 or 0.80 mmol/kg daily) for 7 days. Phenobarbital (40 mg/kg) was used as an internal control. Control and treated groups of animals were allowed free access to food and water and were fasted overnight after the last dose. Experiments were initiated 14–16 hr after the final dose.

Animals were sacrificed; the livers were excised, weighed, and homogenized with a Teflon-glass homogenizer in 4 vol of 20 m*M* Tris-HCl buffer, pH 7.4, containing 1.15% KCl. The homogenates were centrifuged at 9000g for 20 min at 4° in a refrigerated centrifuge. The 9000g supernatant was carefully decanted and recentrifuged at 105,000g for 1 hr in a Model L Beckman ultracentrifuge. The microsomal pellet was resuspended in 20 m*M* Tris-HCl-KCl buffer, pH 7.4, and stored on ice for further use. Experiments were completed within 6 hr. Reaction mixtures contained the following components in a final volume of 3 ml: 5 mg of microsomal protein, a NADPH generating system (consisting of 2 eu glucose 6-phosphate dehydrogenase, 15 μmol of $MgCl_2$, 15 μmol of glucose 6-phosphate, and 2.4 μmol of $NADP^+$), 150 μmol of Tris-HCl buffer, pH 7.4, and substrate. Concentrations of substrates used were 10 μmol of aniline and 5 μmol of ethylmorphine.

Ethylmorphine N-demethylation was assayed by the method of Nash.[18] **Conversion of aniline to *p*-aminophenol** was estimated as described by Kato and Gillette.[19] **Cytochrome P-450** was determined by the procedure of Omura and Sato[20] and **microsomal protein** assayed by the method of Lowry, *et al.*[21] Statistical comparisons were made using the Student's t test.

Results

Initial experiments indicated that the maximal effect of **1** on liver weight, microsomal protein, and certain drug

Table II. Effect of Pretreatment with Phenobarbital and Analogs **1**–**7** on Ethylmorphine *N*-Demethylase, Aniline Hydroxylase, and Cytochrome P-450 in Liver Microsomes from Male Rats

Compd	Dose,[a] mmol/kg	Ethylmorphine *N*-demethylase[b] (nmol of CH_2O/mg/12 min)		Aniline hydroxylase[b] (nmol/mg/15 min)		Cytochrome P-450[b] ($O.D._{450-490}$/mg/ml)	
		Control	Test	Control	Test	Control	Test
Phenobarbital	0.16	48.9 ± 7.1	116.5 ± 8.9[c]	16.4 ± 0.8	20.4 ± 1.4[c]	0.059 ± 0.006	0.111 ± 0.010[c]
1	0.40	57.2 ± 2.4	77.8 ± 4.5[c]	28.9 ± 0.2	29.2 ± 0.1	0.071 ± 0.002	0.093 ± 0.004[c]
	0.80	55.5 ± 1.2	76.4 ± 0.9[c]	12.6 ± 2.2	13.8 ± 0.3	0.075 ± 0.005	0.098 ± 0.003[c]
2	0.40	51.0 ± 0.3	38.1 ± 5.0[c]	23.7 ± 2.3	23.7 ± 1.7	0.077 ± 0.011	0.082 ± 0.005
	0.80	26.0 ± 2.0	26.1 ± 2.0	21.7 ± 2.0	22.3 ± 0.7	0.075 ± 0.001	0.083 ± 0.003[c]
3	0.40	77.5 ± 2.5	77.5 ± 3.8	21.7 ± 1.3	18.0 ± 0.5	0.072 ± 0.003	0.053 ± 0.001
	0.80	48.0 ± 1.3	62.5 ± 5.4[c]	17.7 ± 0.4	18.3 ± 1.4	0.069 ± 0.003	0.055 ± 0.004
4	0.40	58.8 ± 5.0	90.0 ± 3.8[c]	36.0 ± 3.0	39.3 ± 2.0	0.042 ± 0.007	0.057 ± 0.004[c]
	0.80	50.0 ± 5.0	65.0 ± 3.8[c]	24.0 ± 3.0	35.3 ± 2.0[c]	0.042 ± 0.003	0.052 ± 0.003[c]
5	0.40	63.8 ± 2.0	67.5 ± 2.5			0.059 ± 0.010	0.060 ± 0.007
	0.80	45.0 ± 1.4	45.5 ± 2.5	16.2 ± 1.2	18.5 ± 1.3	0.070 ± 0.015	0.072 ± 0.009
6	0.40	28.8 ± 1.2	37.5 ± 2.5[c]	21.7 ± 1.0	26.7 ± 0.7[c]	0.061 ± 0.005	0.076 ± 0.007[c]
	0.80	41.2 ± 1.2	37.5 ± 2.5	16.7 ± 2.0	23.0 ± 2.7	0.073 ± 0.005	0.075 ± 0.007
7	0.40	73.8 ± 8.1	90.0 ± 24.1			0.093 ± 0.031	0.062 ± 0.017
	0.80	60.0 ± 3.0	45.0 ± 2.5[c]	18.5 ± 2.6	15.7 ± 2.4	0.081 ± 0.011	0.065 ± 0.023

[a]Compounds were given orally in coconut oil once daily for 7 days. [b]Values represent the mean ± S.E. of $n = 3$. [c]Significantly different from control ($p < 0.05$).

metabolizing enzyme systems occurred after 7 days of pretreatment.[10] For these reasons, a 7-day dosing schedule was used for all analogs tested.

The effect of pretreatment with phenobarbital (Pb), clofibrate (**1**) and analogs **2**–**7** on liver weight, liver/body weight, and microsomal protein in male rats is summarized in Table I. Pretreatment with Pb, **1**, and analogs **3** and **4** (0.40 mmol/kg) significantly increased the liver microsomal protein content. It should be noted that like **1** and **3** the more rigid cyclic analog **4** increased microsomal protein at the lower dose while the more flexible cyclic chroman analog **6** showed no effect. Increases in liver weight and liver/body weight were observed with Pb administration whereas no increases were noted in these parameters with analogs **1**–**7**.

The effect of pretreatment with Pb and analogs **1**–**7** on ethylmorphine *N*-demethylase, aniline hydroxylase, and cytochrome P-450 in liver microsomes is presented in Table II. The microsomal rates of ethylmorphine N-demethylation and cytochrome P-450 content were increased by prior administration of **1** or Pb while aniline hydroxylation was elevated only by Pb pretreatment. A comparison of the inductive effects of analogs **2**–**7** showed that the chlorodihydrobenzofuran **4** possessed all of the inductive properties of **1** and in addition exhibited an enhancement of aniline hydroxylation (0.80 mmol/kg). The only other analog studied which enhanced the rate of aniline hydroxylation was the cyclic homolog of **4**, namely chlorochroman **6**. This analog increased the rate of aniline hydroxylation only at the lower dose. Considering those analogs which increased the rates of ethylmorphine N-demethylation the more rigid analog **4**, like clofibrate (**1**), was effective at both doses. The chlorobenzodioxane **3** only was effective at the higher dose and the chlorochroman **6** only was effective at the lower dose. The lack of activity observed for **6** at the higher dose is likely due to hepatotoxicity. Moreover, the increases in rates of ethylmorphine N-demethylation observed for Pb and analogs **1** and **6** correlate to elevations of cytochrome P-450, whereas the chlorobenzodioxane **3** did not show any significant change in cytochrome P-450 even though it enhanced ethylmorphine *N*-demethylase activity.

The cyclic deschloro analogs **5** and **7** were unable to produce any changes in specific activities of the hepatic drug metabolizing enzyme systems, cytochrome P-450, microsomal protein, or liver weight after chronic administration. Similarly, the deschlorobenzodioxane analog **2** was ineffective as a hepatic enzyme inducer. At low doses this analog caused a significant decrease in the rate of ethylmorphine N-demethylation while a small increase in cytochrome P-450 was observed at the higher dose. These findings indicate that the presence of a chloro group is required for the inductive properties possessed by these analogs.

Discussion

In recent years, many compounds have been shown to stimulate the metabolism of drugs, foreign compounds, and normal body constituents by inducing the activity of the hepatic microsomal monooxygenase enzyme system.[11] Enzyme inducers have been divided into two general categories: (1) the barbiturate class including Pb and (2) the polycyclic hydrocarbons as exemplified by 3-methylcholanthrene (3-MC). Pb is characterized by an ability to stimulate the metabolism of a wide variety of drug substrates (type I and II substrates) whereas 3-MC increases the hepatic metabolism for a more limited group of compounds (mainly type II substrates).[22,23] Earlier reports[9,24] have shown that the hepatic microsomal N-demethylation of aminopyrine, a type I substrate, is elevated in clofibrate pretreated animals. We recently found that clofibrate (**1**) pretreatment enhanced the hepatic microsomal metabolism of type I substrates but not of type II substrates.[10] These findings were corroborated by the results obtained in the present study (Table II). Prior administration of Pb induced the metabolism of ethylmorphine (a type I substrate) and aniline (a type II substrate) and increased the liver weight and microsomal protein (Tables I and II). These inductive properties exhibited by Pb differed both qualitatively and quantitatively from those obtained with clofibrate (**1**) and analogs **2**–**7**. No increases in liver weight were noted with any of the analogs studied (Table I). Pretreatment with clofibrate (**1**) or analog **4** significantly elevated microsomal protein and the rate of ethylmorphine N-demethylation. Among the analogs investigated by us, only **4** and **6** caused an increased rate for the metabolism of the type II substrate, aniline. The inductive effects possessed by certain analogs, especially **4**, are of comparable magnitude to the parent drug (**1**), although differential effects on aniline metabolism can be noted. Moreover, the results indicate that these analogs cannot be adequately characterized in the barbiturate or

polycyclic hydrocarbon classes of enzyme inducers.

The changes in activities of hepatic drug metabolizing enzyme systems may be correlated to changes in the concentration of the terminal oxidase, cytochrome P-450.[11] This hemoprotein also is involved in the metabolism of a wide variety of steroids[25] which includes the biosynthesis and metabolism of cholesterol.[15,16] In the present work, pretreatment of rats with Pb, clofibrate (1), and analog 4 significantly elevated the level of cytochrome P-450 in liver microsomes from drug-treated animals at all doses used. Recently, Salvador and coworkers[26,27] showed that chronic administration of clofibrate (1) or the enzyme inducers, Pb or chlorcyclizine, significantly lower serum cholesterol and other lipids *in vivo*. These investigators also reported that Pb and, to a much lesser degree, clofibrate (1) increased the rates of testosterone hydroxylation in liver microsomes. In preliminary experiments, we have observed that the hepatic microsomal oxidation of cholesterol is greater in animals pretreated with clofibrate (1) and Pb. In view of these results, further studies will be necessary to evaluate the role of hepatic enzyme induction on the hypolipemic actions possessed by this class of agents, *in vivo*. Whereas White[12] recently demonstrated that a major biochemical site of hypocholesterolemic action by clofibrate (1) may be mediated by the inhibition of 3-hydroxy-3-methylglutaryl CoA reductase, a rate-limiting step in the biosynthesis of cholesterol, it should be noted that elevations in cytochrome P-450 may be related to an increased catabolism of cholesterol and thereby contribute, in part, to the hypocholesterolemic and/or hypotriglyceridemic actions of this drug. Indeed, clofibrate (1) is a hypolipidemic agent which possesses multiple modes of action.[1,11,13,14,28]

In addition to the potential clinical significance of these studies, the differential effects of the various analogs on hepatic drug metabolism may be discussed in relation to the parent drug (1) and the structural requirements for biological activity. Biological studies from our laboratory[1] have previously indicated that removal of the chloro group from cyclic analogs afforded inactive compounds in hyperlipemic rats. Grisar and coworkers[29] also found their deschloro analogs, which represent still another series of clofibrate related compounds, to be much less active as hypolipemic agents. Moreover, the comparative analysis of these cyclic analogs in the hyperlipemic animal model[1] revealed that none of the synthesized derivatives were more effective than clofibrate (1). Nearly identical findings were observed in the present work. Similarly, the chloro analogs **3**, **4**, and **6** exhibited greater inductive properties than their corresponding deschloro analogs **2**, **5**, and **7**. Only the 5-chlorodihydrobenzofuran (4) was found to possess inductive properties comparable to clofibrate (1). The qualitative and/or quantitative differences between the deschloro and chloro analogs observed in this study would not appear to be due to differential rates of enzymatic hydrolysis or to ionization constants of the free carboxylic acids formed. Previous reports have demonstrated that clofibrate (1) undergoes rapid hydrolysis *in vivo* and *in vitro* to form the free acid.[2,30,31] Nazareth, *et al.*,[32] have shown that the esters of analogs **2**-**7** are nearly completely hydrolyzed in rat plasma within 5 min and that the pK_a's of the free carboxylic acids are similar and range from 4.14 to 4.46. One physical parameter which could contribute to the overall differential effects observed between the chloro and deschloro analogs is their lipid solubility. Remmer[33] indicated that the effectiveness of hepatic enzyme inducers is dependent upon their lipid solubility. For example, tolbutamide possesses more lipid solubility and is more potent as an inducer than carbutamide and related sulfonamides. The log P values for the chloro analogs **3**, **4**, and **6** were 2.00 (calculated by addition of 0.60 to the experimentally determined log P value of the deschloro analog),§ 2.11, and 2.40, respectively, and are larger than the log P values for the corresponding deschloro analogs **2**, **5**, and **7** which were found to be 1.40, 1.50 (calculated by subtraction of 0.60 from the experimentally determined log P value for the chloro analog),§ and 1.90, respectively. Clofibrate (1) exhibited a log P value of 2.57 which is greater than any of the other analogs. Thus, there seems to be a favorable correlation between lipid solubility and the ability of these analogs to produce enzyme induction. However, structural features also play a role in enzyme induction since differential effects were noted in the metabolism of type I and type II drug substrates (Table II).

Finally, it should be emphasized that when these compounds were assessed for their hypolipemic activity utilizing Triton WR-1339 treated rats, drugs were given 48 and 24 hr prior to serum lipid analysis. Under these conditions, the influence of these analogs on hepatic enzyme induction would probably not contribute to their hypolipemic properties. Further, using this limited series of analogs there was no apparent correlation of log P values with differential hypolipemic activity. It seems, therefore, that these analogs likely exert their acute effects in the hyperlipemia rat model by other mechanisms. With chronic administration, it is likely that increased hepatic enzyme induction by the chloro analogs may contribute to an enhanced hypolipemic effect as compared to deschloro analogs. However, it should be pointed out that biotransformation or other sites of loss, *e.g.*, deposition and distribution, remain to be assessed and may, in the final analysis, explain differences observed for the effect of these analogs on hepatic enzyme induction and/or their hypolipemic action.

References

(1) H. A. I. Newman, W. P. Heilman, and D. T. Witiak, *Lipids*, **8**, 378 (1973).
(2) D. T. Witiak, T. C. L. Ho, R. E. Hackney, and W. E. Connor, *J. Med. Chem.*, **11**, 1086 (1968).
(3) D. T. Witiak, R. E. Hackney, and M. W. Whitehouse, *ibid.*, **12**, 697 (1969).
(4) D. T. Witiak, D. R. Feller, E. S. Stratford, R. E. Hackney, R. Nazareth, and G. Wagner, *ibid.*, **14**, 754 (1971).
(5) D. T. Witiak, E. S. Stratford, R. Nazareth, G. Wagner, and D. R. Feller, *ibid.*, **14**, 758 (1971).
(6) T. F. Whayne, Jr., and D. T. Witiak, *ibid.*, **16**, 228 (1973).
(7) R. A. Salvador, S. Haber, C. Atkins, B. W. Gommi, and R. M. Welch, *Life Sci.*, **9**, 397 (1970).
(8) R. Hess, W. Staubli, and W. Riess, *Nature (London)*, **208**, 856 (1965).
(9) A. Kaneko, S. Sakamoto, M. Morita, and T. Onoe, *Tohoku J. Exp. Med.*, **99**, 81 (1969).
(10) N. J. Lewis, D. T. Witiak, and D. R. Feller, *Proc. Soc. Exp. Biol. Med.*, in press.
(11) A. H. Conney, *Pharmacol. Rev.*, **19**, 317 (1967).
(12) L. W. White, *J. Pharmacol. Exp. Ther.*, **178**, 361 (1971).
(13) M. E. Maragoudakis, *J. Biol. Chem.*, **245**, 4136 (1970).
(14) M. E. Maragoudakis, *ibid.*, **244**, 5005 (1969).
(15) F. Wada, K. Hirata, K. Nakao, and Y. Sakamoto, *J. Biochem. (Tokyo)*, **66**, 699 (1969).
(16) F. Wada, K. Hirata, and Y. Sakamoto, *ibid.*, **65**, 171(1969).
(17) S. Atkin, E. D. Palmer, P. D. English, B. Morgan, M. A. Cawthorne, and J. Greene, *Biochem. J.*, **128**, 237 (1972).
(18) T. Nash, *ibid.*, **55**, 416 (1953).
(19) R. Kato and J. R. Gillette, *J. Pharmacol. Exp. Ther.*, **150**, 279 (1965).

§C. Hansch, personal communication. Hansch recommends 0.7 for the π value for *p*-chloro in the phenoxyacetic acid series. We observed 0.5 for the difference in log P for chloro analog **6** and deschloro analog **7**. For these reasons we employed 0.6 to obtain calculated log P values.

(20) T. Omura and R. Sato, *J. Biol. Chem.*, **239**, 2370 (1964).
(21) O. Lowry, N. Rosebrough, A. Farr, and R. Randall, *ibid.*, **193**, 265 (1951).
(22) P. L. Gigon, T. E. Gram, and J. R. Gillette, *Mol. Pharmacol.*, **5**, 109 (1969).
(23) G. J. Mannering, "Pharmacological Testing Methods," Vol. 3, A. E. Berger, Ed., Academic Press, London and New York, 1968, p 51.
(24) D. S. Platt and B. L. Cockrill, *Biochem. Pharmacol.*, **18**, 429 (1969).
(25) R. Kuntzman, *Annu. Rev. Pharmacol.*, **9**, 21 (1969).
(26) R. A. Salvador, S. Haber, C. Atkins, B. W. Gommi, and R. W. Welch, *Life Sci.*, **9**, 397 (1970).
(27) R. A. Salvador, C. Atkins, S. Haber, and A. H. Conney, *Biochem. Pharmacol.*, **19**, 1463 (1970).
(28) H. J. Fallon, L. L. Adams, and R. G. Lamb, *Lipids*, **7**, 106 (1972).
(29) J. M. Grisar, R. A. Parker, T. Kariya, T. R. Blohm, R. W. Fleming, and V. Petrow, *J. Med. Chem.*, **15**, 1273 (1972).
(30) D. T. Witiak and M. W. Whitehouse, *Biochem. Pharmacol.*, **18**, 971 (1969).
(31) J. M. Thorp, *Lancet*, **1**, 1323 (1962).
(32) R. I. Nazareth, T. D. Sokoloski, D. T. Witiak, and A. T. Hopper, *J. Pharm. Sci.*, in press.
(33) H. Remmer, *Proc. Eur. Soc. Study Drug Toxicity*, **4**, 57 (1964).

Disposition and Metabolism of 4-Methyl-2-(4-phenylbenzyl)-2-oxazoline-4-methanol in the Rat and Dog

Lennox B. Turnbull,*† Christopher P. Johnson, III, Ying Ho Chen, Lawrence F. Sancilio, and Robert B. Bruce

Research Laboratories, A. H. Robins Company, Inc., Richmond, Virginia 23220. *Received May 29, 1973*

Following oral administration of [2-^{14}C]-4-methyl-2-(4-phenylbenzyl)-2-oxazoline-4-methanol (**1**), a synthetic anti-inflammatory substance, to rats and dogs the drug is eliminated rapidly in the urine and feces. The major metabolite (50–70%) in the rat is 4-(4-hydroxyphenyl)phenylacetic acid (**6**), whereas the dog eliminates primarily the unusual taurine conjugate of biphenylylacetic acid **4** and biphenylylacetic acid **5**. Six minor metabolites have been characterized. The synthesis of the ^{14}C-drug sample and three of the metabolites **2**–**4** is described.

The biphenylylacetic acid derivative, 4-methyl-2-(4-phenylbenzyl)-2-oxazoline-4-methanol (**1**, AHR-5318), has recently been developed and studied in this laboratory as an antiinflammatory substance. A study of its disposition and metabolic fate is the subject of this article and its pharmacology and more detailed chemistry will be reported at a later date. The metabolic fate of this ring system has not been reported in detail previously although Cressman[1] has reported the use of a ^{14}C-labeled sample of 2-amino-5-phenyl-2-oxazoline (Aminorex) in a pharmacokinetic study. Turnbull[2] and Morrison[3] describe the metabolism of 2-oxazolidinone derivatives in rats and dogs; however, this ring system is stable *in vivo* and its fate quite different compared with the 2-oxazoline system.

Experimental Section

Radioassay Methods. Analysis for ^{14}C activity was carried out in a Packard Tri-Carb liquid scintillation spectrometer in an aqueous phosphor mixture of diphenylyloxazole (5 g, PPO) dissolved in 1 l. of toluene-dioxane-fotocol (200 proof), 4:4:2.5. Freeze-dried feces samples were assayed on a Packard sample oxidizer, Model 305 Tri Carb.

Chromatography. Thin-layer chromatography was performed on a precoated, 250-mm thickness, silica gel plate (Analtech, Inc., Silica GF). The following systems were used to develop the plates: A, formic acid (88%)-ethanol-chloroform, 5:5:90; B, methanol-chloroform, 1:9; C, acetic acid-water-1-butanol, 11:25:50; D, ammonium hydroxide (concentrated)-methanol-chloroform, 2:30:70; E, formic acid (88%)-ethanol-chloroform, 2:2:96; F, ammonium hydroxide (concentrated)-methanol-chloroform, 1:10:90; G, 2 *N* ammonium hydroxide-ethanol-1-butanol, 1:1:4; H, methanol-benzene, 1:9. For radioactivity assay the active zones were scraped from the plates and extracted with methanol or 5% ammoniacal methanol and aliquots were counted. The spots were visualized as quenching zones under short-wave uv light.

Mass Spectrometry. Analyses were performed on a Hitachi RMU-6 mass spectrometer using the solid inlet system. Samples obtained from thin-layer plates were first extracted from the scraped off silica with methanol. After evaporation of the solvent the solid residue, consisting of a small amount of silica that had dissolved in the methanol plus the sample, was reextracted with chloroform. After removal of the chloroform the resulting sample gave satisfactory fragmentation patterns. Usually 20-μg samples or larger were extracted from the thin-layer plates in this way.

Hydrolysis of Conjugates. Potential glucuronide and sulfate conjugates were hydrolyzed by 24-hr incubation with Glusulase (Endo Laboratories) of urine at 37°, pH adjusted to pH 5.0 with acetic acid, and 0.1 ml of enzyme concentrate to 10 ml of urine. Tlc experiments with rat urine indicated that the metabolite spectra were the same before and after Glusulase treatment. Rat feces and dog urine and feces were not examined before Glusulase hydrolysis.

Rats. Three 200-g female rats housed in metabolism cages were given the drug, 200 mg/kg, as a sonified oral suspension in one dose and the urine and feces were collected at intervals for 3 days.

Dog. A 14.2-kg female mongrel dog housed in a metabolism cage was given a single oral dose, 50 mg/kg, in a gelatin capsule. Urine and feces were collected daily and analyzed.

Isolation of Metabolites. Urine samples before or after Glusulase treatment were passed through columns of XAD-2 resin, bed volume approximately one-third the urine volumes, and after a water wash the columns were eluted with methanol. By this procedure 92–98% of the drug-related material (radioactivity) could be concentrated into a fraction suitable for thin-layer chromatography directly or for other fractionation procedures.

Homogenized and freeze-dried rat feces were extracted by overnight refluxing and stirring with ethanol, giving about 50–55% of the radioactivity in the extract. Partition between methanol containing 10% water and low-boiling petroleum ether removed fat and after removal of the methanol the residue in water was passed onto an XAD-2 column. All of the radioactivity was then removed by methanol elution.

The freeze-dried sample of dog feces (24–48-hr collection containing 17% of the dose) was run through a Wiley mill and preextracted with petroleum ether to remove fat. It was then extracted successively with acetonitrile, hot water, and refluxing 1:1 ethanol-water to finally extract 91% of the radioactivity in the feces. The total extracts were combined and treated by the same procedure as the rat feces extract to obtain an XAD-2 resin eluate which contained substantially all of the radioactivity in the dog feces extract.

Dog Gastric Juice Incubation. Samples of **1** were dissolved in dog gastric juice at concentrations of 0.5 and 1.0 mg/ml and incubated at 37° for periods of 0.5 and 1 hr. The products were isolated on an XAD-2 resin column and examined by tlc in systems C, E, F, and G.

†This paper is dedicated to my former professor, Alfred Burger.