N, S; C: calcd, 57.49; found, 57.03.

7-[4-(Ethoxycarbonyl)-5-hydroxy-1-oxo-1,2-thiazin-1-yl]xanthone-2-carboxylic Acid (34). Sodium hydride (80% dispersion in oil, 0.30 g, 10.0 mmol) was added in small portions to a stirred solution of methyl 7-[*N*-[2',2'-bis(ethoxycarbonyl)-ethenyl]-*S*-methylsulfonimidoyl]xanthone-2-carboxylate (**35**; 2.5 g, 5.0 mmol) in dry dimethyl sulfoxide (150 mL) at 0 °C. After being stirred for 4 h, the reaction mixture was poured onto ice-cold dilute hydrochloric acid. Chloroform was added, and the chloroform extract was washed with water and dried ($MgSO_4$) before being evaporated to leave a brown semisolid. Trituration with ethanol gave a buff crystalline solid which was recrystallized from chloroform to give white crystals of 7-[4-(ethoxycarbonyl)-5-hydroxy-1-oxo-1,2-thiazin-1-yl]xanthone-2-carboxylic acid (**34**) as the hydrochloride (0.43 g, 18%) melting at 300 °C with decomposition. Anal. ($C_{21}H_{15}NO_8S$·HCl) C, H, N, S.

References and Notes

(1) P. D. Kennewell and J. B. Taylor, *Chem. Soc. Rev.*, **4**, 189 (1975).
(2) G. Satzinger and P. Stoss, *Arzneim.-Forsch.*, **20**, 1214 (1970); (b) The Procter and Gamble Co., U.S. Patent 3 557 206; (c) Warner Lambert, British Patent, 1 168 700; (d) Merck, Sharpe and Dohme Corp., West German Patent, 2 062 017; (e) Beiersdorf A. G., Belgian Patent, 814 400; (f) Warner Lambert, U.S. Patent, 3 803 131; (g) Sandoz, U.S. Patent, 4 031 227.
(3) J. S. Cox, J. E. Beach, A. M. J. N. Blair, A. J. Clarke, J. King, T. B. Lee, D. E. E. Loveday, G. F. Moss, T. S. C. Orr, J. T. Richie, and P. Sheard, *Adv. Drug Res.*, **5**, 115 (1970).
(4) J. R. Pfister, R. W. Ferraresi, I. T. Harrison, W. H. Rooks, A. P. Roszkowski, A. Van Horn, and J. H. Fried, *J. Med. Chem.*, **15**, 1032 (1972).
(5) Z. Ovary in "Immunological Methods", J. F. Ackroyd, Ed., Blackwell Scientific Publications Ltd., Oxford, U.K., 1964, pp 259–283.
(6) SmithKline, Corp., West German Patent 2 549 841.
(7) M. Hojo and R. Masuda, *Tetrahedron Lett.*, 613 (1976).
(8) Syntex, Belgian Patent 823 030.
(9) Syntex, British Patent 1 398 097.
(10) Y. Tamura, K. Lumoto, J. Minamikawa, and M. Ikeda, *Tetrahedron Lett.*, 4173 (1972).
(11) A. C. Barnes, P. D. Kennewell, and J. B. Taylor, *J. Chem. Soc., Chem. Commun.*, 776 (1973).
(12) P. Miller and G. W. L. James, *Arch. Int. Pharmacodyn. Ther.*, **231**, 328 (1978).

Biological Properties of Transition-Metal Organometallic Compounds. 3. β-Ferrocenylalanine[1]

Robert P. Hanzlik,* Phyllis Soine, and William H. Soine

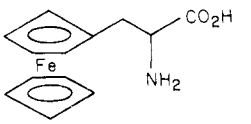
Department of Medicinal Chemistry, School of Pharmacy, University of Kansas, Lawrence, Kansas 66045.

Received August 7, 1978

We have investigated the effects of DL-β-ferrocenylalanine (1), five DL-halophenylalanines (*p*-F, 2; *m*-F, 3; *o*-F, 4; *p*-Cl, 5; *p*-Br, 6), L-β-(2-pyridyl)alanine (7), and DL-β-(6-methyl-2-pyridyl)alanine (8) on the growth of *Leuconostoc meserentoides* and as inhibitors of phenylalanine decarboxylase from *S. Fecalis*, hog kidney aromatic L-amino acid decarboxylase, and rat liver phenylalanine hydroxylase. None of the compounds supported bacterial growth in the absence of L-Phe, but they inhibited growth in the presence of L-Phe in the order $2 \gg 4 > 3 \simeq 7 > 5 > 6$; 8 and 1 were inactive. Both decarboxylases were inhibited by the analogues to a similar extent, the inhibition decreasing in the order $1 > 6 > 5 > 2 > 8 \simeq 7$. Compound 1 was a competitive inhibitor of the hog kidney enzyme with a K_i of 7.2 mM; L-Phe had a K_M of 48.8 mM. Inhibition of the phenylalanine hydroxylase ($DMPH_4$ cofactor) decreased in the order $2 > 1 > 3 > 4 > 5 > 6 > 7 > 8$ over a range from 73 to 2% inhibition. Compound 1 gave *noncompetitive* inhibition with respect to L-Phe and mixed inhibition with respect to $DMPH_4$ cofactor. This pattern of inhibition kinetics is apparently unique, as other known inhibitors either compete for substrate (e.g., 2 and 5) or with cofactor (e.g., norepinephrine or other catechol compounds). It was suggested that 1 may affect the enzyme through interaction at an allosteric or regulatory site previously proposed to account for the activation observed upon preincubation of the enzyme with its normal substrate L-Phe.

Two very well established approaches to modifying the biological activity of molecules containing an aromatic ring involve the placing of substituents at various positions around the perimeter of the ring and/or the introduction of various heterocyclic modifications into the ring itself. An alternative type of substitution which has received relatively little attention involves replacing the aromatic moiety by an aromatic *organometallic* moiety such as ferrocene or benzenechromiumtricarbonyl. The biological activity of ferrocene analogues of several drugs[2-6] has generally been disappointing from a pharmacological or therapeutic point of view. However, ferrocene itself is known to be an effective hematinic agent having very low toxicity in animals and man.[7-11] Recent studies in our laboratory have shown that ferrocene is extensively metabolized in rats by "aromatic hydroxylation" followed by conjugation with glucuronic acid or sulfate. The release of iron in vivo for hemoglobin synthesis apparently occurs via spontaneous decomposition of hydroxyferrocene prior to conjugation.[1a]

Because the enzymatic hydroxylation of phenylalanine to tyrosine shows mechanistic similarities to "aromatic hydroxylation", we have now investigated the interaction of β-ferrocenylalanine (1) with phenylalanine hydroxylase.



1

A series of halophenyl- and pyridylanines were included in the testing, and two other phenylalanine-utilizing test systems, *Leuconostoc mesenteroides* and phenylalanine decarboxylase, were also investigated with these compounds.

As detailed below, 1 behaved similar to other "organic" analogues of phenylalanine in the latter two systems but was found to have unique kinetic properties as an inhibitor of phenylalanine hydroxylase.

0022-2623/79/1822-0424$01.00/0

Table I. Inhibition of Phenylalanine Decarboxylation and Hydroxylation by Phenylalanine Analogues

analogue	% inhibn		
	PD[a]	ALAD[b]	PH[c]
1	28	28	56
6	30	17	10
5	21	16	24
2	0	14	73
8	0	8	2
7	0	5	8
3	*d*	*d*	31
4	*d*	*d*	31

[a] Phenylalanine decarboxylase from *S. fecalis*; [L-Phe] = 10 mM, [analogue] = 2.5 mM. [b] Hog kidney aromatic L-amino acid decarboxylase; [L-Phe] = 9 mM, [analogue] = 3 mM. [c] Rat liver phenylalanine hydroxylase; [L-Phe] = 1.0 mM; cofactor used was $DMPH_4$. [d] Not tested.

Results

The compounds investigated included DL-1, five DL-halophenylalanines (*p*-F, 2; *m*-F, 3; *o*-F, 4; *p*-Cl, 5; *p*-Br, 6), L-β-(2-pyridyl)alanine (7), and DL-β-(6-methyl-2-pyridyl)alanine (8).

Bacterial Growth Assay. The phenylalanine-requiring bacterium *Leuconostoc meserentoides* was used to evaluate β-ferrocenylalanine and several other phenylalanine analogues for their ability to (1) inhibit bacterial growth in the presence of phenylalanine and (2) support growth in the absence of phenylalanine. None of the compounds (1–8) showed any ability to support bacterial growth at several concentrations up to 1.3 mM. However, a number of these compounds interfered *competitively* with the phenylalanine-dependent growth of the organism. Concentrations of 2 as low as 0.18 mM completely inhibited growth at 24 and 36 h at all phenylalanine levels studied, while compounds 8 and 1 caused no detectable inhibition of growth at concentrations up to 3 mM. With the other compounds in Table I, intermediate results were obtained, and from the growth curves at various times and phenylalanine concentrations the following order of potency was apparent: $2 \gg 4 > 3 \gtrsim 7 > 5 > 6 \gg 8 \simeq 1$.

Inhibition of Phenylalanine Decarboxylase. The ability of ferrocenylalanine and several other analogues to inhibit the decarboxylation of phenylalanine was evaluated using two different enzyme preparations, phenylalanine decarboxylase (PD) from *Streptococcus* and aromatic L-amino acid decarboxylase (ALAD) from hog kidney; the results are presented in Table I. As a control, 0.05 mM $FeSO_4$ was tested with the *S. Fecalis* enzyme; 4% stimulation was observed. Since ferrocenylalanine was one of the most active inhibitors in both assays, the kinetics of its inhibition of the mammalian enzyme was investigated and is presented in Figure 1. With the partially purified enzyme preparation used in these studies, L-phenylalanine had a K_M of 48.8 mM, which agrees favorably with the value of 42 mM determined by Christenson et al., using a highly purified enzyme preparation.[13] That 1 is a competitive inhibitor of this enzyme is suggested by the fact that the V_{max} values remain essentially constant at 929, 930, and 932 nmol of CO_2/20 min, while the K_M values increase steadily with increasing concentrations of 1 (42, 45, and 53.5 mM at 0.5, 1.0, and 3.0 mM 1, respectively). Furthermore, a replot of the slopes from Figure 1 against inhibitor concentration gives a straight line, consistent with competitive inhibition, and extrapolating to a K_i value of 7.2 mM.

Inhibition of Phenylalanine Hydroxylase. The ability of 1 and several other phenylalanine analogues to inhibit rat liver phenylalanine hydroxylase (PH) was determined using the procedure reported by Ayling et al.[14] During the preliminary phase of this study, it was noted that the inhibitory effect of 1 was time dependent, maximal inhibition being obtained after preincubation of substrate, enzyme, and inhibitor for 2.5 min prior to initiating the reaction by addition of the cofactor, 6,7-dimethyltetrahydropteridine. Therefore, this preincubation period was used with all of the kinetic experiments. The results obtained are reported in Table I.

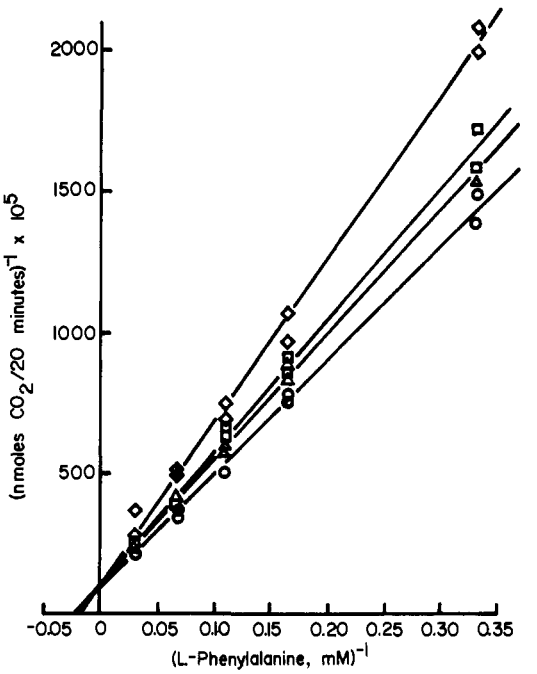


Figure 1. Inhibition of hog kidney aromatic L-amino acid decarboxylase by DL-β-ferrocenylalanine: (○) control; (△) 0.5 mM; (□) 1.0 mM; (◇) 3.0 mM.

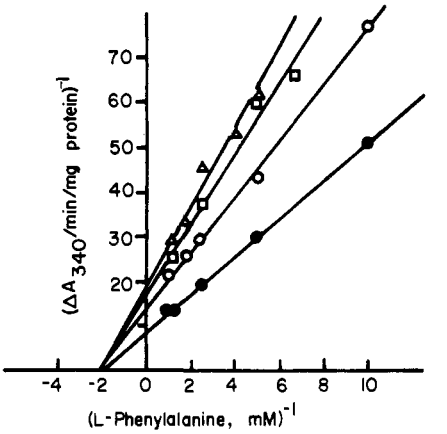


Figure 2. Inhibition of rat liver phenylalanine hydroxylase by DL-β-ferrocenylalanine: (●) control; (○) 0.5 mM; (□) 0.75 mM; (△) 1.0 mM. In this study, the concentration of $DMPH_4$ cofactor was held constant at 0.17 mM while the substrate concentration was varied.

Since ferrocenylalanine was one of the best inhibitors, the kinetic pattern of its inhibition was investigated. In the absence of inhibitors, the K_M and V_{max} values determined for L-phenylalanine were 0.66 mM and 47.3 nmol min^{-1} (mg of protein)$^{-1}$, respectively, which agree well with the reported[14] values of 0.76 mM and 37.5 nmol min^{-1} (mg of protein)$^{-1}$. Lineweaver–Burk plots given in Figures 2 and 3 indicate that ferrocenylalanine is a noncompetitive inhibitor with respect to $DMPH_4$ cofactor. A replot of the *y* intercepts from Figure 2 against inhibitor concentration gave a straight line extrapolating to a K_i value of 0.89 mM

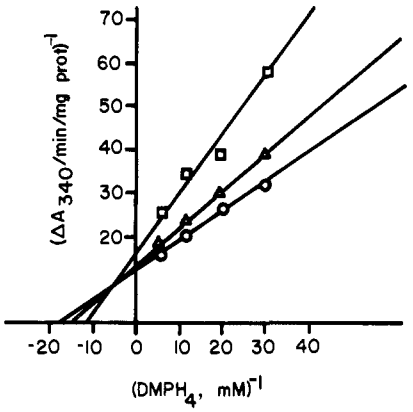


Figure 3. Inhibition of rat liver phenylalanine hydroxylase by DL-β-ferrocenylalanine: (○) control; (△) 0.5 mM; (□) 0.75 mM. In this study, the concentration of L-phenylalanine was held at 0.8 mM while the concentration of $DMPH_4$ cofactor was varied.

for **1** at optimum concentrations of L-Phe and $DMPH_4$.

Discussion

For several years our laboratory has been involved in an investigation of the metabolism and biological properties of transition-metal organometallic compounds, particularly π-arene complexes such as ferrocene and its derivatives.[1] Because these organometallic "aromatic" systems have an elongated cylindrical shape rather than the planar disklike shape of conventional aromatic systems, we felt they might offer an interesting way to probe drug–receptor and substrate–enzyme interactions in a new dimension, i.e., perpendicular to the aromatic ring rather than outwards around its perimeter as is usually done by adding substituents to a conventional benzenoid or heteroaromatic system. One of the simplest biologically active aromatic compounds that has given rise to numerous extensively studied analogues is phenylalanine. Thus, β-ferrocenyl-α-alanine (**1**) was selected for study, along with several halogenated (**2–6**) and heterocyclic (**7** and **8**) analogues of phenylalanine.

Shortly after the development of the antimetabolite concept based on sulfonamide antagonism of *p*-aminobenzoic acid, a variety of β-arylalanines were found to inhibit microbial growth by competitively antagonizing phenylalanine utilization.[12] In the present study, the same general behavior was observed, the most potent inhibitors of *L. mesenteroides* growth being those compounds which bore the closest structural similarity to phenylalanine. While studies of growth-inhibitory effects of phenylalanine analogues have not led to any useful antimicrobial agents, certain analogues have proven useful as tools for investigation of such diverse processes as cell division,[15] antibody biosynthesis,[16] and enzyme turnover in mammalian cells.[17]

With hog kidney aromatic L-amino acid decarboxylase, **1** was the best inhibitor of the eight compounds studied. Because of the instability toward isolation and generally low level of activity of mammalian amino acid decarboxylases relative to the bacterial decarboxylases,[18] the latter commercially available enzymes are often used in the preliminary screening studies for inhibitors.[19] While these two groups of enzymes both utilize pyridoxal and are thought to have similar mechanisms, other properties of the proteins, such as pH optimum, are often substantially different. Thus, it is satisfying to note the similar pattern of inhibition of both enzymes by the compounds studied (Table I). Although enzyme inhibitions of only 20–30% do not at first seem to indicate a very significant degree of interaction of enzyme and inhibitor, it must be remembered that, due to solubility limitations, the substrate concentration in these assays is only approximately one fifth of K_M, and the inhibitor concentrations are even lower. In order to observe maximal effects of inhibitors, the enzyme should be saturated with many times the K_M value of its substrate.

Table II. Kinetic Properties of Inhibitors of Aromatic Amino Acid Hydroxylase Enzymes

inhib	inhibn pattern against[f]		ref
	amino acid	cofactor	
NE[a,b]	NC	C	26
DOPV[c]	NC	C	27
2[b]	C	*d*	28
5[b]	C	M	29
1[b]	NC	M	*e*

[a] NE, norepinephrine. [b] Determined for phenylalanine hydroxylase with L-phenylalanine as substrate. [c] DOPV, α-(3,4-dihydroxyphenyl)valeramide; determined for tyrosine hydroxylase with L-tyrosine as substrate. [d] Cofactor not specified. [e] This work. [f] Abbreviations used are: NC, noncompetitive; C, competitive; M, mixed.

The decarboxylase enzymes used in this study are noted for their general lack of substrate specificity, except for the requirement of an L configuration for both substrates and inhibitors.[13,20,21] The observation that the larger, presumably more lipophilic, compounds (e.g., **1** and **6**) were the best inhibitors suggests that the enzyme possesses a small highly stereoselective active site surrounded by a larger lipophilic side-chain binding site which is relatively nonspecific. (Exceptions may occur, however, when the substrate bears an hydroxyl group in a critical position, as in L-Dopa or 5-hydroxytryptophan.[21]) It was not determined if ferrocenylalanine is decarboxylated by either enzyme, but it was found to be a competitive inhibitor of the hog kidney enzyme, and, with few exceptions, competitive inhibitors of this enzyme are also alternate substrates.[20]

In an earlier study of ferrocene metabolism, we found that ferrocene was efficiently hydroxylated by liver microsomal cytochrome P-450 enzymes.[1a] We therefore felt it would be interesting to study the interaction of ferrocenylalanine with liver phenylalanine hydroxylase (PH), a nonheme iron-containing mixed-function oxygenase also capable of inducing NIH-type shifts of substituents concomitant with ring hydroxylation.[22] A number of ring-halogenated and heterocyclic analogues of phenylalanine have been studied as inhibitors and substrates of PH. Our results with the halogenated compounds are similar to those[23] of Counsell et al., despite the fact that two different types of assays were used. In fact, analogues **2** and **5** are particularly well-studied inhibitors of PH. Considering the major structural differences between these compounds and **1** and the relatively weak inhibition by **6–8**, we were somewhat surprised when **1** was found to be a rather potent PH inhibitor.

Inhibitors of PH generally fall into one of two chemical groups, amino acid substrate analogues and *o*-dihydroxy aromatic compounds and certain congeners. As indicated in Table II, substrate analogues show competitive inhibition with respect to phenylalanine and mixed inhibition with respect to cofactor. On the other hand, compounds of the second type (which are thought to act as chelating agents or as cofactor analogues) show competitive inhibition with respect to cofactor and noncompetitive inhibition with respect to phenylalanine. The results with **1** do not conform to either of these patterns and may be indicative of a unique mechanism of inhibition.

Several lines of evidence suggest that PH may be an allosteric protein or, at least, is susceptible to activation by its substrate or substrate analogues such as 2 or tryptophan (which is an alternate substrate). In particular, Tourain[24] has demonstrated a time- and temperature-dependent *activation* of PH by phenylalanine, such that maximal activity for tyrosine formation was obtained only after about 1.5 min of incubation. In our study it was noted that maximal *inhibition* of PH by 1 was obtained only after preincubating enzyme, substrate, and 1 for about 2.5 min prior to adding cofactor to initiate the reaction. It is conceivable that 1 could be competing with phenylalanine for binding to this hypothetical "activator site" and thus preventing the full activation of the enzyme. This would be consistent with the atypical kinetic pattern for inhibition of PH by 1 (Table II). At present, it is not known if 1 is hydroxylated by PH, but results of studies of ferrocene metabolism in vitro[1a] suggest that if 1 were hydroxylated the product might not be stable enough to isolate.

Conclusions. Although several previous studies of ferrocene analogues have produced disappointing results in terms of pharmacological activity, ferrocene has proven useful for probing the mechanism of cytochrome P-450 hydroxylations,[1a] and at least one ferrocene derivative has proven clinically useful as a hematinic agent in man.[12] This study with ferrocenylalanine further demonstrates that unique selectivities can be obtained using "thick" organometallic analogues of "thin" aromatic π systems.

Experimental Section

The halogenated phenylalanines 2–6 and $DMPH_4$ cofactor were obtained from Sigma; the pyridylalanines 7 and 8 were a generous gift of Dr. Robert Angelici (Iowa State University). *L. meserentoides* (also known as *Pediococcus cerevisiae*, ATCC 8042) was reconstituted from a lypophilized culture obtained from the USDA Northern Regional Laboratory, Peoria, Ill.

DL-β-Ferrocenylalanine. This compound was synthesized using the method of Osgerby and Pauson.[25] It was further purified and converted to a soluble HCl salt by a sequence of passage through a column of Dowex 50-X8, elution with 1 M NH_3, lyophylization, dissolution in 1 M HCl, and lyophylization (48% yield): mp >300; IR (KBr) 3200–2200 (s), 1790 (s), 1485 (m), 1170 (m) cm^{-1}; NMR (Me_2SO-d_6) δ 7.6 (br, 3 H), 4.13 (s, 9 H), 3.83 (t, J = 6 Hz, 1 H), 2.96 (d, J = 6 Hz, 2 H). Anal. ($C_{13}H_{16}ClFeNO_2$) C, H, N.

Bacterial Growth Assay.[12] *L. mesenteroides* was grown in phenylalanine assay medium (Difco). Separate solutions of assay medium, L-Phe, test compound, and inoculum were delivered to a 13 × 100 mm screw cap culture tube for incubation at 37 °C. Growth was determined as optical density at 660 nm, measured at 0, 12, 24, 36, and 48 h. Standard inoculum was prepared by growing *L. mesenteroides* in lactobacilli broth AOAC (Difco) for 2–4 days at 37 °C, centrifuging down the bacteria, decanting excess broth, and adding 3 mL of sterile normal saline; 100 μL of this suspension was used as inoculum for each test incubation (3 mL final volume). It was found that growth of *L. meserentoides* was linear with Phe concentration over the range 0–14 μg/3 mL of incubation volume when measured at 24 or 36 h. Therefore, control growth curves were determined routinely at 0, 6, and 10 μg of Phe/tube. Compounds were tested at four concentrations (100, 200, 400, and 800 μg/tube) as inhibitors of phenylalanine utilization at each of five concentrations of phenylalanine (2, 4, 8, 12, and 14 μg/tube). To determine if an analogue could support growth in the absence of L-Phe, separate solutions of assay medium, test compound (100, 200, 400, or 800 μg/tube), and inoculum were combined and incubated at 37 °C.

Phenylalanine Hydroxylase Assay. The assay for inhibition of phenylalanine hydroxylase by phenylalanine analogues was a slight modification of the procedure developed by Ayling and co-workers.[14] The phenylalanine hydroxylase was prepared from rat liver through the first ammonium sulfate fractionation. Approximately 0.42 mg of protein was used in each incubation. Solutions of the phenylalanine analogues were prepared by dissolving approximately 10 μmol in 1.0 mL of 0.1 M Tris-HCl (pH 7.4) buffer so that addition of 0.1 mL of analogue to 0.9 mL of incubation mixture gave final analogue concentrations of 1.0 mM. Cofactor (stored under nitrogen in sealed serum bottles and frozen) was thawed just prior to use and its UV spectrum was taken to determine that no oxidation had occurred during storage. L-Phenylalanine was dissolved in 0.1 M Tris-HCl (pH 7.5) to make a 100 mM solution, of which 10 μL was used in each incubation. Catalase was not included in the incubations. A blank reaction containing all the components of the assay system except phenylalanine was run simultaneously. A control incubation, which monitored the hydroxylation of phenylalanine in the absence of any inhibitor, was run daily. L-Phenylalanine, phenylalanine hydroxylase, and the phenylalanine analogue to be tested were preincubated together for 2.5 min at 30 °C before initiation of the reaction by the addition of 50 μL of cofactor solution. All assays were run in duplicate, and linear initial rates were observed for up to 1.5 min. The change of absorbance at 330 nm was monitored and the inhibition was measured as the decrease in absorbance in the presence of the analogue relative to the control incubation.

Phenylalanine Decarboxylase Assay. Phenylalanine decarboxylase activity and inhibition were determined by the procedure described by Smissman and co-workers.[19]

Aromatic L-Amino Acid Decarboxylase Assay. The assay for inhibition of aromatic L-amino acid decarboxylase was a slight modification of the procedure used to assay phenylalanine decarboxylase. Aromatic L-amino acid decarboxylase was obtained from two hogs' kidneys and purified according to the procedure of Christenson et al., through the dialysis step.[13] The enzyme was divided into 1.5-mL aliquots and stored at −20 °C; the solution contained 280 mg of protein/mL. The evolution of $^{14}CO_2$ was linear as a function of time and enzyme concentration. The standard reaction mixture (1.0 mL) contained the following: 0.08 M Tris-HCl, pH 8.5; 10 mM 2-mercaptoethanol; 0.07 mM pyridoxal 5′-phosphate; 0.5 μCi of L-[1-^{14}C]phenylalanine; 3–30 mM L-phenylalanine. After a 15-min preincubation at 37 °C, the reaction was started by the addition of 14 mg of enzyme through the side arm. After incubation for 20 min at 37 °C, the reaction was stopped by the addition of 0.25 mL of 20% trichloroacetic acid through the side arm. The flasks were incubated for 30 min at 37 °C to allow the hydroxide of hyamine in the center well to absorb the CO_2 evolved. The plastic center wells were then transferred to scintillation vials and the amount of $^{14}CO_2$ was counted. The blanks contained denatured enzyme.

Acknowledgment. We thank the National Institutes of Health for their generous support of this work (GM-1341 and ES-01375). We are also grateful to Drs. S. Osaki and Charles Decedue for invaluable advice and assistance with various aspects of the biological testing and to Dr. R. Angelici for samples of the pyridylalanines.

References and Notes

(1) For previous papers in this series, see (a) R. P. Hanzlik and W. H. Soine, *J. Am. Chem. Soc.*, **100**, 1290 (1978); (b) R. P. Hanzlik, R. Tessell, and V. Kishore, *Xenobiotica*, **8**, 752 (1978).
(2) B. Loev and M. Flores, *J. Org. Chem.*, **26**, 3595 (1961).
(3) R. Kalish, T. V. Steppe, and A. Walser, *J. Med. Chem.*, **18**, 222 (1975).
(4) F. D. Popp and E. B. Moynahan, *J. Heterocycl. Chem.*, **7**, 351 (1970).
(5) F. D. Popp and E. B. Moynahan, *J. Med. Chem.*, **13**, 1020 (1970).
(6) J. W. Burnhart, J. A. Sefranka, and D. E. Bablitz, *Proc. Soc. Exp. Biol. Med.*, **130**, 1161 (1969).
(7) A. F. Dratz, J. C. Coberly, and J. H. Goldstein, *J. Nucl. Med.*, **5**, 40 (1964).
(8) L. Goldberg and L. E. Martin, *Life Sci.*, **3**, 1465 (1964).
(9) R. A. Yeary, *Toxicol. Appl. Pharmacol.*, **15**, 666 (1969).
(10) J. T. Suh, U.S. Patent 3 408 376 [*Chem. Abstr.*, **71**, 50229*t* (1969)]; U.S. Patent 3 382 267 [*Chem. Abstr.*, **69**, 59378*g* (1968)].
(11) A. N. Nesmeyanov, L. G. Bogomolova, I. G. Andrianova, V.

D. Vilichevskoya, and N. S. Kochetkova, *Khim.-Farm. Zh.*, 6, 61 (1972).
(12) (a) E. M. Lansford, Jr., and W. Shive, *Arch. Biochem. Biophys.*, 38, 347 (1952), and reference therein. (b) W. Shive and C. G. Skinner, in "Metabolic Inhibitors", Vol. I, R. M. Hochster and J. H. Quastel, Eds., Academic Press, New York, 1963, pp 2–74.
(13) J. G. Christenson, W. Dairman, and S. Udenfriend, *Arch. Biochem. Biophys.*, 141, 356 (1970).
(14) J. Ayling, R. Pirson, W. Pirson, and G. Boehm, *Anal. Biochem.*, 51, 80 (1973).
(15) D. N. Wheatley and J. Y. Henderson, *Nature (London)*, 247, 281 (1974).
(16) A. Misefari, G. Costa, and A. L. Costa, *Pharmacol. Res. Commun.*, 9, 785 (1977).
(17) M. R. Miller, D. McClure, and R. Shiman, *J. Biol. Chem.*, 251, 3677 (1976).
(18) E. A. Boeker and E. E. Snell, *Enzymes, 3rd Ed.*, 6, 217–254. (1972).
(19) E. E. Smissman, R. L. Inloes, and S. El-Antably, *J. Med. Chem.*, 19, 161 (1976).
(20) T. R. Bosin, A. R. Buckpitt, and R. P. Maickel, *Life Sci.*, 14, 899 (1974).
(21) W. Lovenberg, H. Weissbach, and S. Udenfriend, *J. Biol. Chem.*, 237, 89 (1962).
(22) S. Kaufman, *Adv. Enzymol.*, 35, 245 (1971).
(23) R. E. Counsell, P. Desai, T. D. Smith, P. S. Chan, P. A. Weinhold, V. B. Rethy, and D. Burke, *J. Med. Chem.*, 13, 1040 (1970).
(24) A. Tourain, *Biochim. Biophys. Acta*, 242, 345 (1971).
(25) J. M. Osgerby and P. L. Pauson, *J. Chem. Soc.*, 656 (1958).
(26) C. Bublitz, *Biochem. Pharmacol.*, 20, 2543 (1971).
(27) S. Udenfriend, P. Zaltzman-Nirenberg, and T. Nagatsu, *Biochem. Pharmacol.*, 14, 837 (1955).
(28) D. D. Watt and J. P. Vandevoorde, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 23, 146 (1964); see also ref 29.
(29) J. Ayling and G. D. Helfand, *Biochem. Biophys. Res. Commun.*, 61, 360 (1974).

Serotonin Receptor Binding Affinities of Tryptamine Analogues

Richard A. Glennon*

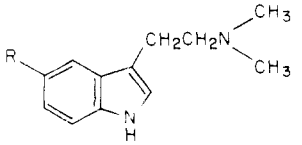
Department of Pharmaceutical Chemistry, School of Pharmacy, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298

and Peter K. Gessner

Department of Pharmacology, School of Medicine, State University of New York, Buffalo, New York 14214.
Received August 3, 1978

Using a rat fundus model, the serotonin (5-HT) receptor binding affinities of 27 tryptamine analogues were determined. Factors which might affect affinity were examined, e.g., lipid solubility, as reflected by partition coefficient, and pK_a. Structure–activity relationships were developed and are discussed in terms of substituents on the terminal amine, the side chain, and the indole 1 position, the 5 position, and at other positions on the indolic nucleus. If lipid solubility and metabolism can be accounted for, there appears to be a parallelism between 5-HT receptor binding affinities and the hallucinogenic (psychotomimetic) potencies of several of these compounds.

As first suggested by Woolley, there may be a relationship between the hallucinogenic (psychotomimetic) activity displayed by various *N,N*-dialkyltryptamine derivatives and their ability to interact with serotonin (5-hydroxytryptamine, 5-HT) receptors in the brain.[1,2] The possible endogenous production of such compounds, via abnormal 5-HT metabolism, might also be related to some of the symptoms of mental illnesses;[2] i.e., various aspects of mental illness and the mechanism of action of the hallucinogenic *N,N*-dialkyltryptamines may share certain common components. In support of this theory, enzyme systems have now been identified which can convert tryptamine and 5-HT into *N,N*-dimethyltryptamine (DMT, 1), 5-hydroxydimethyltryptamine (bufotenine, **2**)



1, R = H
2, R = OH
3, R = OCH_3

and 5-methoxydimethyltryptamine (**3**).[3,5] In addition, attempts have been made to detect these compounds in the urine of schizophrenic patients. While there have been reports that schizophrenics may possess significant levels of the above-mentioned alkylated tryptamines,[6,7] these findings are still controversial.[8,9] For a review see ref 10. Nevertheless, it has been demonstrated that these hallucinogens can interact with serotonergic receptors. Aghajanian and Haigler, employing a microiontophoretic technique, have concluded that low doses of hallucinogenic tryptamines act preferentially upon presynaptic 5-HT receptors to inhibit raphe neurons.[11] Bennett and Snyder, on the other hand, have investigated the binding of tryptamines to calf brain membrane preparations and have suggested that the binding sites involved might be postsynaptic 5-HT receptors.[12,13]

The potencies of tryptamines in causing contractions of the isolated rat stomach fundus strip paralleled their potencies in blocking lysergic acid diethylamide (LSD) binding by rat brain homogenates; thus, the fundus strip appears to be a valid model for brain receptors.[14] An investigation of the structure–activity relationships of tryptamine derivatives which interact with 5-HT receptors of various tissue preparations, for example, the fundus strip, might shed light on the structural requirements of these receptors. Furthermore, such an investigation might assist in elucidating the mechanism of action of hallucinogenic tryptamines in as much as the 5-HT receptors of these preparations might serve as models for central 5-HT–receptor interactions. We have previously reported that analogues **1–3** possess high binding affinities for the 5-HT receptors of the rat fundus preparation.[15] In this present study, we have determined the binding affinities (pA_2 values) of a rather extensive series of *N,N*-dialkyltryptamines and related compounds for the 5-HT receptors of this same model system in order to delineate SAR and

0022-2623/79/1822-0428$01.00/0