New Inhibitors of Steroid 11β-Hydroxylase. Structure–Activity Relationship Studies of Metyrapone-Like Compounds

Joseph L. Napoli and Raymond E. Counsell*

Laboratory of Medicinal Chemistry, College of Pharmacy, The University of Michigan, Ann Arbor, Michigan 48109.

Received July 20, 1976

A series of metyrapone analogues was synthesized for study as inhibitors of steroid 11β-hydroxylase. Racemic mixtures of the new compounds were evaluated in vitro. Preliminary results revealed several analogues to be effective inhibitors of deoxycorticosterone hydroxylation. 2-(3-Pyridyl)propiophenone (**13**) and α,β-diphenyl-3-pyridineethanol (**16**) were the most active new compounds. Each was 65% as potent as metyrapone. 3-Pyridyl α-3-pyridylbenzyl ketone (**3**), 2-phenyl-2-(3-pyridyl)acetophenone (**4**), α-(diphenylmethyl)-3-pyridinemethanol (**17**), and 1,2-di-3-pyridyl-1-propanol (**26**) were 52, 32, 25, and 41% as inhibitory as metyrapone, respectively. Diphenylmethyl 3-pyridyl ketone (**5**), benzyl 3-pyridyl ketone (**10**), 2-(3-pyridyl)acetophenone (**12**), 2-phenyl-1-(3-pyridyl)-1-propanone (**11**), α,β-di-3-pyridylphenethyl alcohol (**15**), and 1,2-di-3-pyridylethanol (**27**) had less than 25% the activity of metyrapone. All compounds displaying a metyrapone-like inhibition contained appropriately substituted alcoholic or ketonic functions. A phenyl or methyl group α to the carbon bearing the oxygen was necessary for appreciable activity. A 3-pyridyl group α to the carbonyl carbon could be replaced by a phenyl group. For optimal activity, however, the other 3-pyridyl group of metyrapone could not be exchanged for a phenyl group.

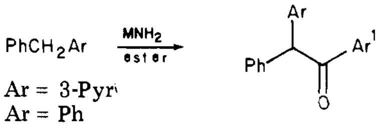
The adrenal cortex steroid hydroxylases have been identified as members of the cytochrome P-450 family of oxidoreductases. These enzymes are lipid-associated hemoproteins which require both a source of reducing equivalents—in the form of NADPH—and molecular oxygen to function in adrenocorticogenesis. Mitochondrial P-450 systems are involved in the conversion of cholesterol to pregnenolone, cortisol, and aldosterone through hydroxylations at the 20, 22, 11β, and 18 positions of the steroid skeleton. Microsomal P-450 systems, on the other hand, biosynthesize precursors to cortisol and aldosterone through 17α and 21 hydroxylations. Each cytochrome P-450 hydroxylase determines the regiospecificity and stereoselectivity of these reactions.[1]

Selective inhibition of specific hydroxylases could conceivably result in willful control of adrenal steroid hormone output. Such control would have important heuristic, diagnostic, and therapeutic implications. One attractive control point is the inhibition of the 11β-hydroxylase. Interference with this enzyme would limit biosynthesis of cortisol and aldosterone without hindering production of pregnenolone or progesterone. Although a number of inhibitors of steroid 11β-hydroxylase are known, no fully acceptable agent is available.

Metyrapone (2-methyl-1,2-di-3-pyridyl-1-propanone, **28**) is perhaps the most prominent steroid hydroxylase inhibitor that has been investigated. This relatively nontoxic, competitive inhibitor of 11β-hydroxylase has an inhibitor constant about 50 times less than the Michaelis constant for the hydroxylation of deoxycorticosterone (DOC).[2] Metyrapone has been widely used in cytochrome P-450 studies[2-5] and in the diagnostic evaluation of "pituitary-adrenal reserve".[6,7] Therapeutically, it has been useful in treating patients with autonomously functioning adrenal neoplasms, Cushing's disease resulting from adrenal tumors, and ectopic ACTH syndrome.[6] The usefulness of this compound, however, is limited by its short half-life in vivo[8,9] and by its nonselectivity—it interacts with all adrenal mitochondrial cytochromes P-450 and also with hepatic microsomal cytochrome P-450.[10-13] Consequently, the need remains for selective inhibitors of specific steroid hydroxylases, both as potential drugs and as biochemical probes.

This paper describes the synthesis and evaluation of a series of metyrapone analogues as inhibitors of steroid 11β-hydroxylase. The purpose of this study was threefold: to conduct a rational structure–activity relationship study of metyrapone; to obtain agents that could ultimately be used to probe the stereochemical requirements of steroid 11β-hydroxylase inhibition; and to establish a basis for approaches to selective inhibitors of steroid 11β-hydroxylase.

Synthesis. 3-Pyridyl α-3-pyridylbenzyl ketone (**3**) and 2-phenyl-2-(3-pyridyl)acetophenone (**4**) were synthesized by condensation of 3-benzylpyridine (**1**) with either methyl nicotinate (**6**) or methyl benzoate. Diphenylmethyl 3-pyridyl ketone (**5**) was obtained by condensation of diphenylmethane (**2**) with **6**. Either sodium or potassium amide was used to effect these reactions. Methyllithium, lithium diisopropylamide (LDA), and lithium diethylamide failed to effect condensation between **2** and **6**. No deuterium was incorporated into **2** upon quenching solutions of **2** and methyllithium or lithium dialkylamide in ether with deuterium oxide. Although LDA did effect condensation between **1** and **6**, there was no apparent advantage in using LDA over the more readily available sodium or potassium amide.



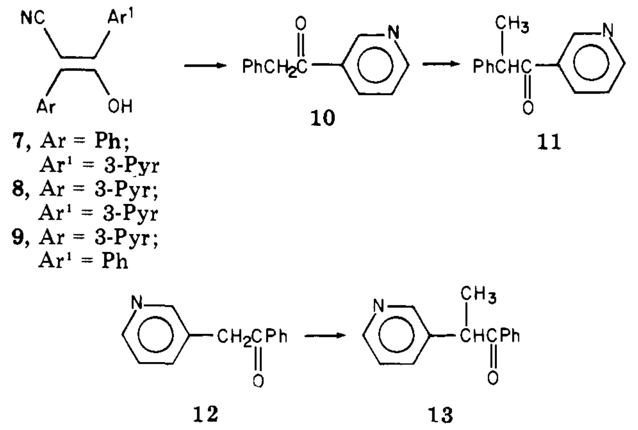
1, Ar = 3-Pyr
2, Ar = Ph

3, Ar = Ar^1 = 3-Pyr
4, Ar = 3-Pyr; Ar^1 = Ph
5, Ar = Ph; Ar^1 = 3-Pyr

Optimal yields were obtained when the ratio base–diarylmethane–ester was 2:2:1.[14] The requirements for 2 equiv of base is understandable, since the methine protons in the ketonic products are more acidic than the benzylic protons of **1** and **2**. However, use of 2 equiv of base and 1 equiv each of diarylmethane and ester decreased yields by about 50%. This phenomenon may result from competition between the diarylmethane anion and the highly nucleophilic amide anion for ester.

Approaches to the remaining ketones envisaged intermediacy of hydroxyacrylonitriles **7**, **8**, and **9** (Scheme I). These compounds were synthesized by known procedures.[15-18] Acid hydrolysis–decarboxylation of **7**, by the published method, provided benzyl 3-pyridyl ketone (**10**).[15] Attempts to recrystallize **10** from EtOH–H_2O, as reported, result in its cleavage to nicotinic acid and phenylacetonitrile. Therefore, **10** was recrystallized from EtOAc. In contrast, attempted hydrolysis–decarboxylation of either **8** or **9** failed to provide the expected 3-pyridyl 3-pyridylmethyl ketone or 2-(3-pyridyl)acetophenone (**12**), respectively. Consequently, **12** was produced by conden-

Scheme I



7, Ar = Ph; Ar¹ = 3-Pyr
8, Ar = 3-Pyr; Ar¹ = 3-Pyr
9, Ar = 3-Pyr; Ar¹ = Ph

10

11

12

13

sation of β-picoline with methyl benzoate by a known procedure.[19,20]

The acid-catalyzed cleavage of 8 and 9, in contrast to the hydrolysis of 7, may result from a weakened carbon–carbon σ bond between the carbon bearing the nitrile and the carbonyl carbon in the keto forms of 8 and 9. The greater inductive effect of the pyridyl ring with respect to the phenyl ring is apparently capable of increasing the polarization of this bond to the extent that cleavage occurs faster than nitrile hydrolysis. The moderate yield (67%) of 10 on hydrolysis and decarboxylation of 7 may result from a competing cleavage reaction. Cleavage of 7 to nicotinic acid and phenylacetonitrile under conditions ($EtOH-H_2O$) which were insufficient to effect nitrile hydrolysis supports this view.

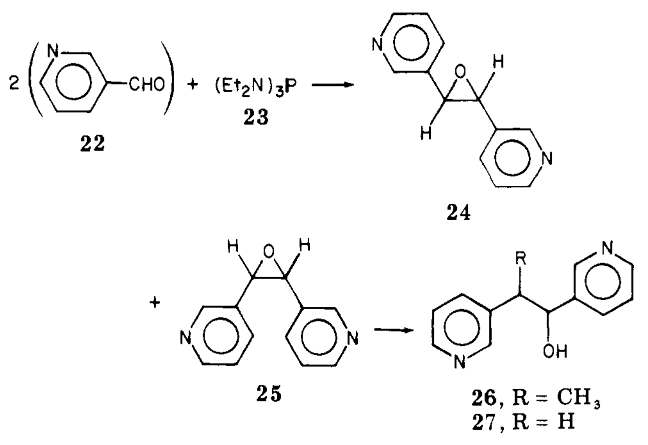
The IR spectra of 7, 8, and 9 exhibited moderate–strong broad absorption characteristic of intramolecularly bonded O–H stretching vibrations (2400–2800 cm^{-1}). There was no absorbance in the region assigned to aryl ketone carbonyl stretching vibrations (1600–1700 cm^{-1}). This was in contrast with the spectra of compounds 3–5 and 11–13 which exhibited strong carbonyl absorption in this region. Moreover, the NMR spectra of 7, 8, and 9 displayed one-proton singlets characteristic of enols between δ 9.0 and 10.8, whereas there was no substantial evidence for the presence of enolic forms in the NMR spectra of 3–5 and 11–13.

Methylation of 10 and 12 gave 11 and 13, respectively. C-Methylation of such compounds may be accompanied by O- and N-methylation. N-Methylation was avoided by using strong bases,[21] and O-methylation did not pose a serious problem. The oils isolated after methylation of 10 and 12 contained both C- and O-methylated products in a 6:1 ratio, as established by NMR. Ketones 11 and 13 were obtained free of the *O*-methyl isomers by fractional crystallization.

Metyrapol (2-methyl-1,2-di-3-pyridyl-1-propanol, 14) and related alcohols 15–21 were obtained by reducing the appropriate ketones with sodium borohydride in absolute ethanol. Completion of the reaction could usually be estimated by observing the disappearance of the yellow borohydride–ketone complex. Quantitative yields were indicated in all cases by TLC. Alcohols 26 and 27 were obtained from epoxide 25.

Treatment of 3-pyridinecarboxaldehyde (22) with hexaethylphosphorus triamide (23) produced a 94% yield of a 1:1 mixture of *trans*- and *cis*-3,3′-(epoxyethylene)dipyridine (24 and 25), as indicated by NMR (Scheme II).[22] From this crude reaction mixture a 30% yield of *cis*-epoxide (25) was isolated. All attempts to isolate pure *trans*-epoxide (24) failed.

Scheme II



22

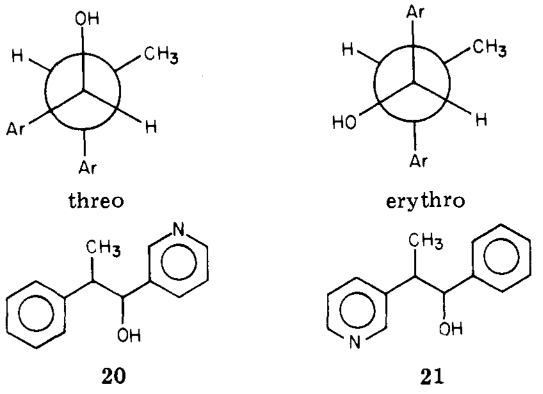
23

24

25

26, R = CH_3
27, R = H

Chart I



20

21

Recent work has shown that lithium diorganocuprates are capable of adding to aliphatic epoxides to produce propanols.[23-27] Although there are no examples of these reagents reacting with aryloxiranes, there was no apparent reason why such a reaction would not succeed. Lithium dimethylcuprate(I) in ether was obtained from the reaction of methyllithium (2 equiv) with purified copper(I) iodide. Treatment of epoxide 25 with a 2 M excess of the lithium–copper reagent gave a 76% yield of 26.

Since such reactions are known to proceed by trans S_N2 additions, the product of nucleophilic attack of *cis*-25 should be *threo*-26.[22,26] Further evidence for the configuration of 26 was provided by comparing the NMR spectra of 20, 21, and 26. In contrast to 26 which represents only one of the possible two pairs of enantiomers, 20 and 21 were a mixture of both threo and erythro forms. NMR spectra of 20 and 21 exhibited two doublets for the methyl group, whereas the spectrum of 26 displayed only one. The chemical shifts of these were 20, δ 1.08 and 1.32; 21, δ 1.10 and 1.31; and 26, δ 1.28. Assigning these chemical shifts to the corresponding diastereomeric forms can be accomplished by considering the environment of the methyl group in each isomer. The rotamers of greatest stability in the isomeric forms of the 1,2-diphenyl-1-propanols are shown in Chart I.[28] One would expect these relationships to be true for alcohols 20, 21, and 26. These projections indicate that the methyl group of the erythro isomer lies slightly below the plane of the aryl group, whereas the methyl group of the threo isomer is not influenced as significantly by the aryl moiety. Consequently, the *threo*-methyl group is deshielded relative to the *erythro*-methyl group. The threo isomer, then, would be the one with the greater chemical shift downfield from tetramethylsilane. This establishes 26 as threo. In further support of this assignment, the chemical shift of the methyl

Table I. Inhibitors of Steroid 11β-Hydroxylase[a]

No.	Compound	% I[b]	Rel potency[c]
3	$ArCH(Ph)COAr$[d]	35	0.52
4	$ArCH(Ph)COPh$	22	0.32
5	$PhCH(Ph)COAr$	15	0.22
10	$PhCH_2COAr$	9	0.13
11	$PhCH(CH_3)COAr$	13	0.19
12	$ArCH_2COPh$	15	0.22
13	$ArCH(CH_3)COPh$	44	0.65
14	$ArC(CH_3)_2CH(OH)Ar$	44	0.65
15	$ArCH(Ph)CH(OH)Ar$	9	0.13
16	$ArCH(Ph)CH(OH)Ph$	44	0.65
17	$PhCH(Ph)CH(OH)Ar$	17	0.25
26	$ArCH(CH_3)CH(OH)Ar$	28	0.41
27	$ArCH_2CH(OH)Ar$	7	0.10
28	$ArC(CH_3)_2COAr$	68	1.00

[a] Inhibitor concentrations were 2 μM relative to a DOC concentration of 22 μM. [b] Inhibition of DOC hydroxylation. [c] Potency relative to metyrapone, 28. [d] Ar = 3-pyridyl.

group of *threo*-1,2-diphenyl-1-propanol was reported to be δ 1.5 whereas that for the erythro isomer was δ 1.2.[29]

Reduction of **26** by lithium aluminum hydride in tetrahydrofuran gave the desired 1,2-di-3-pyridylethanol (**27**) in 89% yield. The yield was lower when ether was employed as solvent.

Biological Evaluation. The efficacy of metyrapone and several of the new compounds to inhibit the transformation of DOC to corticosterone was compared in vitro. An acetone powder of bovine adrenocortical mitochondria was the source of steroid 11β-hydroxylase. Prior to evaluating new compounds, the Michaelis constant (K_M) of DOC hydroxylation was determined to be 25 μM. Moreover, the inhibitor constant (K_I) for metyrapone inhibition was found to be 0.58 μM. These values agreed well with the previously published values of 30 and 0.74 μM, respectively.[2]

In the absence of inhibitor, 2.3 ± 0.04 nmol of corticosterone was biosynthesized from 22 nmol of DOC in 5 min and 1.3 ± 0.04 nmol of corticosterone was produced from 11 nmol of DOC in 5 min. With 2.0 nmol of metyrapone present, the amount of corticosterone produced from 22 nmol of DOC decreased to 0.74 nmol/5 min ± 0.02 (68 ± 1.0% inhibition). Likewise, the amount of corticosterone produced from 11 nmol of DOC decreased to 0.39 nmol/5 min ± 0.03 (70 ± 2.0% inhibition). These results represent the mean of six determinations ± the standard error of the mean.

Results of the inhibition studies are summarized in Table I. Each value, except metyrapone, was the mean of two determinations. The actual values for inhibition varied ±4% from the mean for compounds **11** and **13**, and ±7% for compound **27**. The remainder differed ±2% from the mean.

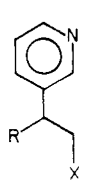
Metyrapol (**14**) was only 65% as active as metyrapone (**28**). Removal of one of the methyl groups from **14** (compound **26**) decreased the efficiency of inhibition. Although *threo*-**26** was only 63% as effective as **14**, it was still a potent inhibitor (28% inhibition at 2.0 μM). This fact suggested the nonessentiality of one of the two methyl groups in **14**. Removal of both methyl groups from **14** (alcohol **27**) radically diminished inhibition potency. Compound **27** was barely at the threshold of activity. Addition of a phenyl group to **27** (analogue **15**) increased activity slightly. Both **15** and **27** were racemic mixtures. *threo*-**26** represented only one of two pairs of enantiomers. The observed inhibition would be greater should one of the optical isomers of these agents bind preferentially to steroid 11β-hydroxylase. Thus, the inhibitory activity expressed by **26** suggests that resolution into its four optical isomers would provide an opportunity to examine the stereochemical requirements of P-$450_{11\beta}$. Compound **16** was equipotent with **14** despite several molecular modifications, the most notable being replacement of the 3-pyridyl ring with a phenyl ring. In contrast, substitution of a phenyl ring for the other 3-pyridyl ring (analogue **17**) caused a decrease in activity.

In the ketone series, replacement of both methyl groups in **28** with a single phenyl group (ketone **3**) reduced the inhibitory activity by one-half. However, some of the observed decrease may result from lesser activity of one of the enantiomers of **3**. In other words, one enantiomer could conceivably be nearly as effective as **28**. Ketone **13** was 65% as effective as **28**, despite loss of one methyl group and exchange of one 3-pyridyl ring for a phenyl ring, relative to **28**. Replacement of the lone methyl group of **13** with a phenyl group (analogue **4**) further decreased activity. But allowing for preferential binding of one enantiomer, **4** is still a potent inhibitor of P-$450_{11\beta}$. Absence of any bulky group α to the carbonyl (ketone **12**) resulted in an inhibitor of lower potency. Exchange of the 3-pyridyl ring α to the carbonyl for phenyl (ketone **13**) caused only minor diminution of activity; however, exchange of the other 3-pyridyl ring (ketone **11**) caused significant loss of potency.

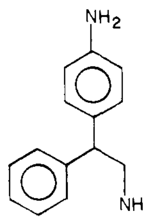
The relationship between oxidation state and biological activity was irregular. Alcohol **16** had twice the activity of the corresponding ketone **4**. On the other hand, alcohol **17** and ketone **5** were nearly equipotent. Unexpectedly, ketone **3** was a more effective inhibitor than **15** by a factor of 4.

The results of these preliminary tests suggest that an appreciable degree of flexibility is possible in the structures of metyrapone-like inhibitors of P-$450_{11\beta}$. Activity was not eliminated by removing a methyl group or by replacing both methyl groups with a phenyl group. Alcohols and ketones were both active. The most active new inhibitors were the monopyridyl derivatives **13** and **16**, while the least active were those in which either the 3-pyridyl α to the bulky center of the molecule was replaced with a phenyl group or both methyl groups were replaced with hydrogen.

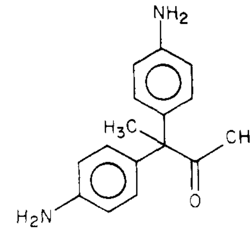
These results suggest that a molecule must contain at least the elements depicted in **29** for appreciable inhibitory activity. (R should not be hydrogen and X represents a heteroatom.) The P-$450_{11\beta}$ inhibitory activities of SKF-12185 and amphenone B can also be rationalized from this formalization—if one accepts replacement of the *p*-aminophenyl group for a 3-pyridyl group. The greater activity of SKF-12185 in rats (220 × **28**) and humans (1.7 × **28**)[30] may lend credence to such a view. Further studies in this direction should eventually provide the structural and stereochemical requirements essential for inhibition of steroid 11β-hydroxylation.[31]



29



SKF-12185



amphenone B

Experimental Section

Infrared spectra were recorded with a Perkin-Elmer Model 337 spectrophotometer as KBr pellets. NMR spectra were obtained with a Varian A-60A spectrometer, in parts per million downfield from Me_4Si in $CDCl_3$, unless otherwise indicated. Ultraviolet spectra were obtained, nm (ϵ), with a Beckman DB-GT grating spectrophotometer. All compounds gave spectra consistent with assigned structures. Melting points were measured with a Mel-Temp apparatus and are uncorrected. Elemental analyses were performed by Midwest Microlab, Ltd., Indianapolis, Ind. Analyses were all within acceptable limits (±0.25%). Thin-layer chromatography was done with polyethylene-supported aluminum oxide plates with fluorescent indicator purchased from Brinkmann, Westbury, N.Y. Eastman polyethylene backed silica gel plates with fluorescent indicator were also used. Column chromatography was performed with standardized aluminum oxide (activity II–III) purchased from Brinkmann. THF was dried by distillation from CaH_2. Peroxides were removed by passing THF through an alumina column. Et_2O was dried by distillation from $LiAlH_4$. For use with $Li(CH_3)_2Cu$ or $LiNR_2$, Et_2O was distilled under an inert gas. Et_2NH and $(i\text{-}Pr)_2NH$ were heated at reflux over sodium for several hours and distilled prior to use. Liquid ammonia used to prepare metal amides was distilled through KOH and condensed directly into the reaction vessel. β-Picoline was refluxed over barium oxide and distilled prior to use. Copper(I) iodide was extracted with dry, peroxide-free THF for 24 h, dried, and stored in an evacuated desiccator. Methyllithium was titrated with *i*-PrOH (1,10-phenanthroline indicator) immediately before use. 3-Benzylpyridine (1) was synthesized by Friedel–Crafts alkylation of benzene by 3-picolyl chloride hydrochloride as reported by Jerchel et al.[32] Commercially obtained starting materials were either recrystallized or distilled before use. Corticosterone and DOC were purchased from Steraloids, Pawling, N.Y. DOC-*4-*^{14}C (specific activity 59.8 nCi/nmol) and corticosterone-*1,2-*^{3}H (specific activity 40 nCi/nmol) were purchased from New England Nuclear, Boston, Mass. DOC-*4-*^{14}C was diluted with DOC to a specific activity of 2.5 nCi/nmol. PCS cocktail was purchased from Amersham/Searle, Chicago, Ill. NADPH was purchased from Sigma Chemical Co.

Synthesis of Diarylmethyl Aryl Ketones 3, 4, and 5. General Method. To a solution of KNH_2, prepared from K (3.1 g, 80 mmol) and liquid NH_3 (200 mL), under a N_2 atmosphere, diphenylmethane (13.4 g, 80 mmol) in Et_2O (40 mL) was added in 15 min. The resulting blood-red mixture was stirred for 10 min and methyl nicotinate (5.5 g, 40 mmol) in Et_2O (40 mL) was added in 5 min. The color changed to yellow. The NH_3 was evaporated by gently heating with a steam bath. As the volume decreased, Et_2O (125 mL) was added. Heating was continued until the Et_2O began to reflux. The reaction was quenched by addition of NH_4Cl (4.4 g, 80 mmol) in H_2O (125 mL). The layers were separated and the aqueous phase was extracted with Et_2O (2 × 200 mL). The combined Et_2O extracts were concentrated to 100 mL under diminished pressure and extracted with 1 N HCl (3 × 100 mL). The organic phase was washed with saturated NaCl and dried (Na_2SO_4). The solvent was evaporated to afford diphenylmethane (8.2 g). The combined acid extracts were neutralized (NaOH) and extracted with CH_2Cl_2 (3 × 200 mL). The combined extracts were washed with saturated NaCl and dried (Na_2SO_4). Solvent was removed under diminished pressure to provide crystalline **5** (8.3 g, 76%). Two recrystallizations from benzene–hexane afforded material for analysis: mp 94–96 °C; IR 1680 cm^{-1}; NMR δ 6.00 (s, 1 H, methine H). Anal. ($C_{19}H_{15}NO$) C, H.

Ketones **3** and **4** were prepared in a similar manner in 62 and 77% yields, respectively. The acid wash was omitted. Instead, fractionation under vacuum provided **3** [bp 194 °C (0.04 mm)] which was crystallized from Et_2O to provide an analytical sample: mp 93.5–95 °C; IR 1685 cm^{-1}; NMR δ 5.94 (s, 1 H, methine H). Anal. ($C_{18}H_{14}N_2O$) C, H.

Ketone **4** was similarly purified by vacuum distillation [bp 185 °C (0.1 mm)]. The distillate crystallized on standing. Recrystallization from Et_2O provided material for analysis: mp 89–91 °C; IR 1670 cm^{-1}; NMR δ 6.02 (s, 1 H, methine H). Anal. ($C_{19}H_{15}NO$) C, H.

2-Phenyl-1-(3-pyridyl)-1-propanone (11). To a solution of KNH_2 prepared from K (400 mg, 10 mmol) and liquid NH_3 (30 mL), **10** (2.0 g, 10 mmol) in Et_2O (35 mL) was added in 5 min. After 10 min of stirring, CH_3I (1.8 g, 13 mmol) in Et_2O (20 mL) was added to the dark solution in 20 min. Stirring was continued for 2 h while the NH_3 was allowed to evaporate. The reaction was quenched by the addition of NH_4Cl (10 mmol) in H_2O (50 mL). The layers were separated and the aqueous phase was extracted with Et_2O (2 × 30 mL). The combined Et_2O layers were washed with saturated NaCl and dried (K_2CO_3), and the solvent was removed under diminished pressure yielding an oil (2 g) which contained 60% *C*-methyl and 11% *O*-methyl **10** (NMR). The oil was crystallized in Et_2O–hexane in a dry ice–*i*-PrOH bath and recrystallized from Et_2O–pentane giving **11** (1 g, 48%). Two more recrystallizations from Et_2O–pentane provided material for analysis: mp 63–65 °C; IR 1675 cm^{-1} (C=O); NMR δ 1.52 (d, *J* = 7 Hz, 3 H, CH_3), 4.6 (q, *J* = 7 Hz, 1 H, CH). Anal. ($C_{14}H_{13}NO$) C, H.

2-(3-Pyridyl)propiophenone (13). To a solution of $NaNH_2$ made from Na (200 mg, 9 mmol) in liquid NH_3 (20 mL), **12** (1.4 g, 7 mmol) in Et_2O (15 mL) was added in 5 min. After 5 min of stirring, CH_3I (1.6 g, 11 mmol) in Et_2O (10 mL) was added. The dark red solution lightened. The reaction was stirred for 1.75 h while the NH_3 was allowed to evaporate. The reaction was quenched with NH_4Cl (50 mg) in H_2O (20 mL). The layers were separated and the aqueous phase was extracted with $CHCl_3$ (3 × 50 mL). The combined organic extracts were dried (K_2CO_3) and the solvent was removed under diminished pressure giving a dark oil. The oil was crystallized by stirring in Et_2O–hexane in a dry ice–*i*-PrOH bath yielding a white solid, 1.1 g (74%). Two recrystallizations from Et_2O–pentane provided material for analysis: mp 58–59 °C; IR 1670 cm^{-1}; NMR δ 1.52 (d, *J* = 6 Hz, 3 H, CH_3), 5.06 (q, *J* = 6 Hz, 1 H, CH). Anal. ($C_{14}H_{13}NO$) C, H.

$NaBH_4$ Reductions. To a stirred solution of ketone in absolute EtOH (10–20 mL), $NaBH_4$ (1.3 molar equiv) was added. The reaction mixture was stirred 10 min after the disappearance of the yellow ketone–borohydride complex. TLC ($CHCl_3$–alumina) indicated a quantitative yield of alcohol in all cases. Water was added, and stirring was continued 0.5 h. If product did not crystallize from this mixture, the solvent was concentrated under diminished pressure and extracted with CH_2Cl_2 (three times). The combined extracts were dried (K_2CO_3) and evaporated under diminished pressure to give the desired alcohol. Data (compound, recrystallization solvent, melting point, analysis): **14**, Et_2O, 100–102 °C, ($C_{16}H_{14}N_2O$) C, H; **15**, EtOH–H_2O, 165–167 °C, ($C_{18}H_{16}NO$) C, H; **16**, Et_2O–pentane, 110–112 °C, ($C_{19}H_{17}NO$) C, H; **17**, EtOH–H_2O, 131 °C, ($C_{19}H_{17}NO$) C, H; **18**, EtOH–H_2O, 105–107 °C, ($C_{13}H_{13}NO$) C, H; **19**, EtOH–H_2O, 125 °C, ($C_{13}H_{13}NO$) C, H; **20**, Et_2O–pentane, 90–91 °C, ($C_{14}H_{15}NO$) C, H; **21**, PhH–cyclohexane, 84–87 °C, ($C_{14}H_{15}NO$) C, H.

***cis*-3,3′-(Epoxyethylene)dipyridine (24).** To nicotinaldehyde (31.2 g, 0.3 mol) purged with dry nitrogen, a solution of $(Et_2N)_3P$ (42.0 g, 0.17 mol) in benzene–hexane (30 mL, 1:1) was added dropwise with stirring in 3 h. After 6 h, 94% of the aldehyde was converted into *cis*- and *trans*-epoxides (1:1, NMR). The crude reaction mixture was dissolved in H_2O (500 mL) and extracted with hexane (5 × 250 mL) and CH_2Cl_2 (5 × 250 mL). The solvent was removed from the combined CH_2Cl_2 extracts under diminished pressure. Benzene was added and distilled several times to remove H_2O. The dark oily residue was triturated with Et_2O–hexane in a dry ice–*i*-PrOH bath to yield a brown solid. This solid was immediately recrystallized from Et_2O to yield discolored *cis*-epoxide (8.5 g, 30%). The trans isomer remained in the mother liquor. Three further recrystallizations from Et_2O provided white needles: mp 87 °C; IR 1025 cm^{-1}; NMR δ 4.41 (s, 2 H, epoxide H). Anal. ($C_{12}H_{10}N_2O$) C, H.

1,2-Di-3-pyridyl-1-propanol (26). To a suspension of CuI (2.85 g, 15 mmol) in Et_2O (40 mL) stirred vigorously under argon, cooled in an ice bath, CH_3Li in Et_2O (15.5 mL of a 1.94 M solution) was introduced portionwise with a syringe. After 15 min of stirring, **25** (1.5 g, 7.5 mmol) in Et_2O (60 mL) was added portionwise. The resulting yellow suspension was stirred for 2.0 h at room temperature. The reaction was quenched by the cautious addition of NH_4OH–NH_4Cl. The Et_2O was decanted and the aqueous phase was filtered and extracted with $CHCl_3$ (5 × 60 mL). The combined organic extracts were filtered through Celite and the solvent was removed under diminished pressure giving an oil which was placed on an alumina column (200 g). Elution with $CHCl_3$ produced **25** (200 mg). Elution with 2% EtOH–$CHCl_3$ (v/v) yielded **26**, which crystallized on standing (1.22 g, 76%).

Three recrystallizations from Et_2O provided material for analysis: mp 88–90 °C; IR 3160, 2870 cm^{-1} (hydrogen bonded OH); NMR δ 1.28 (d, J = 7 Hz, 3 H, CH_3), 3.02 (m, 1 H, methine H), 4.70 (d, J = 6 Hz, 1 H, C*H*OH), 5.53 (br s, 1 H, exchanged with D_2O, OH). Anal. ($C_{13}H_{14}N_2O$) C, H.

1,2-Di-3-pyridylethanol (27). To a suspension of $LiAlH_4$ (100 mg, 2.6 mmol) in THF (10 mL), **25** (1.0 g, 5.0 mmol) in THF (20 mL) was added. The reaction was heated at reflux for 4 h and was quenched by the addition of H_2O (3 drops) and 15% NaOH (4 drops). The mixture was filtered and the residue was washed with Et_2O. Evaporation of the solvents under diminished pressure gave a yellow solid (890 mg, 89%). Two recrystallizations from Et_2O provided pale yellow crystals for analysis: mp 114–115.5 °C; IR 3160, 2850 cm^{-1} (hydrogen bonded OH); NMR δ 2.95 (d, J = 6 Hz, 2 H, CH_2), 4.92 (t, J = 6 Hz, 1 H, C*H*OH), 6.00 (br s, 1 H, exchanged with D_2O, OH). Anal. ($C_{12}H_{12}N_2O$) C, H.

Acetone Powder. Preparation and Reconstitution. Bovine adrenal glands were frozen on dry ice immediately after slaughter. They were brought to the laboratory and partially thawed to facilitate handling. Thereafter, all operations were performed at 0–4 °C. Fat, connective tissue, and the capsule were removed from each gland. The cortex was dissected from the medulla and suspended in buffer solution A (5 mL/g of cortex) of 0.25 M sucrose, 0.5% bovine serum albumin (BSA), and 0.01 M phosphate buffer, pH 7.4. The tissue was thoroughly minced with scissors and homogenized in a Waring blender (2 × 30 s). The homogenate was centrifuged for 10 min (650 g) with a Sorvall RC2-B refrigerated centrifuge. The supernatant was filtered through two layers of gauze. The nuclear pellets were washed with 0.25 times the original volume of buffer solution A. The combined supernatants were centrifuged for 15 min (9200 g). The mitochondrial pellet was washed twice in 0.25 times the original volume of buffer solution A without BSA and suspended in 0.1 times the original volume of buffer solution A without BSA. The suspension was added slowly with efficient stirring to 20 times its volume of cold (−20 °C) acetone. The residue was filtered with suction, washed well with cold (−20 °C) Et_2O, and dried in an evacuated desiccator over KOH at −20 °C for 2 days. Prior to use, the pink acetone powder was homogenized with a mortar and pestle. The acetone powder was reconstituted by suspension in 30% ethylene glycol–H_2O (12 mg of powder/0.4 mL). There was 4.1 mg of protein in 12 mg of powder as determined by the method of Lowry et al.[33]

Inhibition Studies. Incubations were conducted in 15-mL centrifuge tubes at 37 °C for 5 min in a Dubnoff metabolic shaker. Each tube contained: 0.4 M phosphate buffer (0.2 mL), pH 7.4; 12 mg of acetone powder in 0.4 mL of 30% ethylene glycol–H_2O; DOC-^{14}C in EtOH; 2.0 nmol of inhibitor in 0.02 mL of EtOH, or an equivalent amount of EtOH less inhibitor for controls; 1.3 mg of NADPH in 0.1 mL of H_2O; and H_2O to a total volume of 1.0 mL. For the determination of K_M, DOC-^{14}C was used in the concentration range from 5 to 20 μM and added in 0.05 mL of EtOH. In the inhibition studies, DOC-^{14}C (22 nmol) was added in 0.02 mL of EtOH. Incubation was started by addition of substrate after 5 min preincubation with NADPH. The reaction was quenched by 30 s of vigorous stirring (Vortex stirrer) of the incubation mixture with CH_2Cl_2 (3 mL). Blanks contained acetone powder disrupted by 30 s of vigorous stirring with CH_2Cl_2 (3 mL). Upon completion of incubation, 132 000 dpm of [^{3}H]corticosterone was added as an indicator of extraction efficiency, and 100 μg each of corticosterone and DOC was added as chromatographic markers. The aqueous layer was extracted with CH_2Cl_2 (2 × 3 mL). Emulsions were disrupted by centrifugation. The combined organic phases were dried (Na_2SO_4), filtered, and concentrated under vacuum. The remaining solvent was spotted on silica gel strips. Each 4 or 5 by 20-cm strip was developed with $CHCl_3$–acetone (5:1, v:v) and scanned for radioactivity with a Packard Model 7201 radiochromatogram scanner. The observed (UV light) cold substrate (R_f 0.51) and product (R_f 0.31) areas corresponded with the radioactive areas. The product area was cut from the plate and placed in PCS cocktail (10 mL). Radioactivity was measured with a Beckman LS-150 liquid scintillation system and counting efficiency was determined with an automatic external standard. Counts per minute were converted to disintegrations per minute (dpm). Dpm's were adjusted for extraction efficiency and converted to nanomoles of product formed.

Acknowledgment. We are grateful to Drs. Matthias C. Lu, Ahmed El Masry, Souheir E. El Masry, Duane L. Venton, and Vincent G. Zannoni for helpful discussions during the course of this work. We also appreciate financial support from the National Institutes of Health (GM-02010 and HL-11274) and from Horace H. Rackham School of Graduate Studies, The University of Michigan, which provided a dissertation grant.

References and Notes

(1) B. W. Harding, J. J. Bell, L. D. Wilson, and J. A. Whysner, *Adv. Enzyme Regul.*, **7**, 237 (1969).
(2) H. Schleyer, D. Y. Cooper, S. S. Levin, and O. Rosenthal in "Biological Hydroxylation Mechanisms", G. S. Boyd and R. M. S. Smellie, Ed., Academic Press, New York, N.Y., 1972, pp 187–205.
(3) B. W. Griffing, J. A. Peterson, J. Werringloer, and R. W. Estabrook, *Ann. N.Y. Acad. Sci.*, **244**, 107 (1975).
(4) A. G. Hildebrandt in ref 2, pp 79–102.
(5) M. Satre and P. V. Vignalis, *Biochemistry*, **13**, 2201 (1974).
(6) T. E. Temple and G. W. Liddle, *Annu. Rev. Pharmacol.*, **10**, 199 (1970).
(7) A. W. Meikle, S. C. West, J. A. Weed, and F. H. Tyler, *J. Clin. Endocrinol. Metab.*, **40**, 290 (1975).
(8) J. Jenkins, *Proc. R. Soc. Med.*, **60**, 908 (1967).
(9) G. F. Kahl, *Naunyn-Schmiedebergs Arch. Pharmacol.*, **266**, 61 (1970).
(10) K. Griffiths, *J. Endocrinol.*, **26**, 445 (1963).
(11) N. P. Sanzari and F. G. Peron, *Steroids*, **8**, 929 (1966).
(12) F. W. Kahnt and R. Neher, *Experientia*, **27**, 959 (1971).
(13) A. Carballeira, S. C. Cheng, and L. M. Fishman, *Acta Endocrinol.*, **76**, 689 (1974).
(14) R. Levine and S. Raynolds, *J. Org. Chem.*, **25**, 530 (1960).
(15) A. Burger and C. R. Walker, Jr., *J. Am. Chem. Soc.*, **72**, 1988 (1950).
(16) F. J. Villani, U.S. Patent 3 357 986 (1966); *Chem. Abstr.*, **67**, P27262y (1967).
(17) W. L. Bencze and G. N. Walker, U.S. Patent 3 337 567 (1967); *Chem. Abstr.*, **68**, P29609u (1968).
(18) W. L. Bencze and G. N. Walker, U.S. Patent 3 337 568 (1967); *Chem. Abstr.*, **68**, P87179r (1968).
(19) A. D. Miller, C. Osuch, N. N. Goldberg, and R. Levine, *J. Am. Chem. Soc.*, **78**, 674 (1956).
(20) S. Raynolds and R. Levine, *J. Am. Chem. Soc.*, **82**, 472 (1960).
(21) D. T. Hurst and M. Viney, *Tetrahedron Lett.*, **No. 36**, 3895 (1969).
(22) V. Mark, *Org. Synth.*, **46**, 32 (1966).
(23) R. W. Herr, D. M. Wieland, and C. R. Johnson, *J. Am. Chem. Soc.*, **92**, 3813 (1970).
(24) R. W. Herr and C. R. Johnson, *J. Am. Chem. Soc.*, **92**, 4979 (1970).
(25) D. M. Wieland and C. R. Johnson, *J. Am. Chem. Soc.*, **93**, 3047 (1971).
(26) C. R. Johnson, R. W. Herr, and D. M. Wieland, *J. Org. Chem.*, **38**, 4263 (1973).
(27) R. J. Anderson, *J. Am. Chem. Soc.*, **92**, 4978 (1970).
(28) J. L. Mateos and D. J. Cram, *J. Am. Chem. Soc.*, **81**, 2756 (1959).
(29) C. A. Kingsbury and D. C. Best, *J. Org. Chem.*, **32**, 6 (1967).
(30) J. G. Sprunt and D. M. Hannah, *J. Endocrinol.*, 41 (Proc. Soc. Endocrinol., xxvi) (1968).
(31) During preparation of this paper, a report demonstrated that the *R* enantiomer of aminoglutethimide possessed the adrenal steroidogenesis inhibitory activity: N. Finch, R. Dziemian, J. Cohen, and B. G. Steinetz, *Experientia*, **15**, 1003 (1975).
(32) D. Jerchel, S. Noetzel, and K. Thomas, *Chem. Ber.*, **93**, 2967 (1960).
(33) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).