Aromatic Amino Acid Hydroxylase Inhibitors. 1.[1] Halogenated Phenylalanines

R. E. COUNSELL,* P. DESAI, T. D. SMITH, P. S. CHAN,

Laboratory of Medicinal Chemistry, College of Pharmacy, University of Michigan

P. A. WEINHOLD, V. B. RETHY, AND D. BURKE

Department of Biological Chemistry, University of Michigan Medical School, and Veterans Administration Hospital, Ann Arbor, Michigan

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A comparison of all the ring isomeric halophenylalanines as inhibitors of phenylalanine and tyrosine hydroxylase revealed that optimal inhibition was achieved with 3-iodophenylalanine. α-Methylation of the 3-halophenylalanines was found to enhance inhibition of tyrosine hydroxylase, but have no effect on phenylalanine hydroxylase. The implications of these findings are discussed.

One of the approaches to antihypertensive drugs has been to synthesize agents which inhibit the biosynthesis of norepinephrine. Although several enzymatic steps are available for pharmacological control, the demonstration by Udenfriend and coworkers[2] that tyrosine hydroxylase represents the rate-limiting step in norepinephrine biosynthesis has stimulated interest in inhibitors of this enzyme.

Although a number of inhibitors of tyrosine hydroxylase have been described,[3,4] α-methyltyrosine has been the most thoroughly investigated from a clinical standpoint. It has not only become a valuable pharmacological and biochemical tool, but also an effective agent for the treatment of pheochromocytoma.[5] On the other hand, this drug is slowly metabolized to α-methyldopa and α-methylnorepinephrine which may modify its overall pharmacological action particularly upon chronic administration.[6] For this reason, current interest is focused on competitive inhibitors of tyrosine hydroxylase which are not metabolized to catechols.

Moreover, it is important that the inhibitors be selective for tyrosine hydroxylase and not interfere with the other aromatic amino acid hydroxylases. Concomitant inhibition of phenylalanine hydroxylase, for example, would induce a state of phenylketonuria whereas interference with tryptophan hydroxylase would modify brain serotonin levels. It is for this reason that several years ago we initiated a program which would attempt to define and distinguish the structural features required for inhibition of the various aromatic amino acid hydroxylases. Because of the numerous reports dealing with the inhibitory properties of *p*-fluorophenylalanine and *p*-chlorophenylalanine, our first effort involved the comparison of all the isomeric halogenated phenylalanines as inhibitors of the aromatic amino acid hydroxylases. It was hoped that such studies would lead to a better understanding of the nature of the active sites and provide information that would be valuable for the design of selective inhibitors.

The halogenated phenylalanines were obtained commercially or synthesized according to published procedures. Optically active 3-iodophenylalanine was obtained by fractional crystallization of the diastereoisomeric salts formed by treating the *N*-acetyl derivative with *l*-amphetamine. The α-methyl amino acid analogs V were synthesized from the appropriate ketone III *via* hydrolysis of the hydantoin IV. Except for 3′-chlorophenyl-2-propanone (IIIb), the desired ketones had not been described previously. All of the ketones were readily obtained from the corresponding 3-halobenzylcyanides (I). The physical properties for these intermediates and the resulting amino acids are tabulated in Tables I–IV in the Experimental Section.

Experimental Section[7]

α-Acetyl-3-halobenzyl Cyanides (II). General Method.—A mixture of 3-halobenzyl cyanide (0.1 mole) and EtOAc (0.15 mole) was added dropwise to a refluxing soln of NaOEt (3.1 g of Na in 45 ml of EtOH) over a period of 1 hr and the mixture refluxed for 8 hr. The reaction mixture was cooled and poured into ice H_2O and the resulting mixture washed with Et_2O. The aq phase was acidified with AcOH and the product isolated by extraction with Et_2O. Removal of the solvent afforded a solid which was purified by recrystn from dil EtOH (see Table I).

TABLE I

α-ACETYL-3-HALOBENZYL CYANIDES (II)

No.	X	% yield	Mp, °C	Formula	Analyses
IIa	F	80	118–119	$C_{10}H_8FNO$	C,H
IIb	Cl	74	102–103	$C_{10}H_8ClNO$	*a*
IIc	Br	86	100–101	$C_{10}H_8BrNO$	C,H
IId	I	79	105–106	$C_{10}H_8INO$	C,H

[a] Reported mp, 102–103°, U. P. Basy and B. Bhattacharya, *J. Org. Chem.*, **32**, 4108 (1967).

3′-Halophenyl-2-propanones (IIIa–IIId). General Method.—A mixture of II (0.1 mole) in AcOH (35 ml) and concd HCl (75 ml) was refluxed for 24 hr. The reaction mixture was cooled, diluted

* To whom correspondence should be addressed.

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(2) *Cf.* S. Udenfriend, *Pharmacol. Rev.*, **18**, 43 (1966), and ref cited therein.

(3) (a) G. A. Johnson, E. G. Kim, W. Veldkamp, and R. Russell, *Biochem. Pharmacol.*, **16**, 401 (1967); (b) B. N. Lutsky and N. Zenker, *J. Med. Chem.*, **11**, 1241 (1968).

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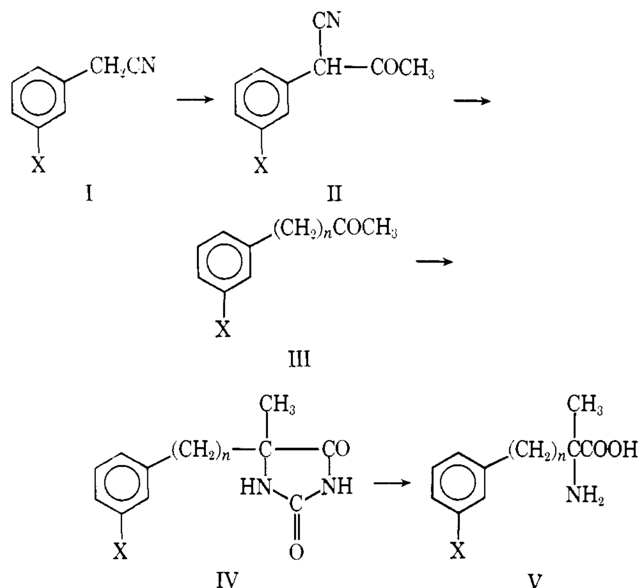
(5) (a) K. Engelman, D. Horwitz, E. Jequier, and A. Sjoerdsma, *J. Clin. Invest.*, **47**, 577 (1968); (b) N. F. Jones, G. Walker, C. R. J. Ruthven, and M. Sandler, *Lancet*, 1105 (1968).

(6) K. Engelman, E. Jequier, S. Udenfriend, and A. Sjoerdsma, *J. Clin. Invest.*, **47**, 568 (1968).

(7) Melting points were taken on a Fisher-Johns hot stage and are corrected. A Perkin-Elmer 141 polarimeter was used to determine optical rotations. Ir spectra are recorded on a Perkin-Elmer 337 spectrophotometer. The nmr spectra were obtained with a Varian A-60A spectrometer (Me_4Si). Elemental analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. Where analyses are indicated only by symbols of the elements, analytical results for those elements were within ±0.4% of the theoretical values.



a, X=F, n=1; b, X=Cl, n=1; c, X=Br, n=1; d, X=I, n=1; e, X=I, n=0; f, X=I, n=2

with twice the vol of H_2O, and extd with C_6H_6. The C_6H_6 ext was washed with 10% aq NaOH and dried ($MgSO_4$) and the solvent removed. The residual oil was purified by distillation *in vacuo* (see Table II).

Table II

3′-Halophenyl-2-propanones (III)

No.	X	% yield	Bp, °C (mm)	Formula	Analyses
IIIa	F	65	60–61 (0.75)	C_9H_9FO	C,H
IIIb	Cl	74	77–79 (0.5)	C_9H_9ClO	*a*
IIIc	Br	57	85–87 (0.2)	C_9H_9BrO	C,H
IIId	I	64	105–107 (0.6)	C_9H_9IO	C,H

[a] Reported bp 84–85° (1 mm), U. P. Basy and B. Bhattacharya, *J. Org. Chem.* **32,** 4108 (1967).

4-(3-Iodophenyl)-2-butanone (IIIf).—Prepared in 54% yield from acetylacetone and *m*-iodobenzyl bromide according to the method of Hauser, *et al.*,[8] bp 96° (0.1 mm). *Anal.* ($C_{10}H_{11}IO$)C, H.

Hydantoins (IV). General Method.—A solution of ketone (III, 0.1 mole), $(NH_4)_2CO_3$ (0.4 mole), and KCN (0.2 mole) in 50% EtOH (170 ml) was stirred and heated at 50–55° for 6 hr. The reaction mixture was dild with H_2O and chilled in an ice bath. The hydantoin IV that pptd was collected by filtration. Purification was achieved by recrystn from dil EtOH (see Table III).

Table III

Hydantoins (IV)

No.	X	n	% yield	Mp, °C	Formula	Analyses
IVa	F	1	78	231–232	$C_{11}H_{11}FN_2O_2$	C,H
IVb	Cl	1	90	239-240	$C_{11}H_{11}ClN_2O_2$	*a*
IVc	Br	1	88	249–250	$C_{11}H_{11}BrN_2O_2$	C,H
IVd	I	1	92	244	$C_{11}H_{11}IN_2O_2$	C,H
IVe[b]	I	0	84	270	$C_{10}H_9IN_2O_2$	C,H
IVf	I	2	81	218–219	$C_{12}H_{13}IN_2O_2$	C,H

[a] Reported mp 240–242°, J. Weinstock and R. Y. Dunoff, *J. Org. Chem.* **33,** 3342 (1968). [b] Starting ketone prepared according to R. E. Bowman, *J. Chem. Soc.* 322 (1950).

α-Methyl Amino Acids (V). General Method.—A solution of hydantoin (IV, 0.1 mole) and $Ba(OH)_2 \cdot 8H_2O$ (0.4 mole) in H_2O (500 ml) was heated at reflux for 4 days. The reaction mixture was cooled, acidified with 6 *N* H_2SO_4, and filtered through Celite. The filtrate was made basic with NH_4OH and concd *in vacuo* until the first sign of pptn. The concentrate was cooled in an ice bath and the resulting ppt collected by filtration. Purification was accomplished by recrystn from dil EtOH (see Table IV).

Table IV

α-Methyl Amino Acids (V)

No.	X	n	% yield	Mp, °C	Formula	Analyses
Va	F	1	76	276–278	$C_{10}H_{12}FNO_2$	C,H
Vb	Cl	1	73	279–280	$C_{10}H_{12}ClNO_2$	*a*
Vc	Br	1	76	270–271	$C_{10}H_{12}BrNO_2$	C,H
Vd	I	1	98	269–270	$C_{10}H_{12}INO_2$	C,H
Ve	I	0	10	267–268	$C_9H_{10}INO_2$	C,H
Vf	I	2	50	272–273	$C_{11}H_{14}INO_2$	C,H

[a] Reported mp 299–301°, J. Weinstock and R. Y. Dunoff, *J. Org. Chem.* **33,** 3342 (1968).

Resolution of 3-Iodophenylalanine.—A mixture of DL-*N*-acetyl-3-iodophenylalanine (6 g) and L-amphetamine (2.4 g) in EtOH (30 ml) and H_2O (15 ml) was warmed on a H_2O bath until solution was effected. The solution was then refrigerated for 12 hr. The ppt was collected, washed with cold aq EtOH, and dried. The product was recrystd from anhyd MeOH until the specific rotation remained constant. This gave 1 g of salt (24%), mp 190–192°, $[\alpha]^{25}D$ +42.4°. *Anal.* ($C_{20}H_{25}IN_2O_3$) C, H. A solution of this salt (180 mg) in H_2O (2 ml) and 2.5 *N* NaOH (2 ml) was extracted with $CHCl_3$. The aq phase was then heated to 70° and treated with 2.5 *N* HCl until the soln was acidic. The mixture was refrigerated and the product that separated collected by filtration and washed with H_2O. Recrystn from dil EtOH gave L-*N*-acetyl-3-iodophenylalanine (80 mg, 63%), mp 124–125°, $[\alpha]^{25}D$ +38.2°. *Anal.* ($C_{11}H_{12}INO_3$) C, H. Hydrolysis of the *N*-acetyl derivative (60 mg) was accomplished by refluxing in 1 *N* HCl (3 ml) for 5 hr. The solution was evapd to dryness under reduced pressure and the residue dissolved in aq EtOH. Treatment with NH_4OH afforded a ppt which was collected by filtration. Recrystn from dil EtOH gave L-3-iodophenylalanine (22 mg, 98%, mp 212–214°, $[\alpha]^{25}D$ −2.1°.[9] *Anal.* ($C_9H_{10}INO_2$) C, H.

Enzyme Inhibition Studies.—Phenylalanine hydroxylase was purified from rat livers according to the method of Kaufman,[10] through the first $(NH_4)_2SO_4$ fractionation. All incubation mixtures contained the following: 1.0 μmole of L-phenylalanine with 0.5 μCi of L-phenylalanine-^{14}C, 1.0 μmole of 6,7-dimethyl-5,6,7,8-tetrahydropteridine ($DMPH_4$), 25 μmoles of 2-mercaptoethanol, 0.08 μmole of Tris-HCl buffer, pH 7.3, and 0.8–1.2 mg of protein in a final volume of 1.0 ml. The $DMPH_4$ and 2-mercaptoethanol were added in 0.05 ml of 0.1 *M* phosphate buffer, pH 7.4. The halogenated amino acids were dissolved in 0.1 *N* HCl at a concentration of 0.02 *M* and 0.05 ml was added to the incubations. Controls were run with 0.05 ml of the 0.1 *N* HCl. The reactions were performed in 10-ml erlenmeyer flasks at 36° with shaking for 10 min, unless otherwise indicated. Reactions were stopped by transferring the reaction mixture to a test tube which was then immersed in boiling H_2O for 4 min. Denatured protein was removed by centrifugation, the samples were evaporated to dryness, and the ppt redissolved in 0.07 ml of distd H_2O. This was applied to Whatman No. 3MM paper. Descending chromatography was performed overnight, in the solvent system *i*-PrOH-concd NH_4OH (2:1). Autoradiograms of the chromatographs were made and used to determine the location of radioactive components. Except for the blanks, 2 radioactive spots showed up in all cases. Their R_f values corresponded to Phe and Tyr control spots. The spots were cut out and counted directly in a Nuclear Chicago scintillation spectrometer. Percent inhibition was determined by comparison to the controls run with each assay. Background counts, which never exceeded 1% of the total, were determined using samples in which the enzyme was denatured before the L-Phen was added. The per cent inhibition caused by the halogenated phenylalanines is listed in Tables V and VI.

Tyrosine hydroxylase was prepared from beef adrenal medulla according to Nagatsu, *et al.*[11] The conditions for the inhibition

(8) S. Boatman, T. M. Harris, and C. R. Hauser, *J. Org. Chem.*, **30**, 3321 (1965).

(9) The configuration was established by the method of O. Lutz and B. Jirgensons, *Ber.*, **63**, 448 (1930).

(10) S. Kaufman, *Methods Enzymol.*, **5**, 809 (1962).

(11) T. Nagatsu, M. Levitt, and S. Udenfriend, *J. Biol. Chem.*, **239**, 2910 (1964).

Table V

Inhibition of Phenylalanine Hydroxylase (PH) and Tyrosine Hydroxylase (TH) by Halogenated Phenylalanines

Halophenylalanine (DL)	% inhibition PH (1×10^{-3} *M*)	% inhibition TH (2×10^{-4} *M*)
2-F[a]	0	0
2-Cl	0	0
2-Br	0	0
2-I	0	0
3-F[a]	20	0
3-Cl	18	26
3-Br	33	41
3-I	56	68[b]
4-F[a]	58	35
4-Cl[a]	31	30
4-Br	19	0
4-I	20	0

[a] Obtained from Aldrich Chemical Company. [b] The resolved L-3-iodophenylalanine gave 78% inhibition at 1×10^{-4} *M*.

Table VI

Inhibition of Phenylalanine Hydroxylase (PH) and Tyrosine Hydroxylase (TH) by 3-Halo-α-methylphenylalanines

Compd	% inhibition PH (1×10^{-3} *M*)	% inhibition TH (2×10^{-4} *M*)
Va	11	23
Vb	16	48
Vc	32	76
Vd	52	86
Ve	0	0
Vf	0	0

studies were described previously.[12] The per cent inhibition of tyrosine hydroxylase by the halogenated phenylalanines at 2×10^{-4} *M* is listed in Tables V and VI.

Results and Discussion

Several investigators have studied certain halogenated phenylalanines for their ability to inhibit aromatic amino acid hydroxylases. No studies, however, have appeared which compare all of the isomeric halogenated phenylalanines for their effects on these enzymes.

DeGraw, *et al.*,[13] evaluated seven halogenated phenylalanines and found only 4-fluoro- and 4-chlorophenylalanine inhibited phenylalanine hydroxylase greater than 50% at 1×10^{-3} *M*. Substitution of halogen at the 3 or 2 position lowered the inhibitory activity. Moreover, conversion of *p*-fluorophenylalanine into its α-Me homolog caused a reduction of the inhibitory activity. Similar structure-activity relationships were observed by Koe and Weissman[14] in their study of the inhibitory effect of several halogenated phenylalanines upon rat liver tryptophan hydroxylase. These workers also noted that *p*-chlorophenylpyruvic acid was fully as potent as *p*-chlorophenylalanine *in vitro* and *in vivo* and suggested that the former may be the metabolite responsible for the action of the latter. If such is the case, this metabolism would be blocked by α-methylation.

These findings are in contrast to those reported for tyrosine hydroxylase inhibitors. Saari, *et al.*,[15] compared the 2-, 3-, and 4-fluorophenylalanines and found that both the 2 and 4 isomers produced >50% inhibition of tyrosine hydroxylase at 1×10^{-4} *M*. In this case, conversion into the α-methylated homolog did not decrease inhibitory activity. The high activity for the 2- and 4-fluoro analogs are in sharp contrast, however, to the finding of McGeer and McGeer[16] who noted that 3-iodophenylalanine is more active than 4-iodophenylalanine as an inhibitor of tyrosine hydroxylase.

The fact that the position and type of halogen are important considerations is further emphasized by the studies of Udenfriend and coworkers[17] with halogenated tyrosine analogs. Comparison of a series of 3-halotyrosine derivatives as inhibitors of tyrosine hydroxylase revealed that (1) the relative order of activity was I > Br > Cl > H > F and (2) the α-methyl amino acids were more potent than the unmethylated analogs.

Our own studies with all of the halogenated phenylalanines confirm and extend the above relationships. The most striking fact which emerges from a comparison of the data in Tables V and VI is that the order of increasing inhibitory activity of all the halogenated phenylalanines is the same for both phenylalanine hydroxylase and tyrosine hydroxylase. For the 3-halogenated analogs the order is I > Br > Cl > F, whereas for the 4-halogenated derivatives the order is F > Cl > Br and I. This order of activity for the 3-halophenylalanines brings to mind the recent findings of Holland, *et al.*,[18] with 3-halophenylcholine ethers. The ganglion stimulant action of these ethers was again in the order I > Br > Cl > F. MO calculations showed a close correlation between the nicotine-like activity and the highest filled orbital (HOMO) and superdelocalizability of atoms 2 and 6 of the aromatic ring. On this basis, it was proposed that the aromatic ring was involved in charge-transfer complex formation with a secondary group on the receptor.[18] Such may also explain the order of activity displayed by the 3-halophenylalanines.

The ability of α-methylation to enhance tyrosine hydroxylase inhibition was observed for all 3-halophenylalanines. This modification did not enhance inhibition of phenylalanine hydroxylase, however. Reduction or extension of the alanine side chain by one methylene destroys inhibitory activity against both enzymes. We are currently utilizing these studies as the basis for the design of potential *in vivo* inhibitors of tyrosine hydroxylase.

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