156 (1978).
(9) J. C. Coll, N. Liyanage, G. J. Stokie, I. Van Altena, J. N. E. Nemorin, S. Sternhell, and R. Kazlauskas, *Aust. J. Chem.*, **31**, 157 (1978).
(10) R. Kazlauskas, P. T. Murphy, R. J. Quinn, and R. J. Wells, *Tetrahedron Lett.*, **1976**, 1333.
(11) R. Kazlauskas, R. O. Lidgard, and R. J. Wells, *ibid.*, **1978**, 3165.
(12) R. S. Norton, R. G. Warren, and R. J. Wells, *ibid.*, **1977**, 3905.
(13) R. Kazlauskas, P. T. Murphy, R. J. Wells, J. J. Daly, and W. E. Oberhansli, *Aust. J. Chem.*, **30**, 2679 (1977).
(14) R. Kazlauskas, P. T. Murphy, R. J. Quinn, and R. J. Wells, *ibid.*, **29**, 2533 (1976).
(15) R. Kazlauskas, P. T. Murphy, R. J. Quinn, and R. J. Wells, *Tetrahedron Lett.*, **1977**, 37.
(16) R. P. Gregson, R. Kazlauskas, P. T. Murphy, and R. J. Wells, *Aust. J. Chem.*, **30**, 2527 (1977).
(17) R. J. Wells, *Pure Appl. Chem.*, **51**, 1829 (1979).
(18) T. C. West, G. Hadden, and A. Farah, *Am. J. Physiol.*, **164**, 565 (1951).
(19) G. V. R. Born and E. Bulbring, *J. Physiol. (London)*, **127**, 626 (1955).
(20) J. L. Barker and H. Levitan, *Membr. Biol.*, **25**, 361 (1975).
(21) P. N. Kaul, S. K. Kulkarni, and E. Kurosawa, *J. Pharm. Pharmacol.*, **30**, 589 (1978).
(22) P. N. Kaul and S. K. Kulkarni, *J. Pharm. Sci.*, **67**, 1293 (1978).

Inhibition of *In Vitro* Cytochrome P-450-Catalyzed Reactions by Substituted Pyridines

JERRY L. BORN ˣ and WILLIAM M. HADLEY

Received August 29, 1979, from the *College of Pharmacy, University of New Mexico, Albuquerque, NM 87131.* Accepted for publication October 25, 1979.

Abstract □ A series of substituted pyridines was investigated as inhibitors of cytochrome P-450-catalyzed reactions. The relative potencies for the *in vitro* inhibition of aminopyrine demethylation and aniline hydroxylation are reported for a series of 2-, 3-, and 4-substituted pyridines.

Keyphrases □ Oxidation—cytochrome P-450, inhibition of *in vitro* metabolism of aniline and aminopyrine, effect of substituted pyridines on inhibition □ Aniline—hydroxylase activity, effect of substituted pyridines on *in vitro* aniline metabolism, cytochrome P-450 oxidation reactions □ Aminopyrine—demethylation activity, effect of substituted pyridines on *in vitro* aminopyrine metabolism, cytochrome P-450 oxidation reactions

Cytochrome P-450 is the terminal oxygenase responsible for the metabolism of many drugs and xenobiotics. Numerous pyridine-containing compounds such as metyrapone and ellipticine are known to inhibit cytochrome P-450-catalyzed reactions (1,2). It has been suggested that potent inhibitors of cytochrome P-450-catalyzed oxidations may be useful therapeutically in the prevention of chemical-induced cancer (2). Another potential use of cytochrome P-450 inhibitors is to increase the half-lives for drugs, thus increasing their therapeutic effectiveness.

The nonselective nature of the known inhibitors of cytochrome P-450-mediated reactions limits their therapeutic application. For example, metyrapone is used to inhibits steroid 11-β-hydroxylase, but it also is a potent inhibitor of many other cytochrome P-450 transformations (1, 3).

To develop leads in designing potent substrate-selective inhibitors of hepatic cytochrome P-450-catalyzed reactions, the ability of some simple pyridine compounds to inhibit the metabolism of aniline and aminopyrine was investigated.

EXPERIMENTAL

Male Sprague–Dawley rats were sacrificed by cervical dislocation, and their livers were excised and placed in ice-cold isotonic 1.15% KCl–tromethamine [tris(hydroxymethyl)aminomethane] buffer (0.1 *M*, pH 7.4). The following steps were performed at 0–4°. The liver was pressed through a tissue press, and the mince was weighed and homogenized[1] in three volumes of ice-cold isotonic potassium chloride–tromethamine buffer.

The homogenate was centrifuged for 20 min at 9000×*g* in an ultracentrifuge[2] using a No. 30 rotor. The supernate was withdrawn and centrifuged at 78,000×*g* for 60 min. Then the supernate was decanted, and the pellet was resuspended in tromethamine buffer so that 1 ml of buffer contained microsomes from 250 mg of liver. The protein content was determined using the method of Lowry *et al.* (4).

Aniline hydroxylase activity was determined by the analysis for *p*-hydroxyaniline. Metabolic reactions were run in 25-ml erlenmeyer flasks. The 5-ml reaction volume contained microsomal protein (10 mg), glucose-6-phosphate (30 μM), nicotinamide adenine dinucleotide phosphate (4 μM), magnesium chloride (50 μM), glucose-6-phosphate dehydrogenase (2 units), inhibitor in tromethamine buffer (pH 7.4), and substrate (1.09 μM) in buffer (pH 7.4). The reaction components, except the substrate, were preincubated for 5 min at 37° in a metabolic incubator under oxygen (flow rate >1000 ml/min) at 100 oscillations/min. Substrate then was added, and the reaction was incubated for 30 min. The production of *p*-hydroxyaniline was analyzed as described by Netter and Seidel (5).

Aminopyrine demethylation was determined by the analysis of formaldehyde. The 5-ml reaction volume contained the same cofactors used for aniline hydroxylation. The aminopyrine concentration was 8 m*M*. Formaldehyde was analyzed by the method of McMahon and Easton (6).

All samples were run in duplicate, and each experiment was repeated. The I_{50} values were calculated from three inhibitor concentrations.

All compounds were prepared by literature methods, and their physical constants corresponded to the literature values (7–9).

RESULTS AND DISCUSSION

The inhibition of aniline and aminopyrine metabolism by the substituted pyridines is shown in Table I. Inhibition of aniline metabolism was highly dependent on the position of substitution on pyridine. The 4-substituted pyridines were the most potent inhibitors of aniline metabolism, and the 3-substituted pyridines were more potent inhibitors than the 2-substituted pyridines. The presence of a hydroxyl group in the inhibitors lowered their ability to inhibit aniline metabolism. The decreased lipid solubility of the hydroxyl-substituted pyridines may be involved in their lower inhibitory activity.

[1] Potter-Elvejhem-type homogenizer.
[2] Beckman L5-40.

0022-3549/80/0400-0465$01.00/0

Table I—Inhibition of *In Vitro* Metabolism of Aniline and Aminopyrine by Substituted Pyridines

Inhibitor	I_{50} of Aniline[a], M	I_{50} of Aminopyrine[b], M	$\frac{I_{50} \text{ of Aminopyrine}^c}{I_{50} \text{ of Aniline}}$
4-(*n*-Pentoxy)pyridine (I)	4.9×10^{-6}	1.1×10^{-4}	22.4
1-(4-Pyridyl)pentane (II)	5.3×10^{-6}	1.0×10^{-4}	18.9
1-(4-Pyridyl)pentanol (III)	1.1×10^{-5}	1.3×10^{-4}	11.8
1-(3-Pyridyl)pentane (IV)	9.6×10^{-5}	1.0×10^{-4}	10.4
1-(3-Pyridyl)pentanol (V)	1.6×10^{-4}	1.35×10^{-4}	0.8
1-(2-Pyridyl)pentane (VI)	3.6×10^{-4}	1.8×10^{-4}	0.5
1-(2-Pyridyl)pentanol (VII)	1.35×10^{-3}	2.7×10^{-4}	0.2

[a] Aniline concentration = 1.09 μM. [b] Aminopyrine concentration = 8 mM. [c] A ratio of >1 indicates that the compound is a more potent inhibitor of aniline hydroxylation than of aminopyrine demethylation.

Aminopyrine demethylation showed much less sensitivity to inhibition by the 4-substituted pyridines than did aniline hydroxylation. In each case, the pyridine compounds that contained an alcohol functional group were less potent inhibitors than the corresponding alkylpyridines. The magnitude of this effect was significantly less than that observed for inhibition of aniline metabolism. The most potent inhibitors of aminopyrine demethylation, 1-(4-pyridyl)pentane (II) and 1-(3-pyridyl)pentane (IV), were only 2.7 times as active as the least potent compound, 1-(2-pyridyl)pentanol (VII). A similar comparison of the most potent inhibitor of aniline hydroxylation, 4-(*n*-pentoxy)pyridine (I), and VII reveals a 275-fold difference in potency.

The most selective inhibitors of this series were the 4-substituted pyridines. Compound I was 22 times more potent an inhibitor of aniline hydroxylation than it was an inhibitor of aminopyrine demethylation. The differential in potency of inhibitors for the two substrates suggests that appropriate molecular modifications on pyridine compounds may produce substrate-selective inhibitors of cytochrome P-450 reactions.

REFERENCES

(1) K. C. Leibman, *Mol. Pharmacol.*, **5**, 1 (1969).

(2) P. Lesca, P. Lecointe, C. Paoletti, and D. Mansay, *Biochem. Pharmacol.*, **27**, 1023 (1978).

(3) G. W. Liddle, D. Island, H. Ester, and G. M. Tomkins, *J. Clin. Invest.*, **37**, 912 (1958).

(4) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

(5) K. J. Netter and G. Seidel, *J. Pharmacol. Exp. Ther.*, **146**, 61 (1964).

(6) R. E. McMahon and N. R. Easton, *J. Pharmacol.*, **135**, 128 (1962).

(7) S. Rocal, French pat. 1,481,022; through *Chem. Abstr.*, **68**, P87188t (1968).

(8) E. Scozewinski and M. Bieganowska, *J. Chromatogr.*, **40**, 431 (1969).

(9) T. Cavigny and H. Normant, *Bull. Soc. Chim. Fr.*, **1965**, 1872.

ACKNOWLEDGMENTS

Supported by the Faculty Research Fund of the University of New Mexico.

Solid Dispersion of Morphine–Tristearin with Reduced Presystemic Inactivation in Rats

KEY S. CHANG and CHARLES I. JAROWSKI ˣ

Received June 15, 1979, from the *Department of Allied Health and Industrial Sciences, College of Pharmacy and Allied Health Professions, St. John's University, Jamaica, NY 11439.* Accepted for publication October 24, 1979.

Abstract □ Solid dispersions of morphine in tristearin, β-sitosterol, and cholesterol were prepared by evaporation of their ethanol solutions. Weight ratios of morphine–lipid of 1:1, 1:3, and 1:4.5 were prepared. Dissolution studies of the solid dispersions and morphine were conducted in a simulated GI medium at 37°. The release rates of morphine from the tristearin dispersions were the slowest. The 1:1 morphine–tristearin dispersion was administered orally to rats. Free and total morphine levels in rat urine were determined by spectrofluorometric and enzymatic immunoassay procedures, respectively. The morphine–tristearin dispersion yielded a higher percentage of free morphine after 24 and 48 hr as compared with morphine and its sulfate.

Keyphrases □ Morphine–tristearin—solid dispersion, reduced presystemic inactivation, rats □ Analgesics—morphine, solid dispersion containing tristearin, reduced presystemic inactivation, rats □ Dissolution—morphine–tristearin solid dispersion, *in vitro*

Orally administered morphine produces a poorer analgesic response as compared with the parenteral route (1). In addition, the LD_{50} of orally administered morphine in rats was 905 ± 144 mg/kg, whereas it was 237 ± 6 mg/kg with the intravenous dose.

In the past, the major organ responsible for first-pass metabolism has been assumed to be the liver. Incubation of morphine with liver tissue was shown to result in inactivation (2). Although the drug-metabolizing capacity of the gut mucosa has been known for many years (3), the involvement of the intestinal wall in the biotransformation of opiates has been observed only recently (4). By using tritium-labeled morphine in rats, approximately two-thirds of the overall first-pass effect (82%) of morphine was estimated to be due to extraction and/or metabolism in the intestine and the remaining one-third occurs in the liver (5).

It was of interest to determine if solid dispersions of morphine in lipids might reduce the extent of first-pass inactivation after oral administration. It was demonstrated previously (6) that orally administered progesterone gave higher blood levels when the hormone was solvent deposited on lactose as a solid solution with cholesterol or its acetate ester. Patel and Jarowski (7) reported that urinary excretion of unmetabolized salicylate was increased when

0022-3549/80/0400-0466$01.00/0