Ligand Interaction of Substituted Pyridines with Cytochrome P-450

JERRY L. BORN [x] and SHERREL EARLY

Abstract [] A series of pyridyl ketones and alkyl pyridines was evaluated as type II ligands for cytochrome P-450. Activity as type II ligands was evaluated in terms of the lipid solubility and the pKa values of the compounds.

Keyphrases [] Ligands—interaction of substituted pyridines and pyridyl ketones with cytochrome P-450, effects of lipid solubility and pKa values on ligand activity [] Pyridines, substituted—ligand interaction with cytochrome P-450, effects of lipid solubility and pKa values on ligand activity [] Ketones, pyridyl—ligand interaction with cytochrome P-450, effects of lipid solubility and pKa values on ligand activity

Cytochrome P-450, the terminal oxidase responsible for many hydroxylation reactions (1, 2), binds to various substrates to produce type II difference spectra (1–6). Compounds that produce type II spectra include alcohols, primary amines, ketones, and pyridines (2).

BACKGROUND

Cytochrome P-450 is an iron-containing porphyrin in which the fifth ligand is a sulfhydryl group and the sixth ligand is a water molecule (7) or an imidazole ring (8). The displacement of the sixth ligand of cytochrome P-450 by pyridine or a pyridine ring of metyrapone[1] (2-methyl-1,2-di-3-pyridyl-1-propanone, I) is thought to result in a pyridine-coordinated cytochrome P-450. The ability of primary amines to produce type II spectra is a function of their ability to bind at a hydrophobic site (5). The base strength of pyridines, as well as an unhindered approach of the pyridine nitrogen to the enzyme, are essential for ligand interaction (9).

Figure 1 shows a proposed (7) binding model for I with cytochrome P-450. The pyridine ring, which is coordinated with the heme site, has a carbonyl attached at position 3; the alkyl-substituted pyridine ring binds at a site removed from the heme portion.

Although this model provides a convenient visualization of the cytochrome P-450 active site, no experimental evidence is available to indicate which pyridine ring of I interacts with the heme site. In addition, the role of secondary binding forces in the interaction of pyridine compounds and cytochrome P-450 has not been investigated.

The difference in the pKa values between the two pyridine rings of I is not likely to be as large as suggested by the pKa values of 5.85 and 3.18 for 3-picoline (II) (10) and 3-pyridyl methyl ketone (III) (11), respectively. Intramolecular steric interactions between the methyl groups of I and the carbonyl-substituted pyridine ring preclude coplanarity of the pyridine ring and the carbonyl group. This situation increases the pKa value of the carbonyl-substituted pyridine ring compared to that of III. Thus, it is not possible to predict which pyridine ring of I coordinates with cytochrome P-450 without considering the role of the carbonyl group in the interaction.

To evaluate further the mode of binding of pyridine compounds to cytochrome P-450, a series of 2-, 3-, and 4-substituted pyridines (Table I) was prepared and evaluated as type II ligands.

EXPERIMENTAL[2]

Pyridyl Ketones—Except for the commercially available pyridyl aldehydes and methyl pyridyl ketones, all pyridyl ketones were prepared

by the addition of the appropriate cyanopyridine at 0.05 M in 75 ml of benzene to the appropriate alkyl magnesium bromide (ethyl, n-propyl, or n-butyl) at 0.1 M in 150 ml of ether. The reaction mixture was stirred and refluxed for 2 hr, 50 ml of 5% HCl was added slowly, and the mixture was refluxed overnight. The reaction mixture then was made basic with 10% NaOH, cooled, and extracted with ether; the extract then was dried under vacuum. The residue was distilled to give the expected pyridyl ketone. In each case, the physical characteristics of the compounds were as reported previously (12, 13).

4-Alkyl Pyridines—The 4-alkyl pyridines were prepared as described by Wibaut and Hey (14), except for the commercially available methyl- and ethylpyridines. The compounds were purified by repeated recrystallization of their picrates. The free base was obtained by treating the picrates with 10% NaOH and extracting the free base with ether. The ether was dried and removed, and the residue was distilled.

3-Alkyl Pyridines—The 3-alkyl pyridines were prepared by Wolff–Kishner reduction of the 1-(3-pyridyl) ketones. The 1-(3-pyridyl) ketone (0.019 M) was dissolved in 25 ml of diethylene glycol, which contained 3.5 g of potassium hydroxide (0.062 M) and 4 ml of 85% hydrazine hydrate, and then was refluxed for 4 hr. Distillation directly from the reaction vessel gave a mixture of water and the 3-alkyl pyridine. The 3-alkyl pyridine was separated from the water by extraction with ether, which then was dried and removed. The residue was distilled to give the expected 3-alkyl pyridine. The observed boiling points, literature (15) boiling points, and yields were: n-propyl, 76°/40 mm, 182–184°/743 mm, 32%; n-butyl, 88°/22 mm, 75–76°/7.5 mm, 41%; and n-pentyl, 115–116°/25 mm, 110–112°/20 mm, 37% (15).

Determination of Spectral Binding Constant (K_s)—Phenobarbital sodium (80 mg/kg) was injected intraperitoneally into male Sprague–Dawley rats for 3 consecutive days. The animals then were fasted for 24 hr and sacrificed by decapitation. During the isolation of microsomes, all tissue was maintained at 0–4°. The liver was removed, washed with 0.25 M sucrose, and then homogenized in 10 volumes of 0.25 M sucrose using a Potter–Elvehjem glass tube and a polytef pestle.

The homogenized material was centrifuged for 15 min at 10,000×g,

Table I—Spectral Binding Constants (K_s) and Relative Activities for 3- and 4-Substituted Pyridines

Compound	$K_s \times 10^{-4} M^a$	SEM	Relative Activity
Isonicotinaldehyde (XIX)	10.4	0.731	1.00
Nicotinaldehyde (XVI)	10.4	0.702	1.00
3-Pyridyl methyl ketone (III)	5.17	0.0402	2.01
4-Pyridyl methyl ketone (IV)	5.04	0.405	2.06
β-Picoline (II)	3.53	0.253	2.94
1-(4-Pyridyl)propanone (XX)	2.21	0.130	4.70
1-(3-Pyridyl)propanone (XVII)	2.17	0.032	4.79
1-(3-Pyridyl)butanone (XVIII)	1.74	0.110	5.97
γ-Picoline (XII)	1.66	0.041	6.26
3-Ethylpyridine (IX)	1.61	0.0945	6.45
1-(4-Pyridyl)butanone (XXI)	1.53	0.113	6.79
1-(3-Pyridyl)pentanone (VI)	1.32	0.0120	8.00
4-Ethylpyridine (XIII)	1.11	0.110	9.36
1-(3-Pyridyl)propane (X)	0.934	0.021	11.13
1-(4-Pyridyl)pentanone (V)	0.753	0.0509	13.81
1-(4-Pyridyl)propane (XIV)	0.655	0.456	15.87
1-(3-Pyridyl)butane (XI)	0.483	0.0353	21.53
1-(3-Pyridyl)pentane (VIII)	0.375	0.0182	27.73
1-(4-Pyridyl)butane (XV)	0.375	0.00841	27.73
1-(4-Pyridyl)pentane (VII)	0.256	0.0153	40.62
2-Methyl-1,2-di-3-pyridyl-1-propanone (I)	0.0135	0.000551	800.00

^{a} Values are means ± *SEM*, n = 3.

---

[1] Ciba-Geigy.

[2] Difference spectra were obtained using a Beckman M-VI Acta spectrophotometer equipped with a scattered transmission accessory.
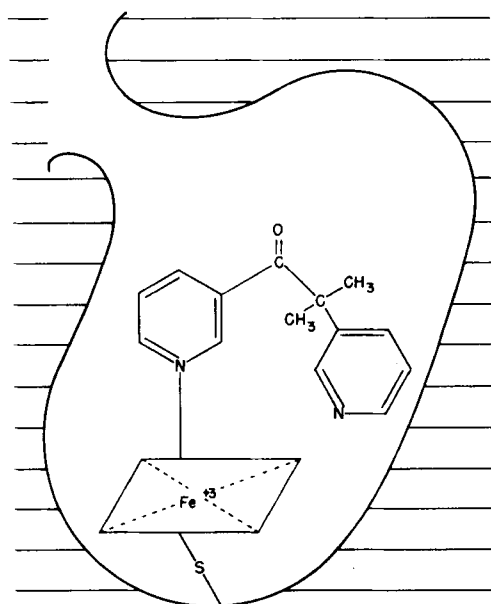
Figure 1—*Proposed interaction (7) of I with the heme portion of cytochrome P-450 via coordination of the carbonyl-substituted pyridine ring of I.*

and the supernate was removed and centrifuged at 100,000×g for 1 hr to produce a microsomal pellet. The microsomal pellet was resuspended in 0.15 M KCl and recentrifuged at 100,000×g for 1 hr. The microsomal pellet obtained by recentrifugation was resuspended in 0.05 M, pH 7.4 tromethamine buffer. Then the protein concentration was determined (16) and adjusted to 1 mg/ml with the buffer.

Difference spectra were recorded by placing 3 ml of the protein solution in both the sample and reference cells and then recording the baseline. After the baseline was established, 3-μl portions of the substrate dissolved in tromethamine buffer were added to the sample cell, and successive spectra were recorded. When the solubility of the substrate in the buffer was low, spectroanalyzed methanol was utilized to dissolve the compounds. Addition of as much as 21 μl of methanol to the sample cell did not change the spectra.

Each compound was examined in triplicate at six concentrations. The spectral binding constant, K_s, was determined by substituting the difference between the maximum (427 nm) and minimum (393 nm) absorbances in the spectrum for V in the classical enzyme substrate model (17). The correlation coefficient for the determination of K_s was >0.96 in each case.

Log P values were calculated utilizing the Hansch method (18, 19). Yeh and Higuchi (20) reported π values for methylene substitution on pyridine of 0.61 ± 0.01 and log P values of 3.750 and 3.128 for VII and XV, respectively.

RESULTS AND DISCUSSION

The 2-pyridyl carbonyl compounds investigated failed to produce typical type II spectra. Picolinaldehyde did not produce a recognizable spectrum while 2-pyridyl methyl ketone, 1-(2-pyridyl)propanone, and 1-(2-pyridyl)butanone gave weak type I spectra. 1-(2-Pyridyl)pentanone gave a weak type I spectrum at concentrations up to 2.7 × 10^{-3} M, but a modified type II spectrum was generated when concentrations exceeded 5.6 × 10^{-3} M. The lack of typical type II spectra for the 2-pyridyl carbonyls was not unexpected since their low pKa values, coupled with the steric inhibition of binding by the 2-substituent, would be expected to be unfavorable to type II binding.

On the basis of the pKa values of 3.18 (11) and 3.51 (21) for III and 4-pyridyl methyl ketone (IV), respectively, the 4-pyridyl ketones were expected to be more effective as type II ligands for cytochrome P-450 than the 3-pyridyl ketones. The spectral binding constants, K_s, of these compounds were quite similar, with only 1-(4-pyridyl)pentanone (V)

exhibiting an increase in binding as compared to its 3-pyridyl analog (VI) (Table I).

The most basic compounds of this series, the 4-alkyl pyridines (10), also were the most potent type II ligands for cytochrome P-450. The K_s value of 1-(4-pyridyl)pentane (VII) was 1.4 times that of 1-(3-pyridyl)pentane (VIII) and 2.9 times that of its parent ketone (V).

A plot of log P versus log K_s for the 3-alkyl pyridines (II and VIII-XI), the 4-alkyl pyridines (VII and XII-XV), the 3-pyridyl ketones (III, VI, and XVI-XVIII), and the 4-pyridyl ketones (IV, V, and XIX-XXI) produced in each case a straight line with a correlation coefficient of >0.93. This result suggests that lipid solubility of pyridine compounds is important in the ligand interaction.

The role of the carbonyl group in the ligand interaction is observed when alkyl pyridines and pyridyl ketones of similar log P values are compared. The strength of the ligand interaction of pyridyl ketones was surprising in view of their low pKa values. When K_s values for compounds with similar log P values are compared, the pyridyl ketones have equal or greater activity compared to that of the alkyl pyridines. Thus, the K_s values of 1-(3-pyridyl)butanone (XVIII) (log P 1.32) and 1-(4-pyridyl)-butanone (XXI) (log P 1.32) are essentially equal to the K_s value for 4-picoline (XII) (log P 1.26) and greater than that of 3-picoline (II) (log P 1.26). Comparison of the K_s values for V (log P 1.93), 1-(3-pyridyl)pentanone (VI) (log P 1.93), 4-ethylpyridine (XIII) (log P 1.87), and 3-ethylpyridine (IX) (log P 1.87) reveals a similar pattern. The 1-(3-pyridyl) ketones are more potent ligands for cytochrome P-450 than the 3-alkyl pyridines of similar log P values, suggesting that the introduction of a carbonyl group in the 3-pyridyl series increases the strength of the ligand interaction. This increase in activity occurs in spite of the fact that the pyridyl ketones are less basic than the alkyl pyridines.

In summary, the data indicate that the carbonyl group in simple pyridine compounds is an important determinant in the strength of ligand interactions. The interaction of I with cytochrome P-450 is supported by the data (Fig. 1).

REFERENCES

(1) J. R. Gillette, *Adv. Pharmacol.*, **4**, 219 (1966).

(2) G. J. Mannering, in "Fundamentals of Drug Metabolism and Drug Disposition," B. N. LaDu, H. G. Mandel, and E. L. Way, Eds., Williams & Wilkins, Baltimore, Md., 1979, chap. 12.

(3) J. B. Cshenkman, H. Remer, and R. W. Estabrook, *Mol. Pharmacol.*, **3**, 113 (1967).

(4) Y. Imai and R. Sato, *J. Biochem.*, **62**, 239 (1967).

(5) C. R. E. Jefcoate, J. L. Gaylor, and R. Calabrese, *Biochemistry*, **8**, 3455 (1969).

(6) J. B. Schenkman, *ibid.*, **9**, 2081 (1970).

(7) B. W. Griffin, J. A. Peterson, J. Werringloer, and R. W. Estabrook, *Ann. N.Y. Acad. Sci.*, **244**, 107 (1975).

(8) M. Chevion, J. Peisach, and W. Blumberg, *J. Biol. Chem.*, **252**, 3637 (1977).

(9) J. L. Born and D. Vaughn, *J. Pharm. Sci.*, **66**, 1046 (1977).

(10) N. Ikekana, Y. Sato, and T. Maeda, *Pharm. Bull.*, **2**, 205 (1954).

(11) "Handbook of Tables for Organic Compound Identification," 3rd ed., Z. Rappoport, Ed., Chemical Rubber Co., Cleveland, Ohio, 1967.

(12) C. R. Warner, E. J. Walsh, and R. F. Smith, *J. Chem. Soc.*, **1962**, 1232.

(13) S. Pavlov and V. Arsenijevle, *Glas. Hem. Drus. Beograd*, **32**, 469 (1967).

(14) J. P. Wibaut and J. W. Hey, *Rec. Trav. Chim.*, **72**, 513 (1953).

(15) A. D. Miller and R. Revine, *J. Org. Chem.*, **27**, 168 (1957).

(16) G. H. Lowry, N. J. Rosenbrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

(17) K. C. Leibman, A. G. Hildebrandt, and R. W. Estabrook, *Biochem. Biophys. Res. Commun.*, **36**, 789 (1969).

(18) J. Iwasa, T. Fugita, and C. Hansch, *J. Med. Chem.*, **8**, 150 (1965).

(19) A. Leo, C. Hansch, and D. Gilkins, *Chem. Rev.*, **71**, 525 (1971).

(20) K. C. Yeh and W. I. Higuchi, *J. Pharm. Sci.*, **61**, 1648 (1972).

(21) A. Fisher, W. J. Galloway, and J. Vaughn, *J. Chem. Soc.*, **1964**, 3521.