# The Interaction of Pyridoxal Phosphate with Tyrosine Analogs[1a]

NICOLAS ZENKER

*Department of Pharmaceutical Chemistry, School of Pharmacy, University of Maryland, Baltimore, Maryland*

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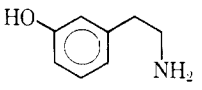
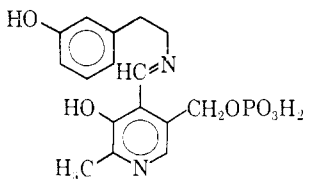
Schiff base formation between pyridoxal phosphate and tyrosine analogs is expressed in terms of affinity constants. The affinity of aromatic amino acids for pyridoxal phosphate is sharply decreased by $\alpha$ methylation; the affinity of phenethylamines for pyridoxal phosphate is decreased by $\alpha$ methylation and increased by $\beta$ hydroxylation. The values obtained for the affinity constants of *meta*-hydroxylated analogs are correlated with their ability to inhibit tyrosine transaminase and to undergo ring closure to tetrahydroisoquinolines.

$\alpha$-Methylated *meta*-hydroxylated tyrosine analogs ($\alpha$-methyl-*m*-tyrosine, and $\alpha$-methyl-DOPA) have been found to be inhibitors of aromatic amino acid decarboxylase[1b] and have furnished a rational approach in the search for antihypertensive agents.[2] $\alpha$-Methylated phenethylamines have played an important role in the development of antidepressant drugs[3] and in the elucidation of the neurohormonal role of catecholamines. Since Schiff base formation between amines and aldehydes is important in the functions of biological amino groups, it seemed of interest to express the affinity of tyrosine and phenethylamine analogs for pyridoxal phosphate (pyridoxal P) in terms of a constant and thereby to estimate the steric effect of the $\alpha$-methyl group.

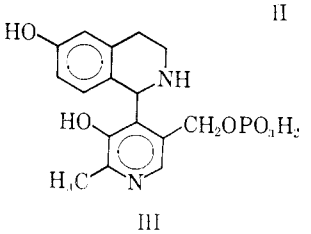
Pyridoxal P has been shown to be the coenzyme necessary for the transamination and decarboxylation of amino acids; in addition, Yasunobu and Yamada[4] have claimed cupric ions and pyridoxal P to be the cofactors necessary for monoamine oxidase activity in a beef plasma enzyme. The formation of a Schiff base (II) between pyridoxal P and a primary amine is a prerequisite for the coenzymic activities of pyridoxal P. The formation of II is, in addition, a prerequisite for the cyclization of *meta*-hydroxylated phenethylamines (I) to tetrahydroisoquinolines (III).[5,6]



I



II



III

(1) (a) Supported by Grant AM-06480 from the National Institutes of Health, U. S. Public Health Service. (b) W. G. Clark in "Metabolic Inhibitors," Vol. I, R. M. Hochster and J. H. Quastel, Ed., Academic Press Inc., New York, N. Y., 1963, Chapter 9, p 363.

(2) C. J. Cavallito in Advances in Chemistry Series, No. 45, R. F. Gould, Ed., American Chemical Society, Washington, D. C., 1964, p 83.

(3) J. H. Biel, ref 2, Chapter 10.

(4) H. Yasunobu and K. T. Yamada, *J. Biol. Chem.*, **238**, 2669 (1963).

(5) H. F. Schott and W. G. Clark, *ibid.*, **196**, 449 (1952).

(6) T. L. Sourkes and A. D'Iorio in "Metabolic Inhibitors," Vol. II, R. M. Hochster and J. H. Quastel, Ed., Academic Press Inc., 1963, Chapter 19, p 86.

Lucas, King, and Brown[7] have measured stability constants characterizing Schiff base formation between aliphatic amino acids and pyridoxal P. Rate constants for the formation of several tetrahydroisoquinoline derivatives have been obtained by Schott and Clark,[5] Sourkes,[8] and the data summarized by Sourkes and D'Iorio.[6] In the present study, affinity constants were obtained for tyrosine analogs; the values obtained were correlated with the inhibition of tyrosine transaminase exerted by related *meta*-hydroxylated phenethylamines and with the rate of tetrahydroisoquinoline formation by such amines.

## Experimental Section

$\alpha$-Methylphenylalanine, $\alpha$-methyltyrosine, and $\alpha$-methyldopamine hydrochloride were donated by Merck Sharp and Dohme Research Laboratories, $\alpha$-methylnorepinephrine hydrochloride was donated by Sterling–Winthrop Research Institute, and $\alpha$-methyltyramine hydrobromide by Smith Kline and French Laboratories; other compounds were obtained from commercial sources.

Rat liver homogenate was used as a source of tyrosine transaminase: the enzyme was induced by the intraperitoneal injection of a suspension of tyrosine and hydrocortisone succinate (Solu Cortef) in male Wistar rats.[9] Five hours after the injection the animals were sacrificed, and the livers were removed and homogenized in isotonic KCl (6 ml/g); the homogenate was centrifuged at 10,000 rpm (11,900 g) for 30 min and the supernatant was stored frozen in small aliquots.

Schiff base formation between pyridoxal P and primary amines results in an increase in ultraviolet absorbance at 275 m$\mu$ and in a shift of the 388-m$\mu$ absorbance maximum of pyridoxal P[1] to about 410 m$\mu$ (Figure 1). By plotting the reciprocal of the increase in 275-m$\mu$ absorbance resulting from the addition of graded concentrations of amino acids to pyridoxal P (maintained at a final concentration of 0.1 m$M$) against the reciprocal of the molar amino acid concentration Lucas, King, and Brown[7] have obtained stability constants which they defined as: $K_1$ = [pyridoxal P][amino acid]/[Schiff base]. The method of Lucas, King, and Brown[7] was used for the determination of stability constants with two major modifications: first, the measurements were made at pH 7.9 (0.2 $M$ phosphate buffer), the pH optimum of tyrosine transaminase;[10] second, Schiff base formation was assessed by measuring absorbances at 388 and 410 m$\mu$ rather than at the 275-m$\mu$ wavelength used by Lucas and co-workers. The procedure used by Lucas and co-workers,[7] illustrated in Figure 2 for phenylalanine and $\alpha$-methylphenethylamine (amphetamine), cannot be used for the strongly ultraviolet-absorbing phenolic analogs of tyrosine. The shift of the pyridoxal P absorbance due to Schiff base formation results in a decrease of 388-m$\mu$ absorbance and in an increase in 410-m$\mu$ absorbance, *i.e.*, in an increase of the absorbance dif-

(7) N. Lucas, H. K. King, and S. J. Brown, *Biochem. J.*, **84**, 118 (1962).

(8) T. L. Sourkes, *Arch. Biochem. Biophys.*, **51**, 414 (1954).

(9) G. Litwack, "Experimental Biochemistry," John Wiley and Sons, Inc., New York, N. Y., 1960, p 281.

(10) E. C. C. Liu, B. M. Pitt, M. Given, and W. E. Knox, *J. Biol. Chem.*, **233**, 668 (1958).

ference $\Delta A = A_{410} - A_{388}$. The equal validity of 275-mμ absorbance and of the $\Delta A$ absorbance difference in the determination of $K_d$ is shown for phenylalanine and α-methylphenethylamine in Figure 2.

Tetrahydroisoquinoline formation between pyridoxal P (at a final concentration of 0.1 m$M$) and tyrosine analogs (at a final concentration ranging from 0.05 to 1.6 m$M$) was followed by recording 327-mμ absorbance change in function of time[6] at 28°.

Tyrosine transaminase activity was assayed by measuring the absorbance of the complex formed between borate and the enol form of *p*-hydroxyphenylpyruvate in the presence of arsenate[10] and also manometrically by following the oxygen uptake resulting from the oxidation of tyrosine to acetoacetate and fumarate.[11]

## Results

Affinity constants obtained by the $\Delta A$ method are listed in Table I; they are listed as $1/K_d$ to better express affinity and to permit correlation with rates of tetrahydroisoquinoline formation. The constants listed for amino acids in Table I show a marked decrease in affinity for the sterically hindered α-methylphenylalanine and α-methyltyrosine. The affinity constants listed for phenethylamines in Table I show a moderate decrease in affinity for α-methylphenethylamines such as amphetamine, α-methyltyramine (paredrine), and α-methyloctopamine [α-(1-aminoethyl)-*p*-hydroxybenzyl alcohol]. The affinity constants further indicate that β-hydroxylated phenethylamines have an increased affinity for pyridoxal P as may be seen by comparing the affinity constant of octopamine (β-hydroxytyramine) with that of tyramine and that of normetanephrine (β-hydroxy-3-methoxytyramine) with that of 3-methoxytyrosine.

The rate constants listed in Table I for the formation of tetrahydroisoquinoline derivatives show that variations in rate constants follow affinity constants for structurally related compounds, suggesting that Schiff base formation is the rate-determining step in tetrahydroisoquinoline formation. The apparent rate constant for epinephrine (a secondary amine which like metanephrine, ephedrine, or N-acetyltyrosine, is unable to form a Schiff base) suggests that the 327-mμ absorbance increase does not assess tetrahydroisoquinoline formation only.

Results describing the effect of some tyrosine analogs on tyrosine transaminase are listed in Table II. The degradation of tyrosine (manometric assay) is markedly inhibited by *m*-tyrosine and DOPA, but not affected by α-methyl-*m*-tyrosine and α-methyl-

TABLE I

AFFINITY CONSTANTS ($1/K_d$)[a] FOR 0.1 m$M$ PYRIDOXAL PHOSPHATE AND RATE CONSTANTS ($k$) FOR TETRAHYDROISOQUINOLINE FORMATION BY TYROSINE ANALOGS

| Analog | $1/K_d$, m$M^{-1}$ | $k$[b] |
|---|---|---|
| Phenylalanine | 0.55 | |
| α-Methylphenalanine | 0.07 | |
| *m*-Tyrosine | | 0.04 |
| α-Methyl-*m*-tyrosine | | 0.003 |
| Tyrosine | 0.55 | |
| α-Methyltyrosine | 0.07 | |
| DOPA | | 0.04 |
| α-Methyl-DOPA | | 0.003 |
| 3-Methoxytyrosine | 0.74 | |
| Tyramine | 0.55 | |
| α-Methylphenethylamine | 0.26 | |
| α-Methyltyramine | 0.32 | |
| Dopamine | | 0.04 |
| α-Methyldopamine | | 0.03 |
| Normetanephrine | 1.0 | |
| Octopamine | 0.96 | |
| α-Methyloctopamine | 0.68 | |
| Norepinephrine | | 0.07 |
| α-Methylnorepinephrine | | 0.03 |
| Epinephrine | | 0.01 |

[a] Affinity constants were obtained from a plot of reciprocal analog concentration against reciprocal $A_{410} - A_{388}$ absorbance increase. [b] Absorbance per micromole per minute.

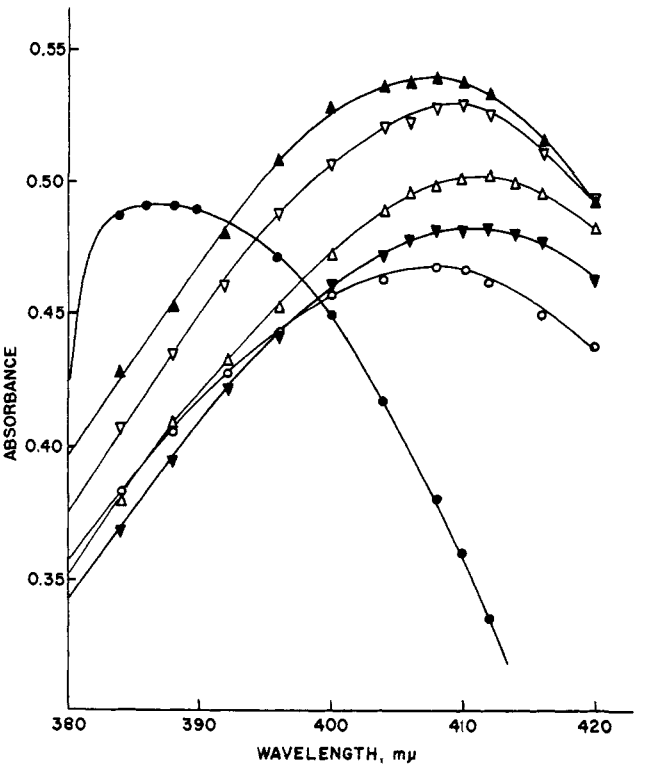



Figure 1.—Absorption spectra of some Schiff bases at pH 7.9: O, phenylalanine; ▽, tyramine; ▲, α-methyltyramine; △, octopamine; ▼, α-methyloctopamine; ●, pyridoxal phosphate.

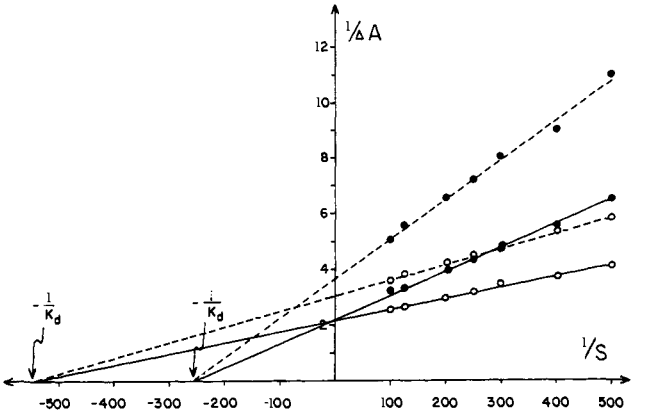



Figure 2.—Determination of the affinity constant, $1/K_d$ for phenylalanine (O) and amphetamine (●). Abscissa, reciprocal molar concentration; ordinate, reciprocal 275 mμ (plain line) and reciprocal $\Delta A = A_{410} - A_{388}$ (dotted line) absorbance increases.

(11) G. Litwack, *J. Biol. Chem.*, **228**, 823 (1959).

TABLE II
INHIBITION OF TYROSINE TRANSAMINASE BY TYROSINE ANALOGS[a]

| Analog | Manometric assay Concn, mM | % inhib | Enol-borate method Concn, mM | % inhib | Lit.[b] |
|---|---|---|---|---|---|
| *dl-m*-Tyrosine | 2 | 66 | 1 | 88 | ... |
| α-Methyl-*m*-tyrosine | 2 | 0 | 1 | 5 | ... |
| *dl*-DOPA | 2 | 70 | 1 | 72 | 86 |
| α-Methyl-DOPA | 2 | 0 | 1 | 17 | 11 |
| Dopamine | 2 | 100 | 1 | 93 | 89 |
| α-Methyldopamine | 2 | 34 | 1 | 72 | ... |
| Norepinephrine | 1 | 74 | 0.5 | 60 | 91 |
| α-Methylnorepinephrine | 1 | 79 | 0.5 | 42 | ... |
| Dopamine | 1 | 74 | 0.5 | 29 | 89 |

[a] The tyrosine concentration was 2 mM in the manometric assay and 1 mM in the enol–borate assay. [b] Results obtained by G. A. Jacoby and B. La Du [*J. Biol. Chem.*, **239**, 419 (1964)] at a tyrosine and inhibitor concentration of 3 mM.

DOPA; dopamine inhibits tyrosine degradation to a higher degree than α-methyldopamine, but α-methylnorepinephrine is as effective an inhibitor of tyrosine degradation as norepinephrine itself. When the effect of tyrosine analogs on tyrosine transaminase is assessed by the more specific enol-borate method (Table II), the inhibitory effect of *meta*-hydroxylated amino acids is seen to be markedly decreased by α methylation, while that of *m*-hydroxyphenethylamines is moderately decreased by α methylation, but increased by β hydroxylation.

## Discussion

The presence of an α-methyl group in tyrosine analogs results in a large amount of steric hindrance to Schiff base formation; it explains the lack of biochemical reactivity of members of this group.

The presence of an α-methyl group in phenethylamines provides a definite but moderate amount of steric hindrance to Schiff base formation. That a similar steric relation holds for rat liver mitochondrial monoamine oxidase, possibly a pyridoxal P[1] enzyme,[12] has been shown by Fuller and Walters.[13] Fuller and Walters state that rat monoamine oxidase was more inhibited by substrate amines than by α-methylated analogs; thus 0.1 mM phenethylamine was able to prevent 74% of kynurenamine oxidation, whereas, at the same concentration, α-methylphenethylamine was 31% and α,α-dimethylphenethylamine (which is sterically similar to an α-methylamino acid) was only 8% effective.

No attempt has been made in the present study to explain the increased affinity of β-hydroxylated phenethylamines for pyridoxal P.

**Acknowledgment.**—The author is indebted to Miss Eileen Ferrin for technical assistance.

(12) S. R. Gitsa, *Biochem. Pharmacol.*, **14**, 159 (1965).
(13) R. W. Fuller and C. P. Walters, *ibid.*, **14**, 159 (1965).

# 2-Aminotyrosine, an Analog of Tyrosine[1,2]

ALVIE L. DAVIS, JOHN W. ZAUN,[3] PERRY C. REEVES, ROBERT L. HANCE, AND TOMMY J. MCCORD

*Department of Chemistry, Abilene Christian College, Abilene, Texas*

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A new tyrosine analog, 2-aminotyrosine, and several related compounds were prepared and tested for growth inhibition of *Escherichia coli* 9723 and *Leuconostoc dextranicum* 8086. Growth inhibition of *E. coli* by 2-aminotyrosine was shown to be reversed in a competitive and specific manner by tyrosine over a 100-fold range of increasing concentrations with an inhibition index of approximately 300. Ethyl 2-acetamido-2-(4-methoxy-2-nitrobenzyl)malonate, resulting from the condensation of 4-methoxy-2-nitrobenzyl bromide with the sodium salt of ethyl acetamidomalonate, was hydrolyzed with hydrobromic and hydrochloric acid to give 2-nitrotyrosine and 4-methoxy-2-nitrophenylalanine, respectively. Catalytic hydrogenation of the free bases of 2-nitrotyrosine and 4-methoxy-2-nitrophenylalanine gave 2-aminotyrosine and 2-amino-4-methoxyphenylalanine, respectively.

Among the previously reported ring-substituted analogs of the aromatic amino acids, some rather interesting biologically antagonistic activities have been observed with the aminophenylalanines in growth inhibition studies. Although no appreciable inhibitory activity was detected using *Leuconostoc dextranicum* as the test organism,[4] *p*-aminophenylalanine caused a growth inhibition of *Escherichia coli* which was reversed by either phenylalanine or tyrosine.[5] *m*-Aminophenylalanine, which is a competitive antagonist of phenylalanine in both *L. dextranicum*[4] and *E. coli*,[5] has also been reported to be a competitive antagonist of lysine in *Saccharomyces cerevisiae*.[6] More recently, *o*-aminophenylalanine has been prepared and found to antagonize specifically and competitively the utilization of phenylalanine for the growth of *E. coli*.[7]

Because of the interesting biological activities of the aminophenylalanines, the synthesis of comparable analogs which are structurally related to other natural amino acids was desirable. Accordingly, in the present investigation, 2-aminotyrosine and several related compounds were prepared, and their biological properties were studied in *E. coli* and *L. dextranicum* as herein presented.

2-Aminotyrosine and related compounds were synthesized through the usual acetamidomalonic ester

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(3) Taken in part from the M.S. Thesis of John W. Zaun, Abilene Christian College, Aug 1965.

(4) A. L. Davis, J. M. Ravel, C. G. Skinner, and W. Shive, *Arch. Biochem. Biophys.*, **76**, 139 (1958).

(5) E. H. Bergmann, S. Sicher, and B. E. Volcani, *Biochem. J.*, **54**, 1 (1953).

(6) K. Dittmer, Abstracts of Papers, 134th National Meeting of the American Chemical Society, Miami, Fla., April 1957, p 37C.

(7) A. L. Davis, R. Lloyd, J. Flercier, L. Bayliss, and T. J. McCord, *Arch. Biochem. Biophys.*, **102**, 48 (1963).