Structure-Activity Relationship Study of the Inhibition of Adrenal Cortical 11β-Hydroxylase by New Metyrapone Analogues[1]

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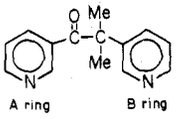
Metyrapone, 2-methyl-1,2-di-3-pyridyl-1-propanone (**1a**), is a potent reversible inhibitor of the cytochrome P-450 11β-hydroxylase enzyme system (P-450$_{11\beta}$) of the adrenal cortex. The structural features of the metyrapone molecule have been systematically altered to determine the requirements necessary for inhibition of P-450$_{11\beta}$ activity. Metyrapone and 14 analogues have been obtained or synthesized and evaluated as inhibitors using a crude, defatted bovine adrenal cortical mitochondrial preparation. The inhibition of P-450$_{11\beta}$ activity with these derivatives demonstrated that (1) the A-ring phenyl derivatives **2a–d** were better inhibitors than the respective dipyridyl analogues, (2) the ketone in the 1-position can be replaced by various functionalities without markedly reducing inhibition, and (3) at least one methyl group should be present in the 2-position to maintain inhibition. The observed inhibition of P450$_{11\beta}$ activity with the metyrapone analogues suggest that A-ring phenyl metyrapone analogues **2a–d** would be candidates for radioiodination and subsequently used as adrenal cortical imaging agents.

Many approaches have been used in the development of tissue-specific or disease-specific radiopharmaceutical agents.[2] One of these, the radiolabeled enzyme inhibitor method, was first investigated in 1968 with the dihydrofolate reductase and antitumor agent [^{131}I]iodoaminopterin.[3] We and others have further developed this approach.[4-9] The fulfillment of at least four criteria are necessary if a γ-labeled enzyme inhibitor is to be an effective imaging agent. There must be (1) a high concentration of the enzyme in the target tissue, (2) a selective concentration of the enzyme in the target tissue, (3) a strong binding of the inhibitor to the enzyme, and (4) an incorporation of a γ-label into the inhibitor without significantly lowering its enzyme binding affinity or altering its biodistribution.[10] Thus, the choice of the target tissue, the target enzyme, and the inhibitor is very important to the success of this approach. This study focuses on the adrenal cortex, the cytochrome P-450 11β-hydroxylase enzyme system (P-450$_{11\beta}$), and the inhibitor metyrapone (**1a**) as a model system.

The adrenal cortex has a unique enzymatic pattern that is directed toward the synthesis of various steroids.[11,12] Of these enzymes in these pathways, P-450$_{11\beta}$ is found almost exclusively in the adrenal cortex and thus represents an excellent target enzyme.[11,12] This membrane-bound mitochondrial enzyme system is composed of (1) adrenodoxin reductase, a flavoprotein; (2) adrenodoxin, an iron–sulfur protein; and (3) cytochrome P-450, a heme-containing protein. P-450$_{11\beta}$ can catalyze the 11β-, 18- and 19-hydroxylations of various steroids; however, P-450$_{11\beta}$ activity is usually characterized with the substrate 11-deoxycorticosterone (DOC).[13-15]

Metyrapone, 2-methyl-1,2-di-3-pyridyl-1-propanone (**1a**),



METYRAPONE (1a)

which is used clinically to test the ability of the pituitary to secrete ACTH in response to a decreased concentration of plasma cortisol,[12,14,16] is a potent competitive inhibitor of P-450$_{11\beta}$ activity. The reversible inhibition of P-450$_{11\beta}$ activity with metyrapone (**1a**) results from its binding to the cytochrome P-450 of this enzyme system.[14,17-22] Cytochrome P-450 is a family of heme-containing proteins that are present in other organs besides the adrenal cortex. The biodistribution of cytochrome P-450 has been reviewed, and, in general, the cytochrome P-450 concentration is higher in the adrenal cortex than in the liver or kidney.[23] Metyrapone (**1a**) has been shown to bind to other P-450's, including the adrenal side-chain cleavage

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(1) A brief report of part of the present study has appeared: Hays, S. J.; Tobes, M. C.; Gildersleeve, D. L.; Wieland, D. M.; Beierwaltes, W. H. "Abstracts of Papers", 183rd National Meeting of the American Chemical Society, Las Vegas, NV, Mar 28–Apr 2, 1982; American Chemical Society: Washington, DC, 1982; Abstr MEDI 91.
(2) Eckelman, W. C.; Reba, R. C. *J. Nucl. Med.* **1978**, *19*, 1179.
(3) Johns, D. G.; Spencer, R. P.; Chang, P. K.; Bertino, J. R. *J. Nucl. Med.* **1968**, *9*, 530.
(4) Wieland, D. M.; Ice, R. D.; Beierwaltes, W. H. *J. Nucl. Med.* **1976**, *17*, 525.
(5) Beierwaltes, W. H.; Wieland, D. M., Ice, R. D., Seabold, J. E.; Sarkar, S. D.; Gill, S. P.; Mosley, S. T. *J. Nucl. Med.* **1976**, *17*, 998.
(6) Wieland, D. M.; Kennedy, W. P.; Ice, R. D.; Beierwaltes, W. H. *J. Labelled Compd. Radiopharm.* **1977**, *13*, 229.
(7) Beierwaltes, W. H.; Wieland, D. M.; Mosley, S. T.; Swanson, D. P.; Sarkar, S. D.; Freitas, J. E.; Thrall, J. H.; Herwig, K. R. *J. Nucl. Med.* **1978**, *19*, 200.
(8) Burns, H. D.; Marzilli, L. G.; Dannals, R. F.; Dannals, T. E.; Trageser, T. C.; Conti, P.; Wagner, H. N., Jr. *J. Nucl. Med.* **1980**, *21*, 875.
(9) Hays, S. J.; Gildersleeve, D. L.; Brown, L. E.; Tobes, M. C.; Wieland, D. M.; Beierwaltes, W. H. *J. Nucl. Med.* **1981**, *22*, P58.
(10) Wieland, D. M. In "Receptor Binding Radiopharmaceuticals"; Eckelman W. C., Ed.; Chemical Rubber Co.: Cleveland, OH, 1982; Vol 1, 1, Chapter 7, p 127.
(11) Temple, T. E.; Liddle, G. W. *Annu. Rev. Pharmacol.* **1970**, *10*, 199.
(12) Gower, D. B. *J. Steroid Biochem.* **1974**, *5*, 501.
(13) Suhara, K.; Gomi, T.; Sato, H.; Itagaki, E.; Takemori, S.; Katagiri, M. *Arch. Biochem. Biophys.* **1978**, *190*, 290.
(14) Sato, H.; Ashida, N.; Suhara, K.; Itagaski, E.; Takemori, S.; Katagiri, M. *Arch. Biochem. Biophys.* **1978**, *190*, 307.
(15) Watanuki, M.; Tilley, B. E.; Hall, P. E. *Biochemistry* **1978**, *17*, 127.
(16) Sprunt, J. G.; Browning, M. C. K.; Hannah, D. M. *J. Endocrinol.* **1968**, *41*, 193.
(17) Liddle, G. W.; Island, D.; Lance, E. M.; Harris, A. P. *J. Clin. Endocrinol. Metab.* **1958**, *18*, 906.
(18) Dominguez, O. V.; Samuels, L. T. *Endocrinology* **1963**, *73*, 304.
(19) Sanzari, N. P.; Peron, F. G. *Steroids* **1966**, *8*, 929.
(20) Gaunt, R.; Steinetz, B. G.; Chart, J. J. *Clin. Pharmacol. Ther.* **1968**, *9*, 657.
(21) Wilson, L. D.; Oldham, S. B.; Harding, B. W. *Biochemistry* **1969**, *8*, 2975.
(22) Satre, M.; Vignais, P. V. *Biochemistry* **1974**, *13*, 2201.
(23) Wickramasinghe, R. H. *Enzymes, 3rd Ed.* **1976**, *19*, 348.

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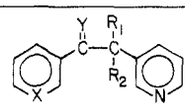
Table I. Inhibition of Bovine Adrenal Cortical Mitochondrial P-450$_{11\beta}$ Activity[a] by Metyrapone Analogues



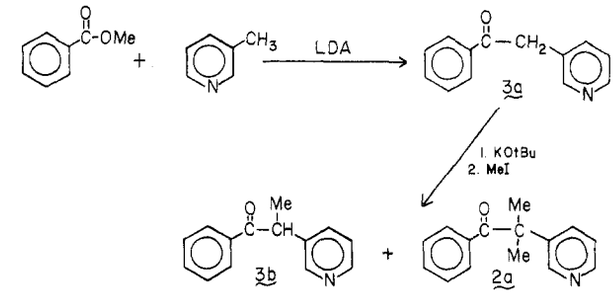
no.	X	Y	R_1	R_2	IC_{50},[b] μM
1a	N	O	Me	Me	7.83
1b	N	NOH	Me	Me	4.78
1c	N	H, NHAc	Me	Me	87.47
1d	N	H, NH_2	Me	Me	>1000
1e	N	H, OH	Me	Me	7.05
1f	N	H, Cl	Me	Me	7.25
1g	N	H, H	Me	Me	2.64
1h	N	H, I	Me	Me	5.36
1i	N	H, OCH_3	Me	Me	7.30
2a	C	O	Me	Me	3.30
2b	C	H, OH	Me	Me	1.53
2c	C	H, Cl	Me	Me	1.63
2d	C	H, H	Me	Me	0.66
3a	C	O	H	H	158.40
3b	C	O	H	Me	7.72

[a] P-450$_{11\beta}$ activity was assayed with DOC as the substrate by a modification of Sato et al.[14] [b] IC_{50} is the micromolar concentration of the inhibitor required to give 50% inhibition. The correlation coefficients were greater than 0.950.

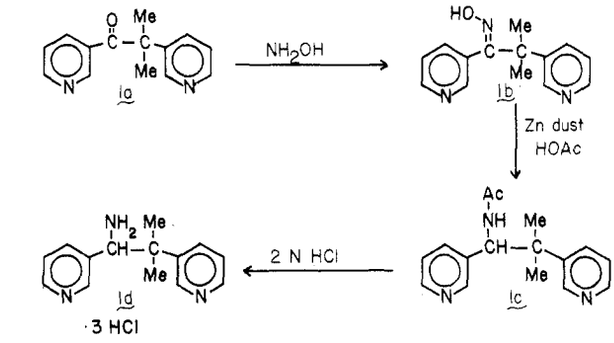
and the liver microsomal enzymes. It's specificity for these P-450's, however, is greatly reduced compared to P-450$_{11\beta}$.[24-28] These observations suggested that radiolabeled metyrapone (**1a**) and its analogues may show selective uptake into the adrenal cortex based on its inhibition of P-450$_{11\beta}$ activity. Tissue distribution studies with tritiated metyrapone (**1a**) have further supported this hypothesis.[5,6,9,29]

We have initiated a program to systematically alter one region of the metyrapone molecule at a time to probe those structural features necessary for inhibition of P-450$_{11\beta}$ activity. The most potent of these inhibitors would be candidates for radioiodination and subsequently studied for potential use as adrenal cortical imaging agents. Our structure–activity relationship (SAR) study differs from a conventional SAR study in that a sufficiently large lipophilic pocket must be identified within the enzyme's active site if a radioiodine atom is to be incorporated into the molecule without concomitant loss of inhibition activity. A previous SAR study of metyrapone derivatives has suggested that the B-pyridyl ring nitrogen of metyrapone (**1a**) is required for binding.[30] Replacement of the A-ring pyridyl by a phenyl group in analogues **2a-d** was undertaken in this study to further assess the binding contribution of the A-ring. In addition, by altering the functionality at the 1-carbon as shown in Table I, we can examine the contribution of various substituents to the binding of the cytochrome P-450$_{11\beta}$. Evaluation of the A-ring phenyl ketone, **2a**, and three additional A-ring phenyl analogues, **2b–d**, would aid in determining whether

Scheme I



Scheme II



similar binding trends exist for 1-substituents of the A-ring 3-pyridyl and phenyl series. Although the demethylated analogues **3a,b** were previously examined to determine the contribution of the geminal methyl groups for inhibition of P450$_{11\beta}$ activity,[30] we have reexamined these compounds in our assay for comparative purposes. The synthesis and enzyme inhibition studies of compounds **1a–i**, **2a–d**, and **3a–b** are described herein.

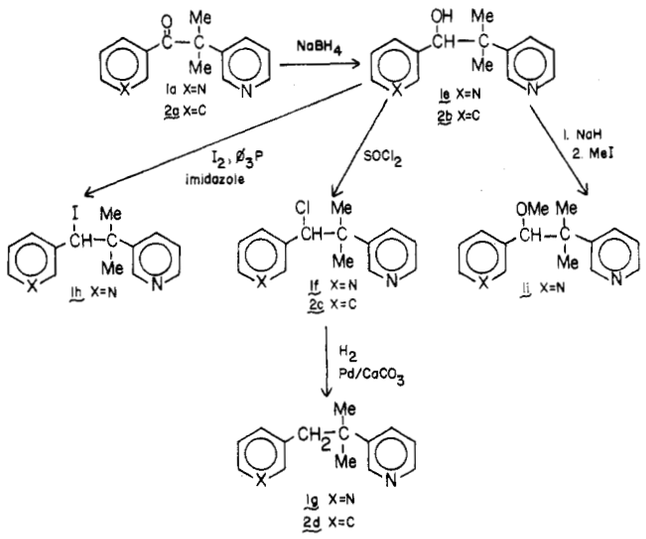
Chemistry. The synthesis of the A-ring phenyl metyrapone derivative **2a** is shown in Scheme I. The starting material, 3-picoline, was treated with lithium diisopropylamide and subsequently reacted with methyl benzoate to yield the known ketone **3a**.[31] Compound **3a** was then alkylated with potassium *tert*-butoxide and methyl iodide to produce the requisite ketone **2a** and the previously reported monomethylated ketone **3b**. The stoichiometry of the base is important, since the product **2a** is a nonenolizable ketone, and an excess of butoxide can result in attack at the carbonyl and a cleavage of product. O-Methylation was not a problem in this synthesis, and N-methylation was avoided by using strong bases[32] and restricting the quantity of methyl iodide.

The analogues **1b–d** containing nitrogenous substituents in the 1-position were synthesized as shown in Scheme II. Metyrapone oxime (**1b**) was synthesized from metyrapone (**1a**) by treatment with hydroxylamine according to the procedure of Bencze and Allen.[33] The oxime **1b** was reduced with zinc dust in acetic acid to form the acetamide **1c**, which was hydrolyzed to the free amine **1d** by refluxing in dilute hydrochloric acid. Attempts to reduce metyrapone oxime **1b** directly to the primary amine with agents such as lithium aluminum hydride, hydrogen with a palladium on carbon catalyst, sodium metal, magnesium metal, titanium trichloride, and diborane at 120 °C resulted in poor yields or no product.

The ketones **1a** and **2a** were reduced with sodium borohydride to yield the hydroxy compounds **1e** and **2b**,

(24) Liebman, K. C. *Mol. Pharmacol.* **1969**, *5*, 1.
(25) Netter, K. J.; Kahl, G.-F.; Magnussen, M. P. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1969**, *265*, 205.
(26) Hildebrandt, A. G. In "Biological *Hydroxylation Mechanisms*"; Boyd, G. S.; Smellie, R. M. S., Eds.; Academic Press: New York, 1972; p 79.
(27) Roots, I.; Hildebrandt, A. G. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1973**, *277*, 27.
(28) Roots, I.; Hildebrandt, A. G. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1973**, *277*, 39.
(29) Hanson, R. N.; Davis, M. A. *J. Pharm. Sci.* **1981**, *70*, 91.
(30) Napoli, J. L.; Counsell, R. E. *J. Med. Chem.* **1977**, *20*, 762.
(31) Miller, A. D.; Osuch, C.; Goldberg, N. N.; Levine, R. *J. Am. Chem. Soc.* **1956**, *78*, 674.
(32) Hurst, D. T.; Viney, M. *Tetrahedron Lett.* **1968**, *36*, 3895.
(33) Bencze, W. L.; Allen, W. J. *J. Am. Chem. Soc.* **1959**, *81*, 4015.

Scheme III



respectively, as shown in Scheme III. The alcohols **1e** and **2b** were conveniently converted to the chlorides **1f** and **2c** after treatment with thionyl chloride. Hydrogenation of **1f** and **2c** in the presence of palladium on calcium carbonate afforded the methylene analogues **1g** and **2d**. Attempts to reduce the ketone directly to the methylene via Wolff–Kischner, Clemmenson, and direct hydrogenation procedures were unsuccessful. Preparation of the 1-iodo derivative **1h** was achieved in one step by treatment of the alcohol **1e** with triphenylphosphine, imidazole, and iodine in refluxing toluene according to the procedure of Garegg and Samuelsson.[34,35] The low yield in this conversion and the inability of the ketone **1a** and oxime **1b** to react with many common reducing agents may reflect the steric crowding between the geminal methyl groups and substituents in the 1-position. The methoxy analogue **1i** was synthesized by treatment of the alcohol **1e** with sodium hydride and methyl iodide as outlined in Scheme III.

Biochemistry and Discussion

We previously observed that the B-ring pyridyl of metyrapone (**1a**) is required for inhibition of P-450$_{11\beta}$ activity, whereas the requirement for the A-ring pyridyl is not stringent. This was demonstrated by comparing the reactivity of **1a** to that of the A-ring phenyl (**2a**), B-ring phenyl, and diphenyl analogues (unpublished results). This investigation suggested that it is the β-ring pyridyl nitrogen of metyrapone (**1a**) that is oriented in close proximity to the hemin iron.

In the present study, a crude, defatted bovine adrenal cortical mitochondrial preparation was used to evaluate metyrapone (**1a**) and 14 analogues as inhibitors of P-450$_{11\beta}$ activity. The IC_{50} (μM) values for the inhibition of P-450$_{11\beta}$ activity are shown in Table I.

When the A-ring phenyl derivatives **2a–d** were compared with their correlative dipyridyl compounds **1a,e–g**, the phenyl analogues were observed to be better inhibitors of P-450$_{11\beta}$ activity. This suggests that a more lipophilic phenyl group in the A-ring position may enhance the activity by possibly interacting with a hydrophobic region at the active site of P-450$_{11\beta}$. This result is not unexpected when one considers the lipophilic character of the steroid substrates that compete at the same active site.

Alteration of the substituent at the 1-carbon of **1a** or **2a** had little effect on the ability of the analogues **1b–i** and **2b–d** to inhibit P-450$_{11\beta}$ activity. Functionalities with diverse electronic and lipophilic properties could be substituted for the ketone in **1a** or **2a**, without significantly affecting the ability to inhibit P-450$_{11\beta}$ activity. Two exceptions were notable. The *N*-acetyl derivative **1c** was a markedly poorer inhibitor of P-450$_{11\beta}$ activity compared to the other analogues, and the amine derivative **1d** appeared to inhibit P-450$_{11\beta}$ activity only at 1-mM concentrations. When analogue **1d** was examined more closely in the inhibition assay, multiple bands were observed in the TLC system used. These bands, which were present even in the absence of enzyme, did not correspond to the elution positions for DOC, the products, corticosterone and 18-hydroxy-DOC, or **1d**. Some interaction between **1d** and DOC, which is independent of P-450$_{11\beta}$ is thus suggested. This phenomenon was not observed with other analogues. The relative order of inhibition potency was found to be the same for the dipyridyl compounds **1a,f–g** and the phenyl derivatives **2a–d** possessing the same 1-substituents. Examination of these derivatives showed that the methylene analogues **1g** and **2d** demonstrated the greatest inhibition. These were followed in potency by the alcohols **1e** and **2b**. The chlorides **1f** and **2c** and ketones **1a** and **2a**, which were equipotent, showed the least relative inhibitory activity. The increased effectiveness of the methylene analogues to inhibit P-450$_{11\beta}$ activity demonstrates that there is no requirement for a heteroatom in the functionality of the 1-carbon, as suggested by a previous SAR study.[30] These observations suggest that there is some tolerance in the region at the active site of P-450$_{11\beta}$ where the functionality of the 1-carbon interacts. However, some limitations exist, possibly due to charge or steric factors.

We investigated the requirement for the geminal methyl groups for P-450$_{11\beta}$ activity by reexamining compounds **3a,b** in our assay. Napoli and Counsel had previously studied **3a,b** and found that they had relative potencies of 0.22 and 0.65, respectively, compared to metyrapone's value of 1.[30] In our system, the monomethylated derivative was equipotent as an inhibitor of P-450$_{11\beta}$ activity compared with metyrapone. The discrepancies observed between the present study and this previous SAR study may reflect differences in either the concentrations of DOC and other components in the assay systems used or experimental design, since the previous SAR study tested inhibitors at only one concentration. Our data, however, do support their initial conclusion that activity was not eliminated by removing one methyl group. This requirement for at least one methyl group may suggest a specific region at the active site of P-450$_{11\beta}$ for binding, or it may reflect a requirement of some conformational rigidity for inhibition. Addition of a second methyl group in the 2-position yielded analogue **2a**, which exhibited approximately a twofold enhancement of inhibition of P-450$_{11\beta}$ activity when compared with metyrapone (**1a**) or the monomethylated derivative **3b**. In summary, the inhibition of P-450$_{11\beta}$ activity with these derivatives demonstrated that (1) the A-ring phenyl derivatives **2a–d** were better inhibitors than the respective dipyridyl analogues, (2) the ketone in the 1-position can be replaced by various functionalities without reducing inhibition, and (3) at least one methyl group should be present in the 2-position to maintain inhibition.

Since the size and substituent effects of the aromatic A ring of metyrapone are presently unexplored, it is our

(34) Garegg, P. J.; Samuelsson, B. *J. Chem. Soc., Chem. Commun.* **1979**, 978.
(35) Garegg, P. J.; Samuelsson, B. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2866.
(36) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

hope that this area of the metyrapone molecule might accommodate an iodide atom. Therefore, A-ring phenyl metyrapone analogues would be candidates for radioiodination and for subsequent use as radiopharmaceutical agents that will selectivity localize in the adrenal cortex due to enzyme inhibition.

Experimental Section

Chemistry. Elemental analyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, MI. Infrared spectra were recorded on a Beckman IR Acculab 8 spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian EM-360A spectrophotometer. Ultraviolet spectra were recorded on a Beckman Model 35 Dual Beam UV/visible spectrophotometer. All melting points were taken on a Laboratory Devices Mel-Temp capillary melting point apparatus and are uncorrected. Metyrapone (**1a**) was a generous gift from Ciba-Geigy, Inc., Summit, NJ.

***N*-(2-Methyl-1,2-di-3-pyridylpropyl)acetamide (1c).** Metyrapone oxime[33] (**1b**; 0.97 g, 4.0 mmol) was dissolved in 12 mL of glacial HOAc. Zinc dust (0.65 g) was added, and the reaction was heated to 70 °C for 20 h. An additional 0.67 g of zinc dust was added, and the reaction was continually heated at 70 °C for 3 days. An additional 0.66 g of zinc dust was added, and the reaction was heated for another 24 h. The reaction was cooled, and concentrated NH_4OH was added to pH 8. The aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL). The CH_2Cl_2 layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated in vacuo. The crude residue (0.97 g) was placed on a silica gel column (30 × 210 mm) and eluted with EtOAc/MeOH (3:2) by the flash chromatographic technique described by Still et al.[36] The product was eluted after the unreacted starting material to yield the desired amide as a clear oil (0.83 g, 76.8%), which was crystallized from EtOAc to form a white solid: mp 133–135 °C; IR (KBr) 1720 (C=O), 1650 (N–H bending); UV (95% EtOH), λ_{max} 267 nm; ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 6 H, CH_3), 1.87 (s, 3 H, Ac), 5.34 (d, 1 H, N*H*Ac), 7.45 (m, 5 H, aromatic), 8.47 (m, 4 H, aromatic). Anal. ($C_{16}H_{19}N_3O$) C, H, N.

β,β-Dimethyl-α-3-pyridyl-3-pyridineethanamine Trihydrochloride (1d). The amide **1c** (0.20 g, 0.74 mmol) was dissolved in 2 mL of 1 N HCl and refluxed for 18 h. The H_2O was azeotroped off with EtOH under reduced pressure, and the solid residue was taken up in a minimum amount of methanol. Anhydrous Et_2O was added dropwise, and a white solid was precipitated and filtered to yield the trihydrochloride salt (0.249 g, 85.5%): mp 299–301 °C dec; IR (KBr) 3430 (NH_2) cm^{-1}; UV (H_2O) λ_{max} 268 nm; ^{1}H NMR ($CDCl_3$–free base) δ 1.41 (s, 3 H, CH_3), 1.47 (s, 3 H, CH_3), 2.13 (br, 2 H, NH_2), 4.23 (s, 1 H, CH), 7.45 (m, 4 H, aromatic), 8.56 (m, 4 H, aromatic). Anal. ($C_{14}H_{17}N_3$·3HCl) C, H, N.

3,3′-(2-Chloro-1,1-dimethyl-1,2-ethanediyl)bis[pyridine] (1f). The alcohol **1e**[37] (28.4 g, 0.107 mol) was dissolved in 350 mL of freshly distilled thionyl chloride, refluxed for 5 h, and then stirred for an additional 18 h at 25 °C. The thionyl chloride was removed under reduced pressure, and the oily residue was partitioned between Et_2O (200 mL) and cold 1 N HCl (400 mL). Solid Na_2CO_3 was added to basify the H_2O layer to pH 8 while the aqueous solution was cooled in an ice bath to 5 °C. The aqueous solution was extracted with CH_2Cl_2 (3 × 125 mL), the CH_2Cl_2 layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated under reduced pressure. Distillation of the residue produced a pale pink oil (16.1 g, 61.0%), bp 143 °C (0.4 mm), which solidified upon standing: mp 71–73 °C; ^{1}H NMR ($CDCl_3$) δ 1.53 (d, 6 H, CH_3), 5.04 (s, 1 H, CH), 7.30 (m, 4 H, aromatic), 8.45 (m, 4 H, aromatic). Anal. ($C_{14}H_{15}N_2Cl$) C, H, Cl, N.

3,3′-(1,1-Dimethyl-1,2-ethanediyl)bis[pyridine] (1g). The chloride **1f** (2.66 g, 10.8 mmol) was dissolved in 50 mL of methanol in a Parr bottle. Palladium on calcium carbonate (1.02 g) was added, and the reaction mixture was hydrogenated at an initial pressure of 40 psi for 18 h. The catalyst was removed by filtration through a Celite pad, and the methanol was removed under reduced pressure. The residue was partitioned between H_2O and EtOAc. The EtOAc layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated under reduced pressure. Distillation of the residue produced a clear oil (1.48 g, 64.6%): bp 138 °C (0.4 mmHg): UV (95% EtOH) λ_{max} 268 nm; ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 6 H, CH_3), 2.87 (s, 2 H, CH_2), 7.30 (m, 4 H, aromatic), 8.32 (m, 4 H, aromatic). Anal. ($C_{14}N_{16}N_2$) C, H, N.

3,3′-(2-Iodo-1,1-dimethyl-1,2-ethanediyl)bis[pyridine] (1h). The alcohol **1e**[37] (0.41 g, 1.8 mmol) was dissolved in 25 mL of toluene. Triphenylphosphine (1.42 g, 5.4 mmol), imidazole (0.37 g, 5.4 mmol), and iodine (0.46 g, 3.6 mmol) were added to the solution, and the reaction was refluxed for 24 h and stirred at 25 °C for an additional 24 h. The toluene layer and reaction flask were washed with 2 N HCl (3 × 30 mL). The aqueous layer was made alkaline to pH 8 with solid Na_2CO_3 and extracted with EtOAc (3 × 30 mL). The EtOAc layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated under reduced pressure. The residue (0.498 g) was purified by preparative thin-layer chromatography on a 2-mm silica gel plate eluted three times with EtOAc and one time with EtOAc/EtOH (5:1). The iodo compound **1h**, R_f 0.32 (silica gel; EtOAc/EtOH, 5:1), was carefully scraped from the plate to separate it from an impurity with a slightly higher R_f. The purified material (0.080 g) was recrystallized from petroleum ether (30–60 °C) to yield a white solid (0.045 g, 7.4%): mp 92–93 °C; ^{1}H NMR ($CDCl_3$) δ 1.57 (d, 6 H, CH_3), 5.21 (s, 1 H, CH), 7.29 (m, 4 H, aromatic), 8.38 (m, 4 H, aromatic). Anal. ($C_{14}H_{15}N_2I$) C, H, N, I.

3,3′-(2-Methoxy-1,1-dimethyl-1,2-ethanediyl)bis[pyridine] (1i). The alcohol **1e**[37] (0.4 g, 1.80 mmol) was dissolved in 5 mL of dry DMF and added to NaH (0.044 g, 1.83 mmol, 1.05 equiv). The solution was stirred for 30 min, and MeI (0.32 g, 2.28 mmol, 0.142 mL, 1.3 equiv) was added. The reaction was stirred at room temperature for 2.7 days. The DMF was removed under reduced pressure, and the residue was partitioned between saturated Na_2CO_3 and CH_2Cl_2. The CH_2Cl_2 was dried ($MgSO_4$) and filtered, and the filtrate was concentrated under reduced pressure. The residue was placed on a silica gel column (30 × 172 mm) and eluted with EtOAc/EtOH (6:1) to yield the methoxy ether **1i** as a clear oil (0.116 g, 27.4%). The remaining material was the starting alcohol **1e**, which could be eluted off after the product: ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 6 H, CH_3), 3.13 (s, 3 H, CH_3), 4.13 (s, 1 H, CH), 7.38 (m, 4 H, aromatic), 8.41 (m, 4 H, aromatic). The ether **1i** was converted to the dipicrate salt, a bright yellow solid, mp 215–217 °C, for elemental analysis. Anal. ($C_{15}H_{18}N_2O$·$C_{12}H_6N_6O_{14}$) C, H, N.

2-Methyl-1-phenyl-2-(3-pyridyl)-1-propanone (2a). The ketone (**3a**;[31] 2.0 g, 10.0 mmol) was dissolved in 20 mL of anhydrous *t*-BuOH in a three-neck flask fitted with a mechanical overhead stirrer, a condenser, and a dropping funnel under an argon atmosphere. KO-*t*-Bu (2.36 g, 0.31 mol, 2.10 equiv) was added in one batch, and the solution was refluxed for 5 h. Methyl iodide (3.12 g, 22.0 mmol, 1.37 mL, 2.2 equiv) was combined with 2 mL of *t*-BuOH in the dropping funnel and added to the reaction vessel dropwise over a 1-h period. The reaction was refluxed for an additional 2 h, cooled to 25 °C, and poured over ice. The pH of the aqueous solution was adjusted to 2 with concentrated HCl and extracted with Et_2O (3 × 40 mL). The H_2O layer was made basic (pH 8) with solid Na_2CO_3 and extracted with CH_2Cl_2 (3 × 40 mL). The CH_2Cl_2 layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated under reduced pressure. The residue was flash chromatographed[36] on a silica gel column (42 × 235 mm) and eluted with EtOAc/hexane (1:1) to yield the dimethylated product **2a** (0.84 g, 37.3%). The oily ketone **2a** could be distilled, bp 118 °C (0.07 mmHg), or crystallized from hexane to produce a white amorphous solid: mp 71–72 °C; IR (KBr pellet) 1668 (C=O) cm^{-1}; UV (95% EtOH) λ_{max} 253 nm; ^{1}NMR ($CDCl_3$) δ 1.69 (s, 6 H, CH_3), 7.31 (m, 7 H, aromatic), 8.50 (m, 2 H, aromatic). Anal. ($C_{15}H_{15}NO$) C, H, N.

The monomethylated ketone **3b** could be eluted from the column after **2a** as a pale yellow oil (0.70 g, 33.2%). This ketone **3b** can also be prepared and crystallized as described previously.[30]

β,β-Dimethyl-α-phenyl-3-pyridineethanol (2b). The propiophenone **2a** (0.18 g, 0.81 mmol) was dissolved in 10 mL of methanol. Sodium borohydride (0.095 g, 2.5 mmol) was added, and the reaction was stirred at 25 °C for 3 h. H_2O (1 mL) was added, and the reaction was stirred an additional 0.5 h. The

(37) Kraulis, I.; Traikov, H.; Li, M. P.; Lantos, C. P.; Birmingham, M. K. *Can. J. Biochem.* **1968**, *46*, 463.
(38) Nedergaard, J.; Cannon, B. *Methods Enzymol.* **1979**, *55*, 3.

methanol was removed under reduced pressure, and the residue was partitioned between Et_2O and aqueous 1 N HCl. The aqueous layer was made basic to pH 8 with solid Na_2CO_3 and extracted with CH_2Cl_2 (3 × 20 mL). The CH_2Cl_2 layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated in vacuo. The crude material was then recrystallized from CH_2Cl_2/petroleum ether (30–60 °C) to yield the alcohol as a white solid (0.103 g, 56.0%): mp 106–108 °C; IR (KBr pellet) 3180 (OH) cm^{-1}; UV (95% EtOH) λ_{max} 267 nm; ^1H NMR ($CDCl_3$) δ 1.36 (s, 6 H, CH_3), 3.07 (br, 1 H, OH), 4.68 (s, 1 H, CH), 7.10 (m, 6 H, aromatic), 7.58 (m, 1 H, aromatic), 8.37 (m, 2 H, aromatic). Anal. ($C_{15}H_{17}NO$) C, H, N.

3-(2-Chloro-1,1-dimethyl-2-phenylethyl)pyridine (2c). The alcohol **2b** (0.39 g, 1.7 mmol) was dissolved in 25 mL of thionyl chloride, refluxed for 6 h, and then stirred at 25 °C for an additional 16 h. The thionyl chloride was removed under reduced pressure, and the oily residue was partitioned between Et_2O (40 mL) and cold 1 N HCl (40 mL). Solid Na_2CO_3 was added to basify the H_2O layer to pH 8 while the aqueous solution was cooled in an ice bath to 5 °C. The aqueous solution was extracted with CH_2Cl_2 (3 × 25 mL), and the CH_2Cl_2 layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated under reduced pressure. Distillation of the residue produced the chloro compound as a clear oil (0.28 g, 67.4%): bp 128 °C (0.4 mm); ^1H NMR ($CDCl_3$) δ 1.55 (s, 3 H, CH_3), 1.62 (s, 3 H, CH_3), 5.07 (s, 1 H, CH), 7.13 (m, 6 H, aromatic), 7.58 (m, 1 H, aromatic), 8.58 (m, 2 H, aromatic). Anal. ($C_{15}H_{16}ClN$) C, H, N, Cl.

3-(1,1-Dimethyl-2-phenylethyl)pyridine (2d). The chloride **2c** (0.150 g, 0.61 mmol) was dissolved in 40 mL of absolute methanol. Palladium on calcium carbonate catalyst (0.065 g) was added, and the reaction was hydrogenated at an initial pressure of 35 psi. The hydrogenation was terminated after 22 h, and the catalyst was filtered through a Celite pad. The solvent was removed under reduced pressure. The residue was taken up in CH_2Cl_2, and the solid $CaCO_3$ was filtered off. The remaining oil was vacuum distilled to yield a clear oil (0.128 g, 87.5%): bp 110 °C (0.4 mmHg); ^1H NMR ($CDCl_3$) δ 1.26 (s, 6 H, CH_3), 2.77 (s, 2 H, CH_2), 7.10 (m, 7 H, aromatic), 8.42 (m, 2 H, aromatic). The product **2d** was converted to the picrate salt, a bright yellow solid, mp 131–133 °C, for elemental analysis. Anal. ($C_{15}H_{17}N$) C, H, N.

Biochemistry. Adrenal cortical mitochondria were isolated, lyophilized, and defatted by a modification of the methods of Nedergaard and Cannon[38] and Napoli and Counsell,[30] and adrenodoxin was purified by a modification of the methods of Kimura and Suzuki and Kumura et al.[39,40] $P\text{-}450_{11\beta}$ activity was assayed with DOC as the substrate by a modification of Sato et al.[14] The reaction mixture, 0.50 mL, was modified to contain 50 mM potassium phosphate buffer, pH 7.40, 200 μM DOC (0.10 μCi of [1,2-^{3}H(N)]DOC), 16 mM $MgCl_2$, 1.5 mM NADPH, 4.6% propylene glycol, 15.0 μM adrenodoxin, and 300 μg of the crude, defatted mitochondrial preparation. The reaction mixture was preincubated at 37 °C for 5 min, and the reaction was initiated by the addition of NADPH and incubated for various times at 37 °C. Reaction controls were done in the absence of NADPH or using a heat-inactivated mitochondrial preparation. The reaction was terminated by the addition of 3.0 mL of methylene chloride containing 5 μg/mL each of DOC and CC and vortexed for 30 s. After the aqueous phase was extracted and discarded, the organic phase was dried down overnight at 30 °C. The residue was reextracted with 100 μL of 100% methanol and applied onto Whatman K6F silica gel glass TLC plates, which were developed in chloroform/acetone (5:1, v/v). Each zone on the plates was scraped, placed into a scintillation vial, extracted with methanol, and counted in a Beckman LS 7500 microprocessor controlled liquid scintillation system after the addition of 10 mL of scintillant.

Stock solutions of the inhibitors were made in 100% ethanol. Inhibitors were added to the reaction mixtures of each assay prior to the addition of NADPH such that a final concentration of 1% ethanol was obtained. The percent inhibition, at varying concentrations of each inhibitor, was calculated by using controls in the presence of 1% ethanol. Characterization of the mitochondrial preparation demonstrated (1) the enhancement of $P\text{-}450_{11\beta}$ activity by the addition of 15.0 μM adrenodoxin, (2) that the addition of 1% ethanol decreased $P\text{-}450_{11\beta}$ activity by less than 10%, and (3) that the apparent K_m of DOC for $P\text{-}450_{11\beta}$ was 6.78 μM. All incubations were run in duplicate, and experiments were repeated one or more times. The IC_{50} (μM) values were obtained from the linear portion of semilogarithmic plots of percent inhibition vs. concentration. Five or more inhibitor concentrations were used in all IC_{50} (μM) determinations. The data were fitted to a least-squares regression analysis, and the point of 50% inhibition was selected. The correlation coefficient indicates the fit of the line to the experimental points.

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Registry No. **1a**, 54-36-4; **1b**, 87372-66-5; **1c**, 87372-67-6; **1d**, 87372-68-7; **1d** (base), 87372-69-8; **1e**, 17159-42-1; **1f**, 87372-70-1; **1g**, 87372-71-2; **1h**, 87372-72-3; **1i**, 87372-73-4; **1i** dipicrate, 87372-74-5; **2a**, 87372-75-6; **2b**, 87372-76-7; **2c**, 87372-77-8; **2d**, 87372-78-9; **2d** picrate, 87372-79-0; **3a**, 1081-48-7; **3b**, 62144-16-5; steroid 11β-hydroxylase, 9029-66-7; cytochrome P-450, 9035-51-2.

(39) Kimura, T.; Suzuki, K. *J. Biol. Chem.* **1967**, *242*, 485.
(40) Kimura, T.; Parcells, J. H.; Wang, H.-P. *Methods Enzymol.* **1978**, *52*, 132.