Coenzyme–Substrate Adducts as Inhibitors of Mouse Liver 3,4-Dihydroxyphenylalanine Decarboxylase

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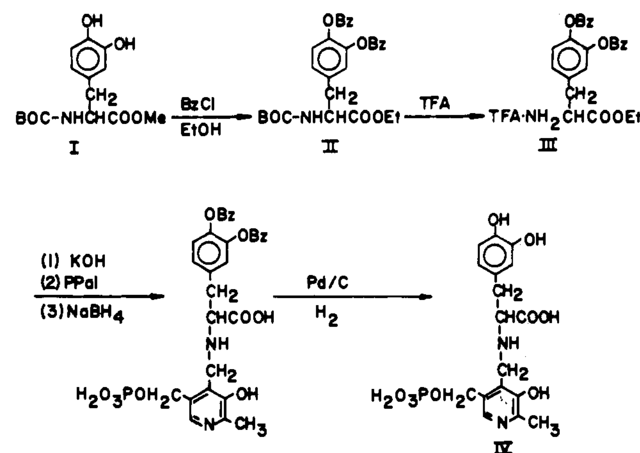
N-(5′-Phosphopyridoxyl) derivatives of several aromatic amino acids have been prepared by conventional methods and tested as inhibitors of mouse liver L-3,4-dihydroxyphenylalanine (Dopa) decarboxylase (EC 4.1.1.26; L-aromatic-amino-acid decarboxylase). The L-tyrosine, L-phenylalanine, and DL-2-hydroxyphenylalanine derivatives were effective inhibitors at concentrations of 10^{-5} M. Because of the spontaneous formation of a tetrahydroisoquinoline cyclic condensation product with pyridoxal phosphate (Pictet–Spengler reaction), the Dopa derivative could not be prepared by the usual procedures. The synthesis of the desired N-(5′-phosphopyridoxyl)-Dopa was accomplished using selective blocking–deblocking methods; its properties are described. This proved to be the most effective inhibitor of those tested. Neither the tetrahydroisoquinoline of L-Dopa and pyridoxal phosphate nor the N-(5′-deoxypyridoxyl)-Dopa was an effective inhibitor of Dopa decarboxylase. These coenzyme amino acid adducts are suggested to act as stage inhibitors of the enzyme.

3,4-Dihydroxy-L-phenylalanine (Dopa[1]) is decarboxylated by a pyridoxal phosphate dependent enzyme that is widely distributed in mammalian tissues, including kidney and liver.[2a] In the adrenals, Dopa decarboxylation is required in the synthesis of the catecholamine hormone, epinephrine.[2b] The enzyme responsible for the decarboxylation of Dopa to 3,4-dihydroxyphenylethylamine (dopamine) in hog kidney has been purified to homogeneity, and such preparations have been extensively studied.[1,3-5] In addition to its action on Dopa, the hog kidney enzyme is capable of effecting the decarboxylation of several other aromatic amino acids such as tryptophan, tyrosine, and phenylalanine.[4] For this reason, the enzyme is best described as an L-aromatic-amino-acid decarboxylase;[6] it is responsible for a significant fraction of the metabolism of the aromatic amino acids.[7]

This enzyme is of particular importance in the central nervous system, since biogenic amines derived from its action can serve as neurotransmitters; its distribution in the brain has been studied.[8] A deficiency of Dopa decarboxylase activity in the nigrostriatial region of human brain results in reduced levels of dopamine, leading to the condition commonly referred to as Parkinson's disease.[9] The clinical management of Parkinsonism is basically one of replacement therapy, i.e., replacement of dopamine. In light of the fact that dopamine itself does not gain access to the central nervous system from peripheral circulating fluids, the strategy of dopamine replacement has involved administration of large oral doses (8–12 g) of L-Dopa, which does cross the so-called blood–brain barrier and thus provides high levels of substrate for the residual Dopa decarboxylase activity in brain.[10] The requirement for such large doses of L-Dopa is a consequence of the fact that there is extensive peripheral decarboxylation of the drug (approximately 90%) by tissues and organs external to the central nervous system such as liver and kidney.[11] Recently, the amounts of L-Dopa used in the treatment of Parkinsonism have been greatly lessened by the availability of drugs which selectively inhibit the peripheral decarboxylation of L-Dopa, thereby making it more available for uptake by the central nervous system.[12]

We describe in this report the inhibitory effects of stable, covalently linked coenzyme–substrate adducts on the Dopa decarboxylase activity in mouse liver homogenates. Included among these inhibitors is N-(5′-phosphopyridoxyl)-L-Dopa, a compound which was prepared in a multistep synthesis made necessary by the fact that the usual procedures[13,14] for the preparation of phosphopyridoxyl amino acid adducts cannot be applied to the synthesis of this material. The compounds tested are suggested to act as stage inhibitors of Dopa decarboxylase, i.e., inhibitors whose action depends upon a structural similarity to a well-defined intermediate or stage in an enzyme-catalyzed reaction pathway.

Scheme I



Results

Synthesis and Properties of N-(5′-Phosphopyridoxyl)-Dopa. The procedure of Ikawa[13] was used to prepare the N-(5′-phosphopyridoxyl) derivatives of L-tyrosine, L-phenylalanine, and DL-2-hydroxyphenylalanine (DL-o-tyrosine). This method involves the reduction of the corresponding Schiff bases. However, this general approach is not applicable to the synthesis of N-(5′-phosphopyridoxyl)phenylalanine derivatives in which there is a strong electron-donating substituent in the 3 position of the amino acid (e.g., –OH, $-NH_2$) because of the activation of such compounds toward Pictet–Spengler condensations. In the case of Dopa, the Pictet–Spengler reaction leads to the rapid formation of the tetrahydroisoquinoline VI under neutral and alkaline conditions. In

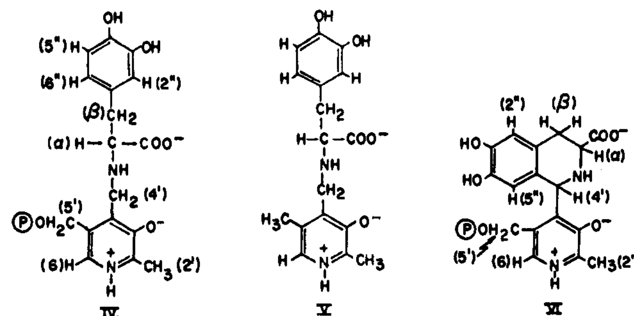


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Table I. Inhibition of Mouse Liver Aromatic-Amino-Acid Decarboxylase by N-(5'-Phosphopyridoxyl) Amino Acids[a]

inhibitor	concn, M	% inhibn ± av dev
N-(5'-phosphopyridoxyl)-L-phenylalanine	1×10^{-3}	83 ± 9 (3)
	1×10^{-4}	57 ± 4 (3)
	1×10^{-5}	17 ± 14 (3)
N-(5'-phosphopyridoxyl)-DL-o-tyrosine	2×10^{-3}	85 ± 4 (1)
	2×10^{-3}	62 ± 1 (2)
	2×10^{-5}	53 ± 2 (1)
DL-IV	2×10^{-5}	63 ± 2 (2)
DL-VI	1×10^{-3}	<10% (3)

[a] Assays were performed as described under Experimental Section. The numbers in parentheses are the number of mouse livers tested; each assay was done at least in duplicate, usually in triplicate.

order to obviate this problem, the synthetic approach outlined in Scheme I was developed. This lead to the preparation of the desired compound IV in 60% yield. The NMR and ultraviolet spectra of IV and VI and their interpretation are completely in accord with those reported by Raso and Stollar who employed similar techniques in the synthesis of N-(5'-phosphopyridoxyl)-3-amino-L-tyrosine and the tetrahydroisoquinoline derivative of 3-amino-L-tyrosine and pyridoxal 5'-phosphate.[15]

It it worthwhile noting that the catalytic hydrogenolysis in Scheme I leads to a mixture of products that can be separated on the carboxylic cation exchanger, Amberlite CG-50. The products are IV, monobenzylated derivatives of IV, and the debenzylated, reductively dephosphorylated derivative V (see Experimental Section). Prolonged hydrogenolysis over Pd/C leads exclusively to V, and, in fact, this procedure is suitable for the preparation of 5'-deoxy derivatives of pyridoxyl amino acids, pyridoxamine, and pyridoxine from the phosphorylated precursors.[16] In a similar reaction, reductive cleavage of benzyl phosphate esters has been used in nucleotide synthesis.[17]

Inhibition of Mouse Liver Dopa Decarboxylase by N-(5'-Phosphopyridoxyl) Amino Acids. Table I provides data on the inhibitory effects of 10^{-5} to 10^{-3} M concentrations of various N-(5'-phosphopyridoxyl) amino acids on the Dopa decarboxylase activity found in mouse liver homogenates. It can be seen in Table I that the most effective inhibitor is the DL-Dopa derivative. An important observation was the finding that the tetrahydroisoquinoline VI is not an inhibitor at concentrations as high as 10^{-3} M. Similarly, N-(5'-deoxypyridoxyl)-DL-Dopa (V) is a poor inhibitor, showing only 15% inhibition at a concentration of 2×10^{-4} M and 50% inhibition at 2×10^{-3} M.

Dose-response curves for the L-tyrosine and L-Dopa derivatives are provided in Figure 1. The concentrations of N-(5'-phosphopyridoxyl)-L-tyrosine and N-(5'-phosphopyridoxyl)-L-Dopa required to cause 50% inhibition are approximately 7×10^{-5} and 1×10^{-5} M, respectively. The inhibition of Dopa decarboxylase by N-(5'phosphopyridoxyl)-L-Dopa was determined to be noncompetitive in nature, as shown in Figure 2. The data in Figure 2 also allow for calculation of the K_m of the enzyme for L-Dopa. This was determined to be 8×10^{-5} M; the value for the K_m of purified hog kidney Dopa decarboxylase for its substrate has been reported to be 1.9×10^{-4} M at pH 7.0;[4] L-Dopa is by far the best substrate for the enzyme.[1,4,6]

Discussion

Reactions catalyzed by pyridoxal phosphate dependent enzymes proceed in "stages" that have been suggested to be accompanied by conformational changes in the structure of these enzymes.[18-20] For example, in trans-

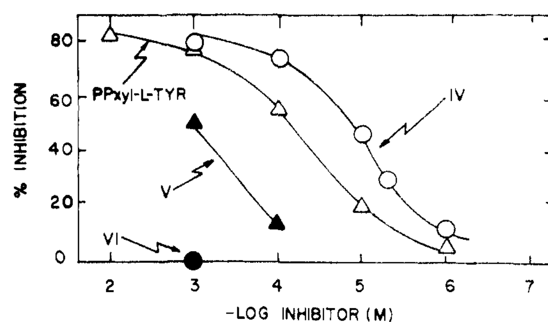

Figure 1. Inhibition of mouse liver Dopa decarboxylase. The effects of varying concentrations of different inhibitors on the enzyme activity were tested: Open circles, N-(5'-phosphopyridoxyl)-L-Dopa (IV); open triangles, N-(5'-phosphopyridoxyl)-L-tyrosine (PPxyl-L-Tyr); filled triangles, N-(5'-deoxypyridoxyl)-Dopa (V); filled circle, tetrahydroisoquinoline (VI). For the L-Dopa derivative IV and the PPxyl-L-Tyr derivative, the data represent the average of five or six separate experiments, each determination being done in triplicate.

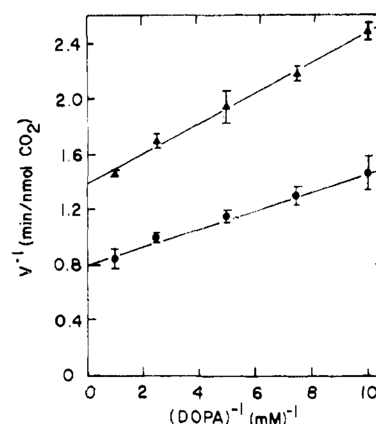

Figure 2. Double-reciprocal plots of Dopa decarboxylase activity in the presence and absence of 1×10^{-5} M N-(5'-phosphopyridoxyl)-L-Dopa (IV). The enzyme preparation (50 μL) used in these experiments was a 1 to 10 dilution of the original homogenate (see Experimental Section). The points shown are the average of the data from two experiments, each determination being done in triplicate; the bars represent the standard error of the six experimental values.

amination reactions, there are both aldimine and ketimine stages, the former resulting from the formation of an imine between the aldehyde form of the vitamin and an amino acid and the latter resulting from the formation of an imine between enzyme-bound pyridoxamine phosphate and a keto acid.[21] The substrate–coenzyme aldimine in vitamin B_6 dependent enzyme catalyzed reactions arises by way of a transaldimination reaction in which the substrate amino group displaces a lysine ε-amino acid group from its linkage with the coenzyme;[22-24] this displacement requires an intermediate having a tetrahedral configuration about C-4' of the coenzyme.[20] N-(5'-Phosphopyridoxyl) amino acid adducts, which are generally prepared by reduction of the corresponding aldimines, also have a tetrahedral configuration about C-4' of the vitamin moiety, and, accordingly, might be expected to act as inhibitors of pyridoxal phosphate dependent enzymes. There are a number of instances of the use of such inhibitors.[14] For example, Dempsey and Snell[25] have established that N-pyridoxylalanine binds to a pyridoxamine–pyruvate transaminase more tightly than the coenzyme itself. Also, Relimpio et al.[26] have reported that N-(5'-phosphopyridoxyl)fluoro amino acids interact stoichiometrically and specifically with glutamic-aspartic transaminase and cannot be displaced from the enzyme by the coenzyme,

substrate, or a combination of the two. In addition, suitable N-(5′-phosphopyridoxyl) amino acids have been shown to inhibit ornithine decarboxylase[27] and 4-aminobutyrate transaminase.[28]

Because of its central importance in the metabolism of aromatic amino acids in general[7] and its particular role in the biosynthesis of dopamine in the central nervous system,[9] we decided to test a number of N-(5′-phosphopyridoxyl) amino acids as inhibitors of a mammalian Dopa decarboxylase. However, the derivative of greatest interest, N-(5′-phosphopyridoxyl)-Dopa (IV), had not been described in the literature, and we found it necessary to devise a method for its preparation. The route shown in Scheme I proved to be satisfactory and involved selective blocking–deblocking procedures, selective reductions, and purification of the desired product by column chromatography. For comparative purposes, the tetrahydroisoquinoline condensation product, VI, was also prepared. All of the physical–chemical data for these compounds are in accord with the proposed structures.

Of the N-(5′-phosphopyridoxyl) amino acid adducts tested, the Dopa derivative proved to be the most effective, showing a 63% inhibition of enzyme activity in mouse liver homogenates at a concentration of 2×10^{-5} M for the DL derivative (Table I). The L derivative showed approximately the same inhibition at one-half the concentration of the DL derivative, indicating that the enzyme has a stereochemical preference for the L isomer. Dose–response curves (Figure 1) for the N-(5′-phosphopyridoxyl) derivatives of L-tyrosine and L-Dopa indicate the latter to be a more effective inhibitor than the L-tyrosine derivative by about an order of magnitude. This parallels the substrate specificity requirements of the enzyme, since L-Dopa is the best substrate for L-aromatic-amino-acid decarboxylase.[4]

The data in Figure 1 also show the concentration of N-(5′-phosphopyridoxyl)-L-Dopa required for 50% inhibition of the enzyme to be approximately 1×10^{-5} M. Borri-Voltattorni et al.[29] have studied the effects of N-(5′-phosphopyridoxyl) amino acids on the recombination of purified hog liver Dopa apodecarboxylase with pyridoxal phosphate. They reported that the inhibition of the combination of apoenzyme and coenzyme was complex and found that the tyrosine and phenylalanine derivatives caused inhibitions of approximately 70–80% at concentrations of 2×10^{-5} M. Kinetic studies with this particular enzyme are made extremely difficult by the nonenzymatic Pictet–Spengler reaction between substrate and coenzyme, by substrate oxidation, particularly at alkaline pH values, and by the fact that there is a decarboxylation-dependent transamination of the substrate causing a loss of coenzyme and, consequently, of enzyme activity. These complications have been discussed fully in a recent article by O'Leary and Baughn.[30]

Of interest in our study is the finding that N-(5′-deoxypyridoxyl)-DL-Dopa is a poor inhibitor of mouse liver Dopa decarboxylase. The concentration of this inhibitor required to cause a 50% inhibition of activity is 2×10^{-3} M, some 100-fold greater than the parent compound. This suggests a requirement for the naturally occurring phosphorylated form of the vitamin moiety for inhibitory efficacy. In addition, the finding that the tetrahydroisoquinoline VI is ineffective as an inhibitor at a concentration as high as 10^{-3} M has implications on the nature of the inhibition caused by IV. As pointed out by Raso and Stollar,[15] cyclic condensation products, such as VI, probably exist in solution with the tetrahydroisoquinoline ring perpendicular to the phosphopyridoxyl ring. On the other hand, the N-(5′-phosphopyridoxyl) derivative IV can exist in an extended or open conformation. Thus, the inference can be made that an extended or open conformation of the reduced coenzyme–substrate adduct IV is the preferred binding form and resembles the form of a coenzyme–substrate stage that is bound to the enzyme during its catalytic sequence. There is ample theoretical and experimental evidence for the requirement of an extended all-planar form of the Schiff bases bound to pyridoxal phosphate dependent enzymes.[31]

Investigations on the nature of the inhibition of Dopa decarboxylase caused by N-(5′-phosphopyridoxyl)-L-Dopa (Figure 2) establish the inhibition as noncompetitive with respect to substrate. A similar finding was reported by Heller et al.,[27] who demonstrated that α-N-(5′-phosphopyridoxyl)-L-ornithine inhibited ornithine decarboxylase in a noncompetitive manner. These investigators proposed that the inhibitor was bound to a conformationally distinct form of the enzyme in place of a Schiff base intermediate. The K_m for L-Dopa obtained from Figure 2 is 8×10^{-5} M and compares to a K_m value of 1.9×10^{-4} M reported for a purified hog kidney enzyme.[4] We have established that the inhibition by IV is essentially instantaneous in that the same percent inhibition is found if the inhibitor is added simultaneously with substrate or if IV is preincubated with enzyme for 15 min prior to substrate addition. In addition, it is noteworthy that the observed inhibitions occur in the presence of a 10 μM concentration of pyridoxal phosphate in the assay system.

The commercially available peripheral Dopa decarboxylase inhibitors used in the treatment of Parkinsonism are benserazide (Ro 44602) and carbidopa (MK-486); these are effective therapeutic agents.[12] However, both of these drugs are hydrazine derivatives and, as such, have the potential for causing adverse effects in vivo. For example, hydrazine derivatives have been suggested to have hepatotoxic and carcinogenic properties.[32] The inhibitors described in this article have the select advantage of not encompassing hydrazine as part of their structure. These N-(5′-phosphopyridoxyl) amino acids may provide useful information on the design of alternate selective peripheral inhibitors of Dopa decarboxylase. Compounds of this type would not be expected to cross the blood–brain barrier, owing to their highly charged nature at physiologic pH values. Furthermore, one would not expect a priori any of their metabolites to be potentially toxic. A study of the effects of ring substitution on N-(5′-phosphopyridoxyl)-aromatic acid adducts may provide valuable information regarding the topography of the active site of Dopa decarboxylase and might lead to the preparation of even more effective inhibitors.

Experimental Section

Materials. Pyridoxal phosphate monohydrate was purchased from Aldrich Chemical Co., as were the deuterated solvents used in NMR measurements. L-Dopa, DL-Dopa, and the other amino acids used in these experiments were obtained from Sigma Chemical Co. The Amberlite CG-50 ion-exchange resin was obtained from Mallinckrodt. [^{14}C]Carboxyl-labeled DL-Dopa was purchased from New England Nuclear. Phosphopyridoxyl amino acids were prepared using the method of Ikawa.[13] All other reagents and chemicals used in these studies were of the highest quality available, usually reagent grade.

Methods. Ultraviolet absorption measurements were made using a Cary Model 14 spectrophotometer or a Beckman DU monochromator in combination with a Gilford Model 2000 multiple sample absorbance recorder. Measurements of pH were performed on a Radiometer pHM 26 meter equipped with a GK 2321 combination electrode. ^{1}H NMR spectra were recorded on a Perkin-Elmer R-12 spectrometer against a Me_4Si standard. This instrument was not equipped with a signal lock device, and the

signals in the spectra are considered to be accurate to ±0.1 ppm. NMR measurements (D_2O) on phosphopyridoxyl derivatives were made on samples that had been dissolved in H_2O with the addition of a minimum volume of 1 N KOH, followed by lyophilization of the water and two more lyophilizations from D_2O. The samples were then dissolved in the desired volumes of D_2O. Radioactivity measurements were made with a Beckman LS-250 liquid scintillation counter. Chemical-ionization and electron-impact mass spectra were obtained on a Finnigan Model 6000 mass spectrometer. We are indebted to Drs. M. Blum and K. Kirk for obtaining these data as well as for the elemental analyses. Elemental analyses for all compounds reported were within ±0.4% of the calculated values.

Dopa decarboxylase activity was determined by the procedure of Tate et al.,[33] except that the $^{14}CO_2$ formed by the decarboxylation of the substrate was trapped by 35 μL of phenethylamine absorbed onto a filter-paper cylinder that was inserted into the center well of the double side-arm Warburg flasks used in these assays. Livers from 6-week-old male ICR Swiss mice were used as the source of Dopa decarboxylase. The animals were killed by cervical dislocation, and the livers were immediately excised and homogenized in a Potter-Elvehjem mortar and pestle with 5 volumes of ice-cold pH 7.0 buffer.[33] The compounds tested as inhibitors were added to the main compartment of the Warburg flasks and were preincubated with the enzyme preparation (50 μL of homogenate) for 15 min at 37 °C prior to tipping in the labeled substrate from one the the sidearms. Heat-denatured controls were regularly run; these had no significant activity compared to unheated liver homogenates. A unit of activity is defined as 1 nmol of CO_2 produced in 15 min. Tate et al.[33] found 10 600 ± 1830 (SE) units of activity/g of liver (eight mice). We found in control assays 14 973 ± 2218 (SD) units/g of liver (ten mice). All of the experimental points reported in the present experiments were the average of triplicate determinations; at least two mice were used for each point.

Synthesis. (a) Boc-L-Dopa-OMe (I). This compound was prepared from Boc azide[34] and L-Dopa-OMe by the procedure of Banerjee and Ressler,[37] except that the free base of L-Dopa-OMe was generated in situ in solvent pyridine by the addition of an amount of redistilled triethylamine equivalent to the L-DOPA-OMe·HCl used in the synthesis. After stirring for 20 min, the triethylamine hydrochloride was removed by filtration, and the protection of the free amino group was carried out as described.[37] The product was obtained in 81% yield and, after recrystallization from methanol/water, the mp was 140–141 °C (lit. 133–135 °C).

(b) *O,O*-Dibenzyl-*N*-Boc-L-Dopa-OEt (II). *N*-Boc-L-Dopa-OMe (4.0 g, 12.8 mmol) was dissolved in 70 mL of dry ethanol. To this solution were added 4.7 g (34 mmol) of potassium carbonate, 156 mg (1.1 mmol) of potassium iodide, and 4.04 g (32 mmol) of redistilled benzyl chloride. This mixture was stirred under nitrogen for 8 h under gentle reflux. After cooling, the entire mixture set to a white slush. After addition of ethyl acetate, the reaction mixture was warmed and the inorganic salts were removed. The solvents were stripped off on a rotary evaporator, and the desired product was obtained as white crystals from ethanol (4.2 g, 67%). A second crop was recovered by concentration and chilling of the liquor (1.3 g, 21%). Recrystallization of the first crop from ethanol gave material melting at 117–119 °C. It should be noted that an ester interchange occurred during the course of the reaction. This was established by both NMR ($CDCl_3$ solvent) and chemical-ionization mass spectra. Anal. ($C_{30}H_{35}NO_6$) C, H, N.

(c) TFA·*O,O*-Dibenzyl-L-Dopa-OEt (III). The Boc group was removed from 1.0 g (1.98 mmol) of II by dissolution in 10 mL of anhydrous trifluoroacetic acid. After stirring for 90 s at room temperature, the trifluoroacetic acid was rapidly removed by means of a rotary evaporator. The resulting light yellow oil was concentrated twice from ethanol and once from acetonitrile. Crystallization was accomplished by dissolving the residue in ca. 4 mL of acetonitrile, followed by the addition of 10 volumes of ether and then petroleum ether (30–60 °C) to turbidity. After refrigeration, the product was filtered, washed with petroleum ether, and dried in vacuo: yield 0.88 g (86%); mp 120–123 °C. The NMR spectrum (CD_3CN) gave the expected signals. The electron-impact mass spectrum had a molecular ion at 405 amu, which is that calculated for the free base form of the product. Anal. ($C_{27}H_{28}NO_6F_3$) C, H, N, F.

(d) *N*-(5′-Phosphopyridoxyl)-Dopa (IV). The following procedures are suitable for the preparation of both the L and DL derivatives.

(e) *N*-(5′-Phosphopyridoxyl)-*O,O*-dibenzyl-Dopa. TFA·*O,O*-dibenzyl-Dopa-OEt (III) (1.038 g, 2 mmol) was added to 8.0 mL of 1.0 N KOH in methanol, and the solution was stirred for 30 min at room temperature. To this was added 0.530 g (2 mmol) of pyridoxal phosphate monohydrate, and the resulting clear bright yellow-orange solution was stirred for another 30 min. Addition of sodium borohydride (38 mg, 1 mmol) in 2.0 mL of ethanol caused a very rapid bleaching of the color. After 45 min, the solvents were removed and the white solid that was obtained was further dried under oil pump vacuum.

Hydrogenolysis of the Benzyl Groups. The protected product obtained in the previous step was dissolved in 25 mL of water, and the pH of the clear, very pale yellow solution was adjusted to approximately 7 with 30% acetic acid, using short-range pH paper. This requires vigorous stirring in order to avoid local precipitation of the starting material. The solution was transferred to a pressure-equalizing dropping funnel that was then incorporated into a system set up for quantitative hydrogenolysis. The catalyst (1.0 g of 10% Pd/C in 10 mL of water) had been previously saturated with hydrogen, and, after the system had been equilibrated with hydrogen and closed, the *N*-(5′-phosphopyridoxyl)-*O,O*-dibenzyl-Dopa was added to the stirred catalyst suspension. Hydrogenolysis was allowed to proceed until 2 molar equiv of hydrogen gas had been consumed (ca. 7 h). The reaction mixture was then rapidly transferred to centrifuge tubes which were gassed with N_2 and capped. After centrifugation, the clear supernatant was pumped on to a 2 × 50 cm column of CG-50 (100–200 mesh) in the hydrogen form. It is necessary at all times to avoid undue exposure to air, since the debenzylated product is very sensitive to oxidation and discolors, especially in alkaline solutions. The column was developed with water at a flow rate of approximately 50 mL/h; fractions having a volume of 9.3 mL were collected. Fractions 23–30 all had white precipitates of the desired product, *N*-(5′-phosphopyridoxyl)-Dopa (IV). Concentration of fractions 16–40 to dryness yielded a powder that was dried in vacuo over P_2O_5 at room temperature, yield 562 mg (60%). For analysis, this material was suspended in water, and 1 N KOH was carefully added with stirring under a nitrogen atmosphere until solution was achieved. Readjustment of the pH to 2.5–3.0 with 4 N HCl yielded an off-white precipitate that was collected by centrifugation and washed by suspension and centrifugation from ice-cold water and ethanol, followed by drying in vacuo at room temperature to constant weight: ^{1}H NMR (D_2O) δ 2.34 (3 H, s, H-2′), 3.0 (2 H, m, H-β), 3.85 (1 H, m, H-α), 4.24 (2 H, s, H-4′), 4.76 (2 H, d, H-5′, ^{31}P-^{1}H coupling constant = 10 Hz), 6.68 (3 H, m, H-2″, -5″, -6″), 7.52 (1 H, s, H-6); UV λ_{max} (pH 7.0) 253 (ε 4510), 285 (ε 3410), 329 nm (ε 7050). Anal. ($C_{17}H_{21}N_2O_9P{\cdot}2H_2O$) C, H, N, P.

Concentration of fractions 41–115 yielded 499 mg of a gray solid. The NMR spectrum (D_2O) of this material showed that this peak consisted of partially debenzylated starting material.

A third product could be obtained from the column by elution with 5% acetic acid. If the hydrogenolysis was allowed to continue for 18 h, the *only* material that could be obtained from the reaction mixture corresponded to this third product, which was identified as *N*-(5′-deoxypyridoxyl)-Dopa (V), a debenzylated and reductively dephosphorylated product of the hydrogenolysis. The NMR spectrum of this material in Me_2SO-d_6 revealed that there was a complete loss of the signal corresponding to the 5′-$CH_2OPO_3H_2$ group and a corresponding gain of a signal in the aromatic methyl region that integrated for three protons. An elemental analysis for phosphorus was negative.

(f) Tetrahydroisoquinoline of Pyridoxal Phosphate and DL-Dopa (VI). This product was prepared by the Pictet–Spengler condensation.[38,39] Thus, 394 mg (2.0 mmol) of DL-Dopa and 557 mg (2.1 mmol) of pyridoxal phosphate monohydrate were dissolved in 10 mL of N_2-saturated water, followed by the addition of 6.2 mL of 1 N KOH. After 30 min, 0.52 mL of concentrated HCl was added, leading to the formation of a copious pale-yellow precipitate. After refrigeration, this material was collected, washed with cold water, and dried in vacuo over P_2O_5, yield 680 mg (79%).

For analysis, the product was dissolved in water by the addition of a minimal amount of 1 N KOH, followed by precipitation with HCl and workup as described above: ^{1}H NMR (D_2O) δ 2.36 (3 H, s, H-2′), 3.2 (2 H, m, H-β), 4.0 (1 H, m, H-α), 4.91 (2 H, d, H-5′, ^{31}P–^{1}H coupling constant = 10 Hz), 5.77 (1 H, s, H-4′), 6.20 (1 H, s, H-5′′), 6.69 (1 H, s, H-2′′), 7.74 (1 H, s, H-6); UV λ_{max} (pH 7.0) 250 (ϵ 6400, s), 292 (ϵ 4630), 326 nm (ϵ 8780). Anal. ($C_{17}H_{19}N_2O_9P\cdot H_2O$) C, H, N, P.

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References and Notes

(1) (a) Abbreviations used are: Dopa, 3,4-dihydroxyphenylalanine; dopamine, 3,4-dihydroxyphenylethylamine; Boc, *tert*-butoxycarbonyl; OMe, methyl ester; OEt, ethyl ester; TFA, trifluoroacetic acid; NMR nuclear magnetic resonance.
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Cyclic Amidine Inhibitors of Indolamine *N*-Methyltransferase

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Syntheses of a large number of mono- and bicyclic, as well as a few tricyclic, amidine derivatives related to 2,3,4,6,7,8-hexahydropyrrolo[1,2-*a*]pyrimidine (DBN) are reported. In vitro potencies for inhibition of the enzyme indolamine *N*-methyltransferase (INMT) from rabbit and human lung are presented. Four bicyclic amidine derivatives and 11 monocyclic derivatives were found to be equal or superior to DBN in in vitro potencies. With the bicyclic amidines, increasing ring size or introduction of substituents reduced activity. Among the monocyclic analogues. the most potent representatives were five- or six-membered systems with an exocyclic imino group, combined with methyl or ethyl substituents on the endocyclic nitrogen. Introduction of additional substituents decreased inhibitory potency. 2,3,5,6-Tetrahydro-8*H*-imidazo[2,1-c][1,4]thiazine and 3-methyl-2-iminothiazolidine have been shown to cause inhibition of lung INMT when administered orally to rabbits.

Although the current treatment of schizophrenia involves the use of neuroleptic agents which are dopamine antagonists, interest persits in other approaches to the therapy of this disease which are based on the theory that the illness is caused by an endogenously produced chemical toxin. There is considerable evidence that *N*,*N*-di-

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