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Aromatic Amino Acid Hydroxylase Inhibitors. 4.[1] 3-Substituted α-Methyltyrosines

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In the present study a series of 3-alkenyl-α-methyltyrosines and their corresponding 3-alkyl- and dihydrobenzofuran analogs was synthesized for potential tyrosine hydroxylase (TH) inhibitory activity. The appropriately substituted hydantoins IIIa and IIIb, which were prepared from the corresponding allyloxybenzylhydantoins IIa and IIb through Claisen rearrangement, served as intermediates for the synthesis of these amino acids. TH inhibition was reduced upon either saturation of the double bond in the side chain or cyclization to form the dihydrobenzofuran analogs. Formation of the epoxide had a similar effect. The inhibitory activity of these compounds against aromatic amino acid decarboxylase (AADC) and dopamine β-hydroxylase (DBH) was also investigated. Unsaturation, in both cases, decreases the inhibitory activity; however, the presence of a free phenolic group appears to be essential for AADC inhibitory activity.

In recent years, numerous agents have been synthesized for the explicit purpose of modulating the central and peripheral biosynthesis of catecholamines. Since tyrosine hydroxylase (TH) is involved in the rate-limiting enzymatic step,[2] it is understandable that many of the investigations have been concerned with regulation of this enzyme.[3]

The α-methylated analogs of phenylalanine and tyrosine (α-MT) are competitive inhibitors of TH[4] and the *in vitro* activity of these compounds was enhanced by introduction of iodine at the 3 position. The relative activity for the halogen derivatives was I > Br > Cl > F.

This order of activity for halogenated analogs has been also noted for thyroxine hormones.[5] In this instance, replacement of the 3′-iodine with alkyl groups led to more potent thyromimetic agents.[6] Unfortunately, this change was not as rewarding when introduced into the TH inhibitors. In this case, the 3-alkylated derivatives showed much less enzyme inhibitory activity than their 3-iodo counterparts.[7]

The 3-alkylated analogs, however, do have an advantage over the 3-iodo derivatives in that they are also active inhibitors of TH *in vivo*. The 3-iodo derivatives lack significant *in vivo* activity because they are rapidly destroyed by tissue dehalogenases and transaminases.[3]

Among the 3-alkylated α-MT derivatives, the 3-methyl, ethyl, and isopropyl were as effective as α-MT as inhibitors of TH. The 3-*tert*-butyl derivative, on the other hand, was completely devoid of inhibitory activity at similar concentrations. While a certain degree of bulk at the 3 position does not interfere with interaction with the enzyme, apparently there is some limitation imposed as to the size of this substituent.

In an effort to derive additional information regarding the steric and structural prerequisites for TH inhibition, this paper describes the synthesis and evaluation of several 3-alkenyl derivatives of α-MT and their corresponding cyclization products.

Since the most widely used synthesis of α-methylamino acids involves hydrolysis of the appropriate hydantoin, the initial goal was the synthesis of hydantoins typified by III. Treatment of *p*-hydroxyphenyl-2-propanone with allyl bromide or methallyl chloride under basic conditions gave the desired ethers Ia and Ib. Treatment of these ketones with ammonium carbonate and KCN in aqueous alcohol afforded the desired hydantoins IIa and IIb in good yield.

On the basis of the extensive study of the Claisen rearrangement by White and Wolfarth,[8] ethylene glycol was initially selected as the most appropriate solvent for converting IIa to IIIa. Unfortunately, isolation of the product from this solvent proved difficult. When octanoic acid was used for the thermal rearrangement, IIIa was isolated in 72% yield. The best yields, however, were achieved when diphenyl ether was used as the solvent (Scheme I).

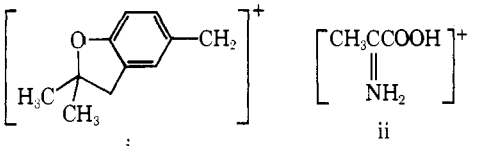
Catalytic reduction of IIIa and IIIb over Pd/C gave the hydantoins IVa and IVb which were readily hydrolyzed with aqueous $Ba(OH)_2$ to 3-*n*-propyl-α-methyltyrosine (VIIa) and 3-isobutyl-α-methyltyrosine (VIIb). Treatment of IIIa and IIIb with 48% HBr in glacial acetic acid[9] cyclized the o-allylphenols to the corresponding dihydrobenzofurans Va and Vb. Hydrolysis of these hydantoins furnished the amino acids VIIIa and VIIIb.

Barium hydroxide hydrolysis of the allyl derivatives was not as straightforward and the nature of the product was found to vary with the reaction temperature. For example, when IIIa was hydrolyzed in a sealed tube at 160°, only the amino acid IX was isolated. When the temperature was lowered to 130°, on the other hand, VIa was obtained in good yield. At temperatures between 130 and 160°, both amino acids were formed. At the reflux temperature, 26 hr was required to effect complete hydrolysis of IIIa, and, in this instance, only VIa was isolated. Isomerization of the allyl double bond[10] of VIa to give IX was achieved by treatment with base at high temperature. The hydantoin IIIb was hydrolyzed similarly, at 130°, and the amino acid VIb was obtained. Treatment of VIa with H_2O_2 in formic acid at 8° afforded the epoxide X in 23% yield.

Nmr and other spectral properties of all the compounds in this study were consistent with the assigned structures except for one case. In this instance, amino acid VIIIb gave a molecular ion peak at *m/e* 231 (P − 18) instead of

†Taken in part from the dissertation presented by L. E. Hare, June 1971, to the Graduate School of The University of Michigan in partial fulfillment of the requirement for the Doctor of Philosophy Degree.

the expected *m/e* 249. However, the base peak at *m/e* 161 (M − 88) and a fragment of *m/e* 88 (M − 161) corresponding to i and ii were present. Moreover, this loss of H_2O during mass spectral analysis of amino acids has been noted previously.[11] Fragmentation at the benzylic carbon (*i.e.*, i and ii) was noted for all the amino acids in this study and correlates with previously reported data.[7]



i ii

Enzyme Inhibition Studies. The 3-alkenyl-α-MT were analyzed for their ability to inhibit the catecholamine biosynthetic enzymes: TH, aromatic amino acid decarboxylase (AADC), and dopamine β-hydroxylase (DBH).

Examination of Figure 1 clearly illustrates that the analogs evaluated in this study fall into two groups with respect to their ability to inhibit TH. Only the 3-allyl derivative VIa, its conjugated isomer IX, and the *n*-propyl derivative VIIa were found to inhibit at approximately the same levels as α-MT, which was used as the standard. These results, along with previous studies in this series, indicate that TH inhibition is markedly reduced when one exceeds a 3-carbon moiety at the 3 position of α-MT. Unsaturation in the alkyl substituent seems to increase the TH inhibitory activity (compare VIa and IX to VIIa, and VIb to VIIb). Alternatively, conversion of the allyl substituent to an epoxide (X) or cyclization to the dihydrobenzofuran derivative (VIIIa and VIIIb) reduced the inhibitory activity.

Although the compounds in this study were synthesized primarily as TH inhibitors, their effect on the other enzymatic steps in the biosynthesis of norepinephrine (NE) was also evaluated (see Table I). Activity against AADC was compared with that of α-methyl-Dopa. In contrast with the results with TH, the most active inhibitor is the 3-isobutyl derivative VIIb. Unsaturation in this instance

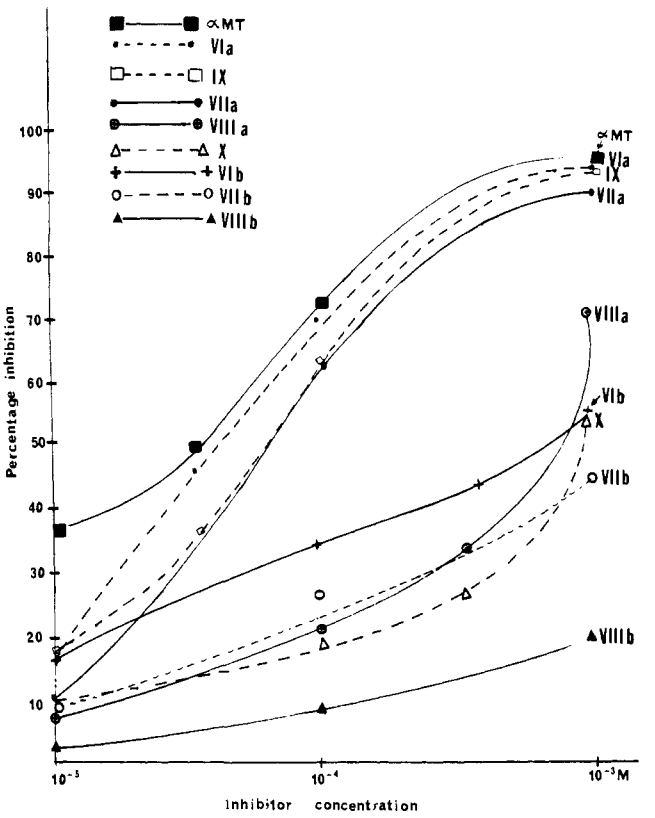
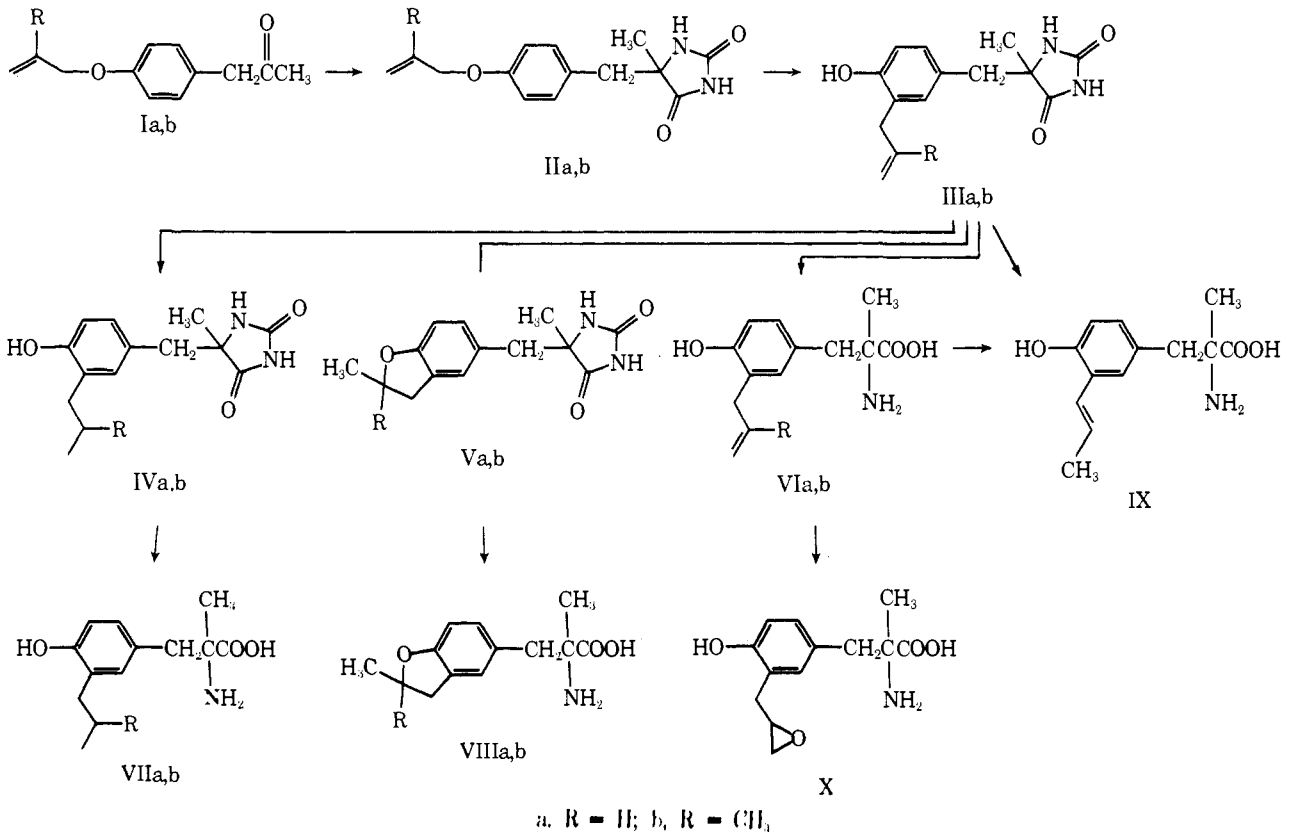


Figure 1. Inhibition of tyrosine hydroxylase (TH) by 3-substituted α-methyltyrosines.

Scheme I



a, R = H; b, R = CH_3

decreases inhibitory activity. The presence of a free phenolic group also appears to be essential.

The spectrophotometric procedure of Creveling, *et al.*,[12] was used for examining DBH inhibition. All compounds showed weak inhibitory activity. However, the 3-isobutyl derivative VIIb possessed the highest activity among the compounds studied. In contrast with the above results with TH and AADC, the presence of the phenolic OH does not appear to be as critical for inhibitory activity. The structure–activity relationships observed in this and previous studies have suggested other pertinent structural modifications which are currently being explored in these laboratories.

Experimental Section‡

1-[*p*-(Allyloxy)phenyl]-2-propanone (Ia). Potassium carbonate (18.0 g, 0.13 mol) was suspended in a solution of *p*-hydroxyphenyl-2-propanone[13] (17.1 g, 0.11 mol) and freshly distilled allyl bromide (15.7 g, 0.13 mol) in acetone (150 ml). The reaction mixture was refluxed 7 hr, allowed to cool, diluted with H_2O, and extracted with Et_2O. The Et_2O phase was washed successively with 10% NaOH and H_2O and dried ($MgSO_4$). Evaporation of the Et_2O gave a yellow oil from which pure Ia (17.2 g, 86%) was obtained by vacuum distillation: bp 111–114° (0.3 mm). *Anal.* ($C_{12}H_{14}O_2$) C, H.

1-[*p*-[(2-Methylallyl)oxy]phenyl]-2-propanone (Ib) was prepared in a manner similar to that described for Ia using methallyl chloride§ instead of allyl bromide. Vacuum distillation afforded Ib (80%): bp 118–120° (0.2 mm). *Anal.* ($C_{13}H_{16}O_2$) C, H; C: calcd, 76.43; found, 75.68.

5-[*p*-(Allyloxy)benzyl]-5-methylhydantoin (IIa). Hydrated $(NH_4)_2CO_3$ (45.5 g, 0.4 mol) and KCN (13.0 g, 0.2 mol) were suspended in a solution of Ia (17.1 g, 0.098 mol) in 50% EtOH (150 ml). The temperature of the stirred reaction mixture was raised to 50° over a 40-min period during which time all the solids dissolved. A precipitate formed after 45 min at 50–60°. Compound IIa (20.3 g, 80%) was collected by filtration and washed with H_2O followed by Et_2O. An analytical sample was recrystallized from EtOH: mp 188–189°. *Anal.* ($C_{14}H_{16}N_2O_3$) C, H.

5-Methyl-5-[*p*-[(2-methylallyl)oxy]benzyl]hydantoin (IIb) was prepared from Ib in a manner similar to that described for IIa. This gave IIb (77%) as white crystals: mp 176–178°. *Anal.* ($C_{15}H_{18}N_2O_3$) C, H.

5-(3-Allyl-4-hydroxybenzyl)-5-methylhydantoin (IIIa). A. Claisen Rearrangement in Ethylene Glycol. Compound IIa (5.0 g, 19.2 mmol) was dissolved in ethylene glycol (50 ml) and heated under N_2 at 170° for 25 hr. The reaction mixture was allowed to cool and diluted with 10% NaOH. The solution was washed with Et_2O to remove the ethylene glycol, and the alkaline solution was acidified with 10% HCl. The aqueous phase was extracted with Et_2O and the organic layer was washed with H_2O and dried ($MgSO_4$). Evaporation of the Et_2O afforded a tan solid. Pure IIIa (2.8 g, 56%) was obtained by recrystallization from MeOH–H_2O: mp 188–190°. *Anal.* ($C_{14}H_{16}N_2O_3$) C, H.

B. Claisen Rearrangement in Octanoic Acid. Compound IIa (31.5 g, 0.12 mol) in octanoic acid (75 ml) was heated at 200° under N_2 for 20 hr. The reaction mixture was allowed to cool and the resulting precipitate was collected by filtration and washed with $CHCl_3$ affording 22.6 g (72%) of IIIa: mp 144–146°; ir and nmr identical with that of an authentic sample of IIIa. The melting point was not raised by recrystallization from MeOH. A small amount of this material was dissolved in 10% NaOH. The alkaline solution was acidified and the crystalline solid collected: mp 182–185°; ir and nmr identical with that of an authentic sample of IIIa. Thus, compound IIIa may exist in two polymorphic forms.

C. Claisen Rearrangement in Diphenyl Ether. Compound IIa (5 g, 19.2 mmol) in $(C_6H_5)_2O$ (20 ml) was heated at 200° under N_2 for 20 hr. The solution was allowed to cool and the resulting gel diluted with $CHCl_3$ (100 ml). The precipitate was collected by filtration and washed with $CHCl_3$ to give IIIa (4.9 g, 98%): mp 143–145°; ir and nmr identical with that of an authentic sample of IIIa.

5-[4-Hydroxy-3-(2-methylallyl)benzyl]-5-methylhydantoin (IIIb) was prepared from IIb in a manner similar to that described for IIIa using method C and a reflux period of only 10 hr. This gave IIIb (95%) as a white crystalline compound: mp 155–157°. *Anal.* ($C_{15}H_{18}N_2O_3$) C, H.

5-(4-Hydroxy-3-propylbenzyl)-5-methylhydantoin (IVa). Compound IIIa (2.0 g, 7.7 mmol) was dissolved in 95% EtOH (50 ml) and hydrogenated over Pd/C (0.2 g) at 20 psi and room temperature for 2 hr. The catalyst was removed and the solution was evaporated to dryness affording a clear oil from which pure IVa (1.7 g, 84%) was obtained by trituration in $CHCl_3$. An analytical sample was prepared by recrystallization from MeOH–benzene: mp 133–135°. *Anal.* ($C_{14}H_{18}N_2O_3$) C, H.

5-(4-Hydroxy-3-isobutylbenzyl)-5-methylhydantoin (IVb) was prepared from IIIb in a manner similar to that described for IVa. This gave IVb (82%) as white needles: mp 116–118°. *Anal.* ($C_{15}H_{20}N_2O_3 \cdot H_2O$) C, H; H: calcd, 7.53; found, 6.99.

5-[(2,3-Dihydro-2-methyl-5-benzofuranyl)methyl]-5-methylhydantoin (Va). A solution of IIIa (3 g) in glacial AcOH (12 ml) and 48% aqueous HBr (6 ml) was refluxed for 0.5 hr under N_2. The cooled reaction mixture was added dropwise to H_2O (150 ml) and the precipitate was filtered, washed with H_2O until free from acid, and dried *in vacuo*. This gave Va (1.9 g, 63%): mp 184–186°. A portion was recrystallized from MeOH–H_2O to give an analytical sample: mp 188–191°. *Anal.* ($C_{14}H_{16}N_2O_3$) C, H.

5-[(2,3-Dihydro-2,2-dimethyl-5-benzofuranyl)methyl]-5-methylhydantoin (Vb). This compound was prepared from IIIb in a manner similar to that described for Va. This gave Vb (77%) as white crystalline powder: mp 235–236.5°. *Anal.* ($C_{15}H_{18}N_2O_3$) C, H.

3-(3-Allyl-4-hydroxyphenyl)-2-methylalanine (VIa). Method A. A mixture of the hydantoin IIIa (10 g, 38 mmol), $Ba(OH)_2 \cdot H_2O$ (40 g, 0.233 mol), and H_2O (150 ml) was refluxed for 26 hr with vigorous stirring. The cooled reaction mixture was brought to pH 1 with H_2SO_4 and filtered. The $BaSO_4$ precipitate was washed with dilute H_2SO_4. The pH of the filtrate and washings was adjusted to pH 6.5 with NH_4OH whereupon VIa precipitated (7.8 g, 83%): mp 297–299° dec. *Anal.* ($C_{13}H_{17}NO_3$) C, H.

Method B. Compound IIIa (1.0 g, 3.8 mmol) and $Ba(OH)_2 \cdot H_2O$ (3.0 g, 15 mmol) were suspended in H_2O (15 ml) and heated in a sealed tube at 130° for 6 hr. The reaction mixture was allowed to cool and was acidified to pH 1 with H_2SO_4 (2 ml). The resulting precipitate was removed by filtration and washed with H_2O. The pH of the filtrate and washings was adjusted to 6.5 with NH_4OH whereupon VIa precipitated (0.73 g, 81%). It was identical in all respects (melting point, ir, nmr, mass spectra) with that prepared by method A.

3-[4-Hydroxy-3-(2-methylallyl)phenyl]-2-methylalanine (VIb). This compound was prepared from IIIb in a manner similar to that described in method B above. This gave VIb (52%) as a white crystalline powder: mp 304–306° dec. *Anal.* ($C_{14}H_{19}NO_3$) C, H.

α-Methyltyrosines VII–IX. General Method. A mixture of hydantoin IIIa (3 g) and $Ba(OH)_2 \cdot H_2O$ (9 g) was suspended in H_2O (45 ml) and heated in a sealed tube at 160° for 4.5 hr. The reaction mixture was cooled and acidified to pH 1 with H_2SO_4 and the precipitate was removed by filtration and washed with H_2O. The filtrate was made slightly alkaline with NH_4OH and concentrated to *ca.* 100 ml. The precipitate (2.1 g, 74%) was collected by filtration and washed with H_2O. Recrystallization from H_2O gave IX as a colorless solid, mp 298–300° dec (see Table II).

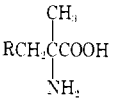
Isomerization of VIa. 3-Allyl-α-methyltyrosine (VIa, 0.47 g) was dissolved in H_2O (4 ml) containing KOH (0.3 g) and most of the H_2O removed *in vacuo*. To the orange residue was added diethylene glycol (2 ml) and triethanolamine (0.6 ml) and the thick syrup was heated under N_2 at 170–180° for 0.5 hr. The reaction mixture was allowed to cool and H_2O (30 ml) was added, followed by H_2SO_4 until pH 2 was reached. NH_4OH was added until the solution was faintly alkaline. The solution was chilled in ice and the precipitate was collected (0.28 g, 57%), mp 298–300° dec, whose ir and nmr spectra were identical with IX obtained in the general method for preparation of α-methyltyrosines described above.

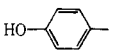
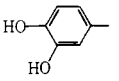
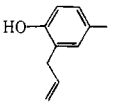
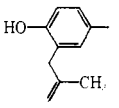
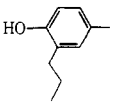
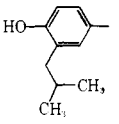
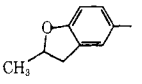
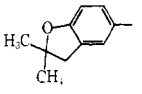
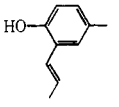
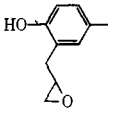
3-[3-(2,3-Epoxypropyl)-4-hydroxyphenyl]-2-methylalanine

‡Melting points were taken on a Thomas-Hoover apparatus and are corrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. Analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich., and Midwest Microlab, Ltd., Indianapolis, Ind. Ir spectra were recorded on a Perkin-Elmer 337 spectrophotometer. Nmr spectra were obtained with a Varian A-60A spectrometer (Me_4Si or DDS). Mass spectra were recorded on Du Pont 21-490 and AEI MS 30 mass spectrometers with DS 30 data system.

§Aldrich Chemical Co., Milwaukee, Wis.

Table I. Inhibition of Catecholamine Biosynthetic Enzymes[a]



Compd	R	Per cent inhibition				
		TH			AADC	DBH
		1×10^{-3} *M*	1×10^{-4} *M*	1×10^{-5} *M*	1×10^{-3} *M*	1×10^{-3} *M*
α-MT		95.6	73.4	37.7	11.0	39.9
α-Methyl-Dopa					75.9	*b*
VIa		94.3	69.8	17.2	30.5	24.9
VIb		52.6	34.7	17.2	21.8	45.6
VIIa		90.3	63.5	11.9	31.6	47.8
VIIb		44.4	26.3	9.6	50.7	68.2
VIIIa		69.0	20.4	8.5	11.6	42.0
VIIIb		19.9	8.9	3.2	9.9	36.3
IX		93.2	64.1	19.3	14.5	*b*
X		52.4	19.1	10.0	8.1	12.7

[a] For details of these determinations see the Experimental Section. [b] Inhibitors interfered with spectrophotometer assay.

(**X**). To a solution of 3-allyl-α-methyltyrosine (VIa, 3 g) in 88% HCOOH (15 ml) was added 30% H_2O_2 (1.6 ml) at a temperature less than 5°. The solution was kept at 8° for 3 days and then crystals of Na_2SO_3 were added until a negative test with KI-starch test paper was obtained. The HCOOH was removed *in vacuo* at room temperature and the semisolid residue was dissolved in MeOH (200 ml). A solution of $Ba(OH)_2 \cdot 8H_2O$ (10 g) in MeOH (150 ml) was added dropwise, with stirring, until the solution became definitely alkaline. The mixture was warmed gently on a steam bath for 10 min and cooled. A solution of oxalic acid (1 g) in MeOH (10 ml) was added dropwise until the mixture became faintly acidic; then the mixture was rendered faintly alkaline with NH_4OH. The mixture was filtered and the filtrate was evaporated *in vacuo* at room temperature. The residue that remained was dried *in vacuo* and crystallized from MeOH. The white crystals (0.7 g, 23%) were collected: mp 266–268° dec. A portion was recrystallized from MeOH to give an analytical sample, mp 275–277° dec. *Anal.* ($C_{13}H_{17}NO_4 \cdot H_2O$) C, H; C: calcd, 57.98; found, 58.56.

Enzyme Inhibition Studies. Materials and Methods. Reagent chemicals and glass-distilled water were used in all enzyme preparations and assays. L-Tyrosine-*carboxyl*-^{14}C and DL-3,4-dihydroxyphenylalanine-*carboxyl*-^{14}C were purchased from New England Nuclear Corp. L-Tyrosine and 6,7-dimethyl-5,6,7,8-tetrahydropterine hydrochloride ($DMPH_4$) were obtained from Calbiochem. 3-Iodotyrosine, DL-2-methyl-3-(3,4-dihydroxyphenyl)alanine (α-methyl-Dopa), DL-2-methyl-3-(3-hydroxyphenyl)alanine (α-methyltyrosine, α-MT), DL-3,4-dihydroxyphenylalanine (Dopa), fumaric acid, tyramine hydrochloride, and catalase as a purified powder from bovine liver were purchased from Sigma Chemical Co. NCS was obtained from Amersham/Searle Co.

Table II. α-Methyltyrosines VII–IX

Compd	R	Mp, °C	% yield	Formula[a]
VIIa	H	295–297 dec	84	$C_{13}H_{19}NO_3$
VIIb	CH_3	301–302 dec	73	$C_{14}H_{21}NO_3$
VIIIa	H	273–275 dec	71	$C_{13}H_{17}NO_3 \cdot 0.5H_2O$
VIIIb	CH_3	312–314 dec	80	$C_{14}H_{19}NO_3$
IX		298–300 dec	74	$C_{13}H_{17}NO_3$

[a] All compounds were analyzed for C and H.

A. Aromatic Amino Acid Decarboxylase Assay. The nonspecific aromatic amino acid decarboxylase (AADC) was isolated

from fresh hog kidneys and assayed as reported in the literature.[14] Dopa (50 nmol) was incubated with 0.5 unit of AADC in a 0.5-ml incubation mixture and the evolved ^{14}C-labeled carbon dioxide trapped in NCS solubilizer and counted in a toluene cocktail with a 94% efficiency.

B. Tyrosine Hydroxylase Assay. Tyrosine hydroxylase (TH) was isolated from bovine adrenals by the method of Nagatsu, *et al.*[15] Its activity was determined by the coupled decarboxylase assay[14] as modified previously.[1] $DMPH_4$ was dissolved in 0.005 *N* HCl and the inhibitors were dissolved in the phosphate buffer. An excess of AADC (7.5 units per 0.5-ml incubation mixture) was used to decarboxylate the Dopa-*carboxyl*-^{14}C formed from tyrosine-*carboxyl*-^{14}C. Under these conditions no limitation on decarboxylation was observed even with compounds which in the assay of AADC were good inhibitors.

C. Dopamine β-Hydroxylase Assay. Dopamine β-hydroxylase (DBH) was isolated from bovine adrenal medullary tissue as described by Friedman and Kauman[16] and modified by Kuzuya and Nagatsu.[17] It was assayed by the spectrophotometric method reported in the literature.[12]

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Effect of Acylation with Eleostearic Acids on the Monoamine Oxidase Inhibitory Potency of Some Hydrazine Antidepressants in Mice

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The effect of incorporation of an eleostearoyl group into molecules of aralkylhydrazines on their monoamine oxidase inhibitory potency was investigated *in vitro* and *in vivo*. The results showed that on a molar basis the hydrazides possessed an *in vitro* potency lower than and an *in vivo* potency and acute toxicity comparable to those of the corresponding aralkylhydrazines. The sequence of the relative potency of aralkylhydrazines and their hydrazides was similar. The overall pharmacological profile indicated that these aralkylhydrazines retained their monoamine oxidase inhibitory properties when the free hydrazino nitrogen was acylated with an eleostearoyl group.

Pheniprazine (PIH), phenelzine (PEH), benzylhydrazine (BZH), and isopropylhydrazine are among the most potent monoamine oxidase (MAO) inhibitors,[1] yet only phenelzine has been used as a safe antidepressant. Acylation of these hydrazines with various acyl groups has led, in some instances, to hydrazides possessing comparable potency, greater organ specificity, and reduced toxicity.[1,2] The unsaturated long-chain fatty acids such as oleic, linoleic, and linolenic acid inhibit mitochondrial MAO to some extent *in vitro* whereas the saturated fatty acids such as palmitic and stearic acid are ineffective.[3] These fatty acids are taken up by the brain without prior oxidation to acetate.[4-6] This property would furnish a possibility of transport of the hydrazide molecules made from similar fatty acids and aralkylhydrazines to the brain for action. The present study was undertaken to determine whether these aralkylhydrazines retain their potency when the free hydrazino nitrogen is acylated with eleostearic acids. Syntheses of these hydrazides have been described previously.[7]

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Pharmacological Results. Acute Toxicity. Neither a lethal effect nor toxic symptoms other than diarrhea were observed for eleostearic acid isomers at 10.8 mmol/kg (3.0 g/kg) over a period of 2 weeks. The acute toxicity as indicated by LD_{50} over a 72-hr period of the hydrazides was equal to that of the corresponding aralkylhydrazines (Table I). The neuropharmacologic symptoms at 0.70 mmol/kg were sedation induced by benzylhydrazine and its hydrazide and central stimulation induced by phenelzine, pheniprazine, and their hydrazides. The central stimulant effects of the four inhibitors were qualitatively similar. The prominent symptoms included enhanced spontaneous motor activity, jumping and squeaking, and stereotyped behavior. The onset of central stimulation was 8–15 min after injection of phenelzine and pheniprazine and 2–2.5 hr after their acylated derivatives. It lasted 0.5–2 hr in the case of hydrazines, whereas it persisted for 5–7 hr in the case of hydrazides.

Inhibition of MAO Activity *in Vitro*. The inhibitory