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Dual Enzyme-Activated Irreversible Inhibition of Monoamine Oxidase

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(*E*)-β-Fluoromethylene-*m*-tyrosine and related amino acids were synthesized from acetophenone derivatives and shown to be dual enzyme-activated inhibitors of monoamine oxidase. These substances are decarboxylated by hog kidney aromatic L-amino acid decarboxylase liberating (*E*)-β-fluoromethylene-*m*-tyramine derivatives which, in turn, are enzyme-activated inhibitors of rat brain mitochondrial monoamine oxidase.

Over the past decade the use of enzyme-activated irreversible inhibitors to selectively inhibit target enzymes has been an extremely fruitful approach, leading to the design and synthesis of a number of therapeutically useful compounds (*1*). A major attraction of this type of enzyme inhibitor is the high degree of target enzyme specificity that can be achieved in most cases. It could also be useful (but is rarely possible) to direct enzyme-activated inhibitors to certain key sites where enzyme inhibition is most desirable. An example where such dual specificity would be advantageous is the inhibition of monoamine oxidase (MAO; EC 1.4.3.4) (*2, 3*). MAO is a membrane bound, flavin-dependent mitochondrial enzyme which oxidatively deaminates a large variety of biogenic and exogenous amines in both the brain and peripheral organs of animals and man. Based on studies with selective inhibitors and on the differences in rates at which substrates are oxidized, two forms of the enzyme have been identified. Type A MAO selectively deaminates norepinephrine and serotonin and is inhibited by clorgyline while Type B preferentially oxidizes benzylamine and phenethylamine and is selectively inhibited by L-deprenyl and MDL 72145 (*4, 5, 19*).

Inhibitors of MAO have long been recognized as effective antidepressants though today they are rarely used in the clinic because of adverse side effects. It is generally accepted that MAO inhibitors exert their beneficial effect by inhibiting the brain enzyme contained in monoamine nerve endings (*6*). On the other hand, side effects, such as hypertensive crises following the ingestion of tyramine-containing foodstuffs, can usually be attributed to inhibition of Type A MAO in the gut, liver, and peripheral monoamine nerve terminals (*7*). Selective inhibition

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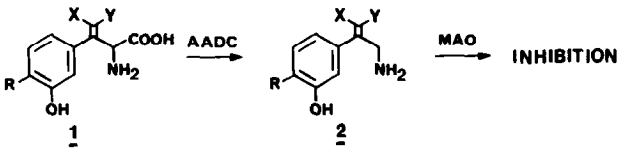
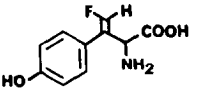


a X = F; Y,R = H
b Y = F; X,R = H
c X = Cl; Y,R = H
d X,Y,R = H
e X = F; R = CH_3; Y = H
f X = F; R = OCH_3; Y = H



1g

of the brain enzyme would thus appear to be an attractive approach to overcome the shortcomings of classical MAO inhibitors while retaining, or even enhancing, the therapeutic benefits of such treatment.

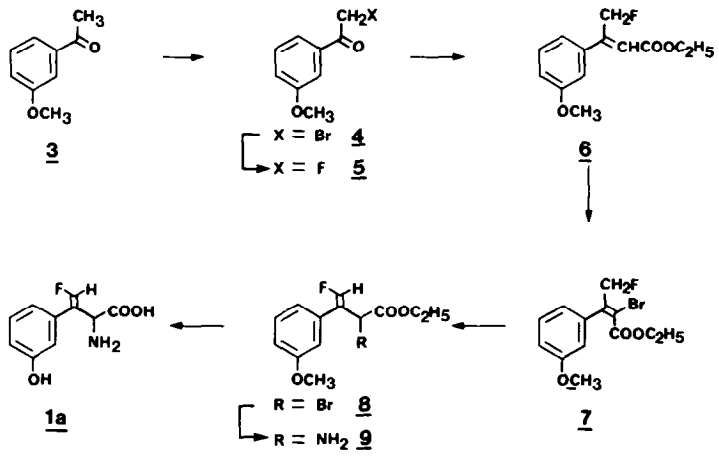
β-Fluoromethylenephenethylamine derivatives (**2**) are potent, enzyme-activated, irreversible inhibitors of MAO (*8*). These compounds are structurally related to naturally occurring phenethylamines, many of which are involved in neuronal transmission in monoamine nerves. An essential step in the biosynthesis of these neurotransmitters is the enzyme-catalyzed decarboxylation of the corresponding ring hydroxylated phenylalanine precursor. This conversion is performed by aromatic L-amino acid decarboxylase (AADC; EC 4.1.1.26) (*9*), a widely distributed enzyme which in the brain is predominantly located in monoamine nerve endings (*10*). Thus, it was conceived that if β-fluoromethylenephenylalanine derivatives (**1**) were also substrates for AADC such a dual enzyme-activated approach could offer a means of restricting MAO inhibition to monoamine nerve endings. Moreover, if a peripherally selective inhibitor of AADC was coadministered with **1**, this could potentially lead to selective inhibition in the brain. To test this hypothesis, and since *m*-tyrosine is a good substrate of AADC (*11*), we have prepared (*E*)-β-fluoromethylene-*m*-tyrosine (**1a**)[2] and related amino acids (**1b–g**).

SYNTHESIS

The syntheses of the allylamines **2a** and **d** have been reported previously (*8*); the preparation of **2c** and **e** followed standard procedures which are described under Experimental.

Two general routes were used to prepare the amino acids. The first (Scheme I), which incorporates a Wittig-type reaction to construct the carbon skeleton (*12, 13*), led to virtually pure *E*-isomers while the second (Scheme II), relying upon an isocyanoacetate condensation and reductive ring opening of the oxazoline intermediates (*14*), afforded mixtures of both isomers. Both routes used *m*-methoxy-

[2] A part of this work has been published as a preliminary communication (*12*).

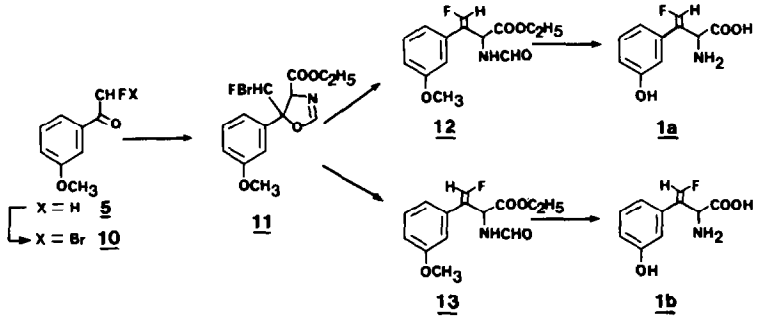


SCHEME I. Wittig route.

acetophenone derivatives as starting materials. The following description relates to the preparation of (*E*)- and (*Z*)-β-fluoromethylene-*m*-tyrosine (**1a** and **b**, respectively); the synthesis of the related amino acids **1c–g** can be found under Experimental.

Wittig route (*12, 13*). Bromination of **3** with Br_2 in $CHCl_3$ afforded the bromoketone **4** in 88% yield. Of the many procedures investigated, fluoride exchange was best achieved using KHF_2 in hot triethylene glycol followed by distillation from K_2CO_3. In this way fluoroketone **5** could be readily obtained in yields greater than 50%. Condensation of **5** with the anion derived from triethyl phosphonoacetate afforded **6** in 80% yield as a mixture of isomers (*Z*/*E* = 8/1). Bromine addition followed by dehydrobromination using piperidine led to the bromoester **7** (*Z*/*E* = 95/5) in good yield. Deconjugation (LDA) afforded the α,β-unsaturated bromoester **8** as the major isomer (>99%). Without purification **8** was reacted with NH_3 in dimethyl sulfoxide (DMSO) whereupon the amine **9** was isolated as its hydrochloride salt in 29% yield from **7**. Deprotection with refluxing aqueous HBr followed by treatment with propylene oxide in CH_3OH afforded **1a** as a colorless powder in 86% yield.

Isocyanoacetate route (*14*). Bromination of **5** with phenyltrimethylammonium tribromide in CH_2Cl_2 gave **10** in 87% yield. Condensation of **10** with ethyl isocyanoacetate using the Cu_2O-catalyzed procedure (*14, 15*) afforded an 82% yield of the oxazoline **11** as a mixture of diastereomers. Reductive ring opening followed a modification of the published method (*14*). The reaction proceeded smoothly when CF_3COOH was added to protonate the oxazoline prior to the addition of Zn. In this way, equal amounts (28% yields) of **12** and **13** were obtained. In the case of the (*E*)-isomer **12**, deprotection and liberation of the free amino acid **1a** proceeded as above in 62% yield. On the other hand, while deprotection of (*Z*)-**13** with aqueous HBr and subsequent treatment with propylene oxide gave chromatographically and spectroscopically pure **1b** (59% yield), this substance did not give a satisfactory combustion analysis.



SCHEME II. Isocyanoacetate route.

X-RAY STRUCTURAL ANALYSES

Confirmation of the (*E*)-geometry of **1a**, and hence of the other amino acids, was established by X-ray structural analysis of the partially protected derivative **9** (Fig. 1). An unusual feature is the large dihedral angle (98.7°) between the planes of the phenyl ring and the double bond. Unfortunately, it has not yet been possible to undertake a similar study with (*Z*)-**1b** due to poor crystal forms and general stability problems.

EVALUATION OF DUAL ENZYME-ACTIVATED INHIBITORY PROPERTIES

The amino acids (**1a–e**) were found to be substrates of partially purified hog kidney AADC (*16*) and aliquots taken from the incubation medium at various times inhibited MAO (Fig. 2). The degree of MAO inhibition increased progressively and was related to the rate at which **1** was decarboxylated. Since racemic mixtures were used, decarboxylation proceeded smoothly until 50% of the compound was consumed, which is in agreement with the stereoselectivity of AADC. The appearance of the amines (**2a–e**, respectively) and the disappearance of the amino acids (**1a–e**) were monitored by HPLC. In one case (**1a** → **2a**), the structure of the new product was confirmed to be **2a** by gas chromatography/mass spectrometry analysis of the *N,O*-trifluoroacetyl derivative. Otherwise, the amines were identified by comparison of the retention times with those of authentic samples, the one exception being **2b**. In this case the peak appearing on the chromatogram was assumed to be that of **2b** and it was also assumed that the detection response of **2b** was the same as that of **2a**. If AADC was omitted from the incubation medium, or if the potent AADC inhibitor α-monofluoromethyldopa (*17, 18*) was included with the AADC, no decarboxylation occurred and no amine could be detected. (*E*)-β-Fluoromethylene-*m*-tyrosine (**1a**) is not an inhibitor of AADC. When tested as a time-dependent inhibitor up to a concentration of 2 mM

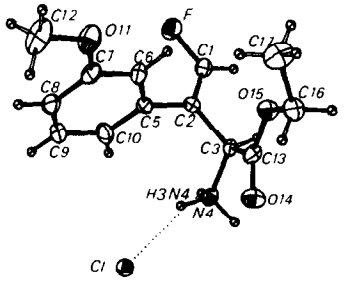


FIG. 1. X-ray structural determinations. ORTEP plot of the hydrochloride salt of ethyl 2-amino-3-(3-methoxyphenyl)-4-fluoro-3-butenoate (**9**).

no inhibition was observed whereas under the same conditions α-monofluoromethyldopa produced 94% inhibition within 4 min at 10 μM. We have previously reported (*25*) that **1a** (100 μM) does not inhibit AADC in isolated synaptosomes *in vitro* or after ip injection into rats at 0.5 mg/kg.

The substrate properties of the (*E*)- and (*Z*)-fluoro-isomers **1a** and **1b**, respectively, were studied in comparison to those of L-Dopa; K_m, V_{max}, and V_{max}/K_m ratios determined from Lineweaver–Burk plots are shown in Table 1.

The pure amines (**2a, c–e**), when tested (*8*) as selective (Type A or B) inhibitors of MAO, were found to be time-dependent inhibitors, the inhibition following pseudo-first-order kinetics for at least two half-lives. The fluorine-containing compounds (**2a** and **e**), as expected (*8*), were potent inhibitors, the others much less so. The selectivity (*8*) for the A or B form of MAO, shown in Table 2, while not very marked, varied from compound to compound.

The amino acids were proven to be dual enzyme-activated inhibitors of MAO as follows. When tested as time-dependent inhibitors of MAO directly, the amino acids (**1**) were inactive. If AADC was omitted from the first incubation medium, or if the AADC was inhibited, aliquots failed to inactivated MAO. Finally, we

TABLE 1

K_m, V_{max}, AND V_{max}/K_m VALUES FOR THE DECARBOXYLATION OF THE AMINO ACIDS **1a**, **1b**, AND L-DOPA BY AROMATIC L-AMINO ACID DECARBOXYLASE

Compound	K_m (μM)	V_{max} (nmol/min/mg protein)	$\frac{V_{max}}{K_m} \times 100$
1a	600	41	6.8
1b	75	2	2.7
L-Dopa	55	99	180

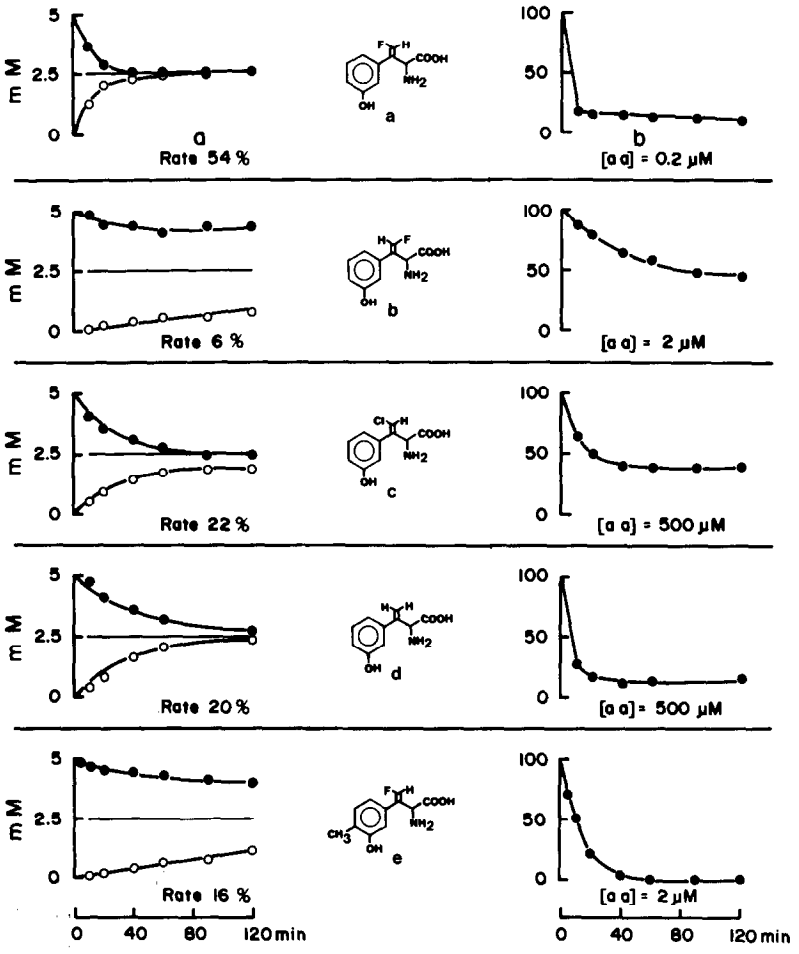


FIG. 2. The left-hand-side graphs show the AADC-mediated conversion of the racemic amino acids (**1**, ●) into amines (**2**, ○). The rate of decarboxylation of the L-isomer is compared to the rate of conversion of L-dopa → dopamine (100%). The appearance of the amines as a function of time is also analyzed by testing aliquots of the incubation medium as inhibitors of MAO (shown on the right hand side; ordinate shows % control MAO activity). aa = final amino acid concentration used in the MAO inhibition assay.

have previously demonstrated that allylamines of structure **2** satisfy all requirements (*8*, *19*) to be considered as enzyme-activated inhibitors of MAO.

DISCUSSION

It was not clear at the outset of this work whether **1** would be a substrate, an inhibitor, or both for AADC. Looking at the possible mechanisms involved in the conversion of **1** to **2** on the one hand and in the inactivation of MAO by **2** on the other (Scheme III), it appears that very similar activated intermediates **14** and **15**,

TABLE 2

AADC SUBSTRATE PROPERTIES OF AMINO ACIDS **1a–g** AND MAO INHIBITORY KINETICS AND SELECTIVITY OF AMINES **1a** AND **c–e**

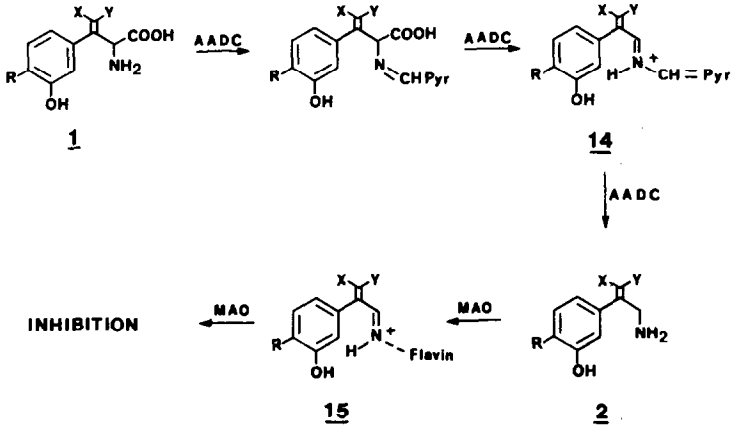
Compound	R	X	Y	AADC	MAO inhibitory potency of amines **2**				
					Type A		Type B		
				Formation of **2**[a]	$t_{1/2}$ (min)	I (M)	$t_{1/2}$ (min)	I (M)	Type A selectivity[b]
1a	H	F	H	54	2	1×10^{-7}	15	1×10^{-7}	10
1b	H	H	F	6	ND	ND	ND	ND	ND
1c	H	Cl	H	22	6.5	5×10^{-5}	6.5	5×10^{-4}	0.1
1d	H	H	H	20	5	5×10^{-5}	5	2.5×10^{-4}	5
1e	CH_3	F	H	16	16	5×10^{-9}	4.5	5×10^{-9}	0.2
1f	CH_3O	F	H	—					
1g				—					

[a] Relative to the rate of decarboxylation of L-dopa → dopamine (100%) and based on 50% conversion of racemic **1**.

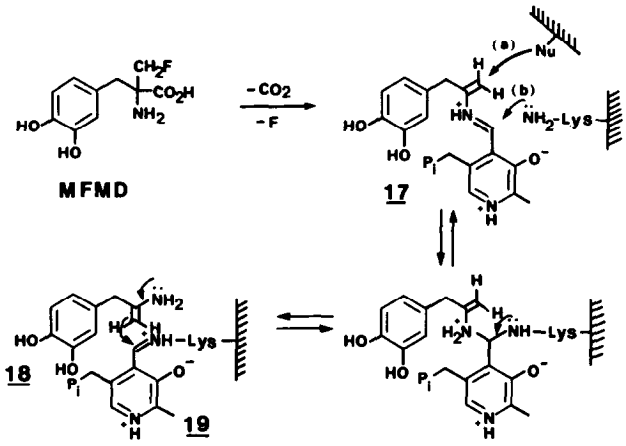
[b] The selectivity for the A form relative to the B form of MAO was estimated from the ratio of the concentrations of the inhibitors needed to decrease the activity of both forms of the enzyme at the same rate.

respectively, could be formed. Nevertheless, for **1** to act cleanly as a dual enzyme-activated inhibitor of MAO, **14** is required to undergo subsequent transformations leading to **2** while **15** must eventually interact irreversibly with MAO.

The complete lack of AADC inhibitory properties of **1** is somewhat surprising in view of the structural similarities between **14** and the reactive intermediate **17** (*18*) (Scheme IV) which has been postulated to alkylate a nucleophilic enzyme residue (pathway a) during the inactivation of AADC by the extremely potent enzyme-activated irreversible inhibitors α-monofluoromethyldopa (MFMD) and α-monofluoromethyl-*m*-tyrosine (MFM-tyrosine). This lack of inhibition of **1** could reflect either an absence of a nucleophilic enzyme residue suitably positioned relative to



SCHEME III. Schematic representation of the dual enzyme-activated inhibition of MAO by the amino acids (**1**).



SCHEME IV. α-Monofluoromethyl dopa inhibition of AADC.

the activated intermediate **14** (Scheme III) or a mechanism for the inactivation of AADC by MFMD and MFM-tyrosine which is different from the one postulated. Along these lines it is interesting to consider the mechanism demonstrated by Metzler and co-workers (*20*) for the inactivation of L-glutamic acid decarboxylase by *O*-sulfate-L-serine. The Metzler mechanism has been recently shown to apply to the inactivation of the PLP-dependent enzymes alanine racemase (*21*) and amino acid racemase (*22*) by β-substituted alanines. If such a mechanism (Scheme IV) were to apply to the inhibition of AADC by MFMD and MFM-tyrosine, the intermediate **17** would not directly interact irreversibly with an enzyme residue (pathway a) but would be converted to the enamine **18**, with concomitant formation of a Schiff base (**19**) between the pyridoxal phosphate cofactor and the lysine residue (pathway b). Subsequent alkylation of **19** by **18** would be the critical step leading to irreversible inactivation. In the case of the putative intermediate **14** (Scheme III), chemical transformations such as these would be unlikely.

A qualitative appraisal of the ability of AADC to decarboxylate the tyrosine derivatives **1** was obtained by measuring the rate of appearance of the corresponding products **2** when AADC was incubated with 5 mM of **1**. It was assumed that the amino acid concentrations used in these assays were much greater than K_m and that consequently the rate of appearance of product would approximate V_{max}. From the results presented in Table 2 it can be seen that (*Z*)-**1b** was decarboxylated at approximately one-ninth the rate of **1a**, while the non-halogen-substituted compound **1d** and the (*E*)-chloro derivative **1c** were decarboxylated at approximately one-half the rate. The *p*-CH_3 analog **1e** was one-third as good a substrate as **1a** but the *p*-OCH_3 compound **1f** was not converted to **2f** at a measurable rate. The latter result is difficult to put into perspective because although 3-hydroxy-4-methoxyphenylalanine has been reported to be a good substrate for guinea pig kidney AADC, it was not decarboxylated by the mouse brain enzyme (*23*). (*E*)-β-

fluoromethylene-*p*-tyrosine (**1g**), as expected (*9*), was not decarboxylated to any extent by AADC under our assay conditions.

These qualitative results were partially substantiated when the kinetic parameters K_m and V_{max} of the isomers **1a** and **1b** were determined and compared to those of L-Dopa (Table 1). At amino acid concentrations less than K_m, however, it can be seen from the V_{max}/K_m ratios that L-Dopa is a somewhat better substrate than these analogs.

It has been known for some time that AADC can tolerate substitution in both the α and β positions of ring-hydroxylated phenylalanine substrates. For example, L-threo-3,4-dihydroxyphenylserine is a relatively good substrate (*24*) although α-methyldopa and α-methyl-*m*-tyrosine are considerably poorer (*9*). To account for the good substrate properties of the β-methylene-substituted phenylalanine derivates **1a–e**, it is tempting to speculate that these substances exist in solution in a conformation approaching that of **9**, i.e., with a dihedral angle between the planes of the phenyl ring and the double bond close to 90° preventing conjugation and modification of the electronic properties of the aromatic ring and perhaps approximating the conformation adopted by L-Dopa and *m*-tyrosine in the active site of AADC. Ultraviolet spectroscopy adds support to this suggestion. The spectra of amino acids **1a–e** show a 10- to 15-nm blue shift of the short wavelength styrene band compared to the spectra of 3-methoxystyrene (λ_{max} = 250 nm) (*29*) which reflect that the plane of the double bond is twisted relative to that of the aromatic ring (*30*). Until more information concerning the conformation of other members of this series is available, however, it would be premature to elaborate further.

From this small group of dual enzyme-activated inhibitors the versatility of such an approach to enzyme inhibition is well illustrated. Small structural modifications can alter AADC substrate properties of the amino acids, the potency of the amines as MAO inhibitors, and, to a lesser extent, the selectivity for one form of MAO or the other. The localization of AADC mainly to nerve endings (in the brain) coupled with the potential to inhibit this enzyme using an AADC inhibitor that acts exclusively extracerebrally (thus leaving the brain enzyme intact) indicates that site selective irreversible inhibition of MAO in brain neurons is attainable and should lead to safe, effective antidepressant therapy. Of the compounds discussed in this paper, **1a** would appear to offer the best chance of success (*25*).

EXPERIMENTAL

Melting points were determined on a Mettler FP5 or a Buchi SMP20 and are uncorrected. Microanalyses were obtained on a Perkin–Elmer 240 CHN analyzer and were within +0.4% of the theoretical value. NMR spectra were recorded on a Varian T-60. Ultraviolet spectra were recorded on a Varian Cary 118 spectrophotometer.

Syntheses of New Allylamines

(E)-2-(3-Hydroxyphenyl)-3-chloroallylamine (**2c**). This substance was prepared via *(E)*-1-chloro-2-(3-methoxyphenyl)-3-phthalimidopropene according to our published procedures (*8*); cream powder (HCl salt), mp 205–206°C. NMR (D_2O): δ4.00 (broad s, 2H); 6.68 (broad s, 1H); 6.75 to 7.55 (m, 4H). uv(H_2O): λ_{max} 235 (sh, 5900), 281 nm (ε 1970).

Anal. ($C_9H_{11}Cl_2NO$) C,H,N.

(E)-2-(3-Hydroxy-4-methylphenyl)-3-fluoroallylamine (**2e**). This compound was prepared from 3-methoxy-4-methylphenylacetic acid (*26*) according to the published methods (*8*); colorless needles (HCl salt), mp 198–199°C, NMR (D_2O): δ2.24 (s, 3H); 3.94 (d, J = 2 Hz, 2H); 7.00 to 7.30 (m, 3H); 7.09 (d, J = 81 Hz, 1H).

Anal. ($C_{10}H_{13}ClFNO$) C,H,N.

Syntheses of the Amino Acids

Wittig Route

α-Fluoro-3-methoxyacetophenone (**5**). KHF_2 (183 g, 2.35 mol) was added in two portions over 45 min to a heated (100°C) and stirred solution of α-bromo-3-methoxyacetophenone (*27*) (178 g, 0.78 mol) in triethylene glycol (1.2 liters). Stirring was continued for 3 h; then the mixture was cooled, poured into ice/water (4 liters), and extracted with ethyl acetate (EtOAc). The EtOAc solution was washed consecutively with water, aqueous $NaHCO_3$, and water, dried, and evaporated to leave an orange oil (152 g). K_2CO_3 (10 g) was added and the mixture was distilled; the fraction, bp 106–110°C/0.02 mm, was further purified by recrystallization from *n*-hexane/$CHCl_3$ whereupon **5** (71.5 g, 54%) was obtained as colorless needles, mp 53–54°C. NMR ($CDCl_3$): δ3.82 (s, 3H); 5.43 (d, J = 46 Hz, 2H); 6.93 to 7.53 (m, 4H).

Anal. ($C_9H_9FO_2$) C,H.

Ethyl 3-(3-methoxyphenyl)-4-fluoro-2-butenoate (**6**). The anion derived from triethyl phosphonoacetate (77.5 g, 0.35 mol) and oil-free NaH (8.3 g, 0.35 mol) in toluene (300 ml) was treated with a solution of **5** (58.0 g, 0.35 mol) in toluene (400 ml) at such a rate (30 min addition time) that the temperature did not rise above 15°C. When all was added, the solution was poured onto ice/water and immediately neutralized with dilute aqueous HCl. Ether extraction afforded an orange oil (86.2 g) which yielded essentially pure product (65 g, 80%) on distillation, bp 112–116°C/0.025 mm. NMR analysis indicated that this material consisted of *Z*-**6** (90%) and *E*-**6** (10%). Silica chromatography (10% ether in petroleum ether) followed by distillation afforded pure isomers: bp 114–116°C/0.025 mm, *Z*-isomer—NMR ($CDCl_3$): δ1.28 (t, J = 7 Hz, 3H); 3.77 (s, 3H); 4.20 (q, J = 7 Hz, 2H); 5.83 (d,d, J = 47 and 1.5 Hz, 2H); 6.15 (broad s, 1H); 6.73 to 7.43 (m, 4H); and bp 103–105°C/0.03 mm, *E*-isomer—NMR ($CDCl_3$): δ1.03 (t, J = 7 Hz, 3H); 3.67 (s, 3H); 3.93 (q, J = 7 Hz, 2H); 5.86 (d,d, J = 47 and 1.5 Hz, 2H); 6.03 (broad s, 1H); 6.56 to 7.40 (m, 4H).

Anal. ($C_{13}H_{15}FO_3$) C,H.

Ethyl 2-bromo-3-(3-methoxyphenyl)-4-fluoro-2-butenoate (**7**). (a) Br_2 (33.6 g, 0.21 mol) in CCl_4 (400 ml) was added to a solution of **6** (50.0 g, 0.21 mol) in CCl_4 (600 ml) over 90 min so that the temperature was kept at −10 to −5°C. The orange solution was stirred for a further 90 min, then it was washed consecutively with aqueous $Na_2S_2O_3$, brine and water, dried, and evaporated to leave essentially pure dibromide (84.2 g, 90% crude). (b) A solution of the dibromide (99.5 g, 0.25 mol) in dry ether (1 liter) at about 5°C was treated for 2.5 h with piperidine (21.5 g, 0.25 mol). The solids were filtered and the filtrate was washed consecutively with water, 5% aqueous HCl, and water, dried, and evaporated to leave an orange oil. Distillation from K_2CO_3 afforded a pale yellow oil (74.5 g), bp 116–120°C/0.02 mm. NMR analysis showed this material to contain approximately 80% of the *E,Z* mixture of **7** and 20% of unidentified, non-fluorine-containing material. It was used in the next step without further purification. An analytical sample was obtained by silica chromatography (10% ether in petroleum ether) during which the mixture isomerized to the *Z*-isomer, almost colorless oil, bp 119–120°C/0.02 mm. NMR ($CDCl_3$): δ0.97 (t, J = 7 Hz, 3H); 3.78 (s, 3H); 4.00 (q, J = 7 Hz, 2H); 5.35 (d, J = 48 Hz, 2H); 6.70 to 7.52 (m, 4H).

Anal. ($C_{13}H_{14}BrFO_3$) C,H.

Ethyl 2-amino-3-(3-methoxyphenyl)-4-fluoro-3-butenoate (**9**). A solution of **7** (38 g, 0.12 mol) in tetrahydrofuran (THF) (150 ml) was added to lithium diisopropylamide [from diisopropylamine (18.4 g, 0.18 mol) and 1.6 M *n*-butyllithium (114 ml, 0.18 mol)] in THF (800 ml) cooled to about −78°C. After 30 min, a solution of concentrated aqueous HCl (16 ml) and ethanol (100 ml) was added, then the reaction mixture was allowed to warm to about 0°C. Water was added and **8** was isolated by ether extraction as a brownish oil (36.72 g). NH_3 gas was bubbled through dimethyl sulfoxide (750 ml) at room temperature for 2.5 h, then a solution of **8** in dimethyl sulfoxide (90 ml) was added slowly. The flask was firmly stoppered and the solution stirred for 2.5 h, then ice water was added and the mixture was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with water, then extracted with 5% aqueous HCl; the aqueous layer was subsequently made alkaline (aqueous $NaHCO_3$) and extracted with CH_2Cl_2, dried, and evaporated to leave a yellow oil (14.35 g). This was dissolved in ether (50 ml) and treated with hydrogen chloride-saturated ether (120 ml) whereupon a colorless solid (13.21 g) precipitated. Recrystallization from ethanol/ether afforded the HCl salt of **9** (10.22 g, 29% from **7**) as colorless needles, mp 141–142°C. NMR (CD_3OD): δ0.73 (t, J = 7 Hz, 3H); 3.40 (s, 3H); 3.93 (q, J = 7 Hz, 2H); 4.63 (s, 1H); 6.93 (d, J = 79 Hz, 1H); 6.33 to 7.17 (m, 4H).

Anal. ($C_{13}H_{17}ClFNO_3$) C,H,N.

(E)-β-Fluoromethylene-m-tyrosine (**1a**). A solution of **9** (7.00 g, 24 mmol) in 47% aqueous HBr was refluxed for 4 h then evaporated to dryness to leave an almost colorless powder (5.58 g). This was dissolved in dry CH_3OH (50 ml) and treated with propylene oxide (4.2 g, 72 mmol). After several hours, **1a** (4.37 g, 86%) was collected as a colorless powder, mp 214–215°C. NMR (CD_3OD): δ4.62 (s, 1H); 6.28 to 7.13 (m, 4H); 6.98 (d, J = 80 Hz, 1H). uv(H_2O): λ_{max} 239 (sh, 4560), 279 nm (ε = 1900).

Anal. ($C_{10}H_{10}FNO_3$) C,H,N.

Isocyanoacetate Route

α-Bromo-α-fluoro-3-methoxyacetophenone (**10**). A solution of **5** (16.80 g, 0.1 mol) and phenyltrimethylammonium tribromide (37.60 g, 0.1 mol) in CH_2Cl_2 (60 ml) was stirred for 20 h at room temperature. The solution was washed with aqueous $Na_2S_2O_3$ and with water, then dried and evaporated to leave an almost colorless oil. Flash chromatography on silica (15% ether in petroleum ether) gave **10** (22.72 g, 92%) as a colorless oil, bp 139–140°C/0.02 mm. NMR ($CDCl_3$): δ3.83 (s, 3H); 7.23 (d, J = 52 Hz, 1H); 7.00 to 7.63 (m, 4H).

Anal. ($C_9H_8BrFO_2$) C,H.

4-Carbethoxy-5-bromofluoromethyl-5-(3-methoxyphenyl)-2-oxazoline (**11**). Cu_2O (0.20 g) was added to a vigorously stirred solution of ethyl isocyanoacetate (8.25 g, 73 mmol) and **10** (18.00 g, 73 mmol) in toluene (150 ml). The mixture warmed up appreciably (40°C) over 15 min. After 1 h, the mixture was filtered (celite) and evaporated to leave a brownish oil (25.90 g). Chromatography on silica (ether) afforded pure **11** (21.50 g, 82%) as a mixture of isomers. NMR analysis indicated the presence of isomers in the ratio 60/40. NMR ($CDCl_3$): δ0.87 and 1.35 (two t, J = 7 Hz for each, 3H total); 3.72 and 4.33 (two q, J = 7 Hz, 2H); 3.75 and 3.79 (two s, 3H); 5;00 (broad s, 1H); 6.30 to 7.50 (m, 6H).

Ethyl 2-formylamino-3-(3-methoxyphenyl)-4-fluoro-3-butenoate (**12** and **13**). A solution of **11** (16.50 g, 46 mmol) in dimethylformamide (250 ml) was cooled to 0°C and treated dropwise with CF_3CO_2H (5.25 g, 46 mmol). The solution was stirred for an additional 15 min then activated Zn powder (9.00 g, 138 mmol) was added and the mixture was vigorously stirred for a further 60 min. The resulting mixture was poured into water/ice and extracted with ether to give a pale yellow oil (13.21 g). Careful flash chromatography on silica (50% ether in petroleum ether) allowed the partial separation of the two isomers of the desired product. Recrystallization of the pure isomers from CH_2Cl_2/hexane afforded: (*E*)-**12** (3.65 g, 28%), colorless needles, mp 71–72°C—NMR ($CDCl_3$): δ1.25 (t, J = 7 Hz, 3H); 3.78 (s, 3H); 4.23 (q, J = 7 Hz, 2H); 5.80 (d, J = 8 Hz, 1H); 6.78 (d, J = 81 Hz, 1H); 6.60 to 7.43 (m, 5H); 8.13 (broad s, 1H); and (*Z*)-**13** (3.60 g, 28%), colorless needles, mp 100–101°C—NMR ($CDCl_3$): δ1.18 (t, J = 7 Hz, 3H); 3.77 (s, 3H); 4.17 (q, J = 7 Hz, 2H); 5.33 (d, J = 8 Hz, 1H); 6.93 (d, J = 80 Hz, 1H); 6.53 to 7.50 (m, 5H); 8.13 (broad s, 1H). Several fractions contained a mixture (4.50 g, 35%) of the two isomers.

Anal. ($C_{14}H_{16}FNO_4$) C,H,N for both compounds.

(*E*)-*β-Fluoromethylene-m-tyrosine* (**1a**). A mixture of **12** (0.14 g, 0.5 mmol) and 47% aqueous HBr (8 ml) was refluxed for 2.5 h, then evaporated to dryness to leave a brown solid (0.095 g). This was directly treated with an excess of propylene oxide in isopropanol (3 ml) to give **1a** (0.065 g, 62%) as a colorless powder identical with **1a** prepared above.

(*Z*)-*β-Fluoromethylene-m-tyrosine* (**1b**). A mixture of **13** (0.84 g, 3 mmol) and 47% aqueous HBr (50 ml) was refluxed for 3 h, then evaporated to dryness. The residue was dissovled in 6 M aqueous HCl, washed with CH_2Cl_2, then reevaporated to leave a pale brown residue. Treatment with propylene oxide (0.52 g, 9 mmol) in isopropanol (10 ml) gave **1b** (0.37 g, 59%) as a yellowish powder, mp

178–180°C. NMR (D_2O + DCl): δ5.33 (s, 1 H), 6.77 to 7.57 (m, 4H), 7.12 (d, J = 81 Hz, 1 H). uv (H_2O): λ_{max} 239 (sh, 4370), 280 nm (ε = 1760).

Preparation of ***1c*** *to* ***1g***

(*E*)-β-*Chloromethylene-m-tyrosine* (**1c**). This was prepared from α,α-dichloro-3-methoxyacetophenone (mp 45–46°C) via the Wittig route; colorless powder, mp 202–203°C. NMR (D_2O + DCl): δ5.17 (s, 1H); 6.80 to 7.66 (m, 4H); 7.05 (s, 1H). uv (H_2O): λ_{max} 237 (sh, 3850), 279 nm (ε = 1860)

Anal. ($C_{10}H_{10}ClNO_3$) C,H,N.

β-*Methylene-m-tyrosine* (**1d**). This was prepared according to a slight modification of the published procedure (*13*) in which the final purification step used propylene oxide in methanol as described above; mp 221–222°C [lit. (*13*) mp 205–206°C]. NMR (CD_3OD): δ4.8 (s, obscured by the solvent peak); 5.33 (s, 1H); 5.47 (s, 1H); 6.47 to 7.13 (m, 4H). uv (H_2O): λ_{max} 239 (sh, 6250), 283 nm (ε = 1830).

Anal. ($C_{10}H_{11}NO_3$) C,H,N.

(*E*)-β-*Fluoromethylene-3-hydroxy-4-methylphenylalanine* (**1e**). This was prepared from α-fluoro-3-methoxy-4-methylacetophenone (mp 63–64°C) via the isocyanoacetoate route; colorless powder, mp 212–213°C. NMR (D_2O): δ2.27 (s, 3H); 5.12 (s, 1H); 6.82 to 7.37 (m, 3H); 7.37 (d, J = 80 Hz, 1H). uv (H_2O): λ_{max} 236 (sh, 5575), 280 nm (ε = 2200).

Anal. ($C_{11}H_{12}FNO_3$) C,H,N.

(*E*)-β-*Fluoromethylene-3-hydroxy-4-methoxyphenylalanine* (**1f**). This was prepared from α-fluoro-3-tetrahydropyranyloxy-4-methoxyacetophenone (mp 78–79°C) via the Wittig route; colorless powder, mp 213–214°C. NMR (D_2O + DCl): δ3.87 (s, 3H); 4.98 (s, 1H); 7.23 (d, J = 80 Hz, 1H); 6.73 to 7.20 (m, 3H). uv (H_2O): λ_{max} 243 (sh, 5975), 282 nm (ε = 3300).

Anal. ($C_{11}H_{12}FNO_4$) C,H,N.

(*E*)-β-*Fluoromethylene-p-tyrosine* (**1g**). This was prepared from α-fluoro-4-tetrahydropyranyloxyacetophenone (mp 179–182°C, dec.) via the Wittig route; colorless powder, mp 212–213°C. NMR (D_2O + DCl): δ5.00 (s, 1H); centered at 7.11 (AA'BB', 4H); 7.28 (d, J = 80 Hz, 1H). uv (H_2O): λ_{max} 228 (sh, 8240), 280 nm (ε = 1160).

Anal. ($C_{10}H_{10}FNO_3$) C,H,N.

Biochemistry

Preparation of Purified Hog Kidney AADC

AADC was extracted from hog kidney and purified through a second $(NH_4)_2SO_4$ precipitation step as described by Christenson *et al.* (*28*). The specific activity was 346 nmol/h/mg protein measured using 1-[^{14}C]Dopa as substrate.

Qualitative AADC Substrate Properties of ***1a–g***

The biochemical methods used to determine substrate properties of **1** for AADC and MAO inhibitory properties of **2** have been previously reported in full (*8, 19, 25*).

The appearance of the amines **2** and the disappearance of the amino acids **1** were monitored by HPLC using the following system: Ultrasphere ion pair column (5-μm particle size, 250 × 4.6 mm i.d.); uv detection at 254 or 280 nm; linear gradient elution (0.05 M NaH_2PO_4/CH_3CN from ratio 88/12 (v/v, pH = 2.30) to 71/29 (v/v, pH = 2.30) over 25 min); octanesulfonic acid (5 mM) as the ion-pairing agent in the eluant; flow rate = 1.5 ml/min; temperature = 40°C. The retention times are listed in Table 3.

Determination of the Inhibitory Effect of ***1a*** *on Hog Kidney AADC*

Hog kidney AADC (11.6 mg protein/ml) was incubated at 37°C with varying concentrations of **1a** (0.05-2 mM). As a control, AADC was also incubated with α-monofluoromethyldopa (10 μM). Incubations were undertaken in phosphate buffer (50 mM, pH 7.2) containing pyridoxal phosphate (125 μM, PLP) and mercaptoethanol (10 mM). At 1 min, and then at 4-min intervals for 20 min, aliquots (20 μl) were removed from the incubation medium and diluted to 2 ml with phosphate buffer/PLP/mercaptoethanol containing L-Dopa (150 μM). Remaining AADC activity was determined after a further 10-min incubation at 37°C when the reaction was stopped by adding 0.2 M $HClO_4$/EDTA/$Na_2S_2O_5$ (2 ml). AADC activity was calculated from the formation of dopamine by HPLC. Control AADC activity, determined as a function of time in the absence of **1a** or α-monofluoromethyldopa, remained constant during the 20-min preincubation.

Determination of K_m *and* V_{max} *for* ***1a***, ***1b***, *and* L-*Dopa*

Preliminary experiments were undertaken to determine if the decarboxylation of **1a** and L-Dopa was linearly related to the concentration of AADC and to the time of incubation. With racemic **1a** (1 mM; i.e., 0.5 mM L-isomer) as substrate, decarboxylation was linearly related to AADC over the concentration range of 30–600 μg protein/ml. Using L-Dopa as substrate, decarboxylation was linear over the range 1–100 μg protein/ml. With either racemic **1a** (0.5 or 5 mM) and 320 μg protein/ml AADC or L-dopa (0.05 or 0.5 mM) and 32 μg protein/ml AADC, decarboxylation was linear for at least 15 min of incubation at 37°C.

TABLE 3

HPLC RETENTION TIMES OF AMINO ACIDS **1a–f** AND AMINES **2a–f**

Amino acid	Retention time (min)	Amine	Retention time (min)
1a	7.0	**2a**	14.90
1b	6.5	**2b**	14.60
1c	10.1	**2c**	18.30
1d	7.6	**2d**	15.20
1e	13.65	**2e**	29.00
1f	8.95	**2f**	19.00
1g	Not analyzed by HPLC	**2g**	Not analyzed by HPLC

For the determination of K_m and V_{max} the following conditions were used: **1a** (0.05 to 5 mM) and AADC (160 μg protein/ml); **1b** (0.1 to 8 mM) and AADC (635 μg protein/ml); and L-Dopa (0.02 to 1 mM) and AADC (13 μg protein/ml). All incubations were conducted at 37°C for 10 min in a shaking water bath using phosphate buffer (50 mM, pH 7.2) containing PLP (125 μM) and mercaptoethanol (10 mM). After 10 min the reaction was stopped by adding an equal volume of 0.2 M $HClO_4$ containing $Na_2S_2O_4$ (500 mg/liter) and Na_2EDTA (100 mg/liter). The samples were left on ice for 1 h before being centrifuged. Aliquots of the clear supernatant were assayed for the amino acid and amine by HPLC (see below). The velocity of the decarboxylation was determined in nanomoles of amine formed per minute per milligram of protein. The kinetic constants were calculated by plotting the results according to the method of Lineweaver and Burk. The line of best fit was obtained by linear regression analysis.

*HPLC Conditions Used for the Determination of K_m and V_{max} for **1a**, **1b**, and L-Dopa*

The disappearance of the amino acids and the formation of the corresponding amine were monitored by reverse-phase HPLC. Several different systems were used:

*Analysis of the decarboxylation of **1a**.* Ultrasphere IP-C_{18} column (250 × 4.6 mm i.d., 5 μm particle size); mobile phase [87.5/12.5 (v/v) mixture of NaH_2PO_4 (0.05 M) and CH_3CN-containing octanesulfonic acid (OSA, 5×10^{-3} M), pH 2.50]; flow rate (1.5 ml/min); 40°C; detection [uv at 254 nm and electrochemical (0.9 V) in series]; retention times [**1a** (5.6 min), **2a** (25.5 min)].

*Analysis of the decarboxylation of **1b**.* Column as before; gradient elution—eluant A [99/1 (v/v) mixture of NaH_2PO_4 (0.05 M) in CH_3CN containing OSA (5×10^{-3} M), pH 2.26]; eluant B [60/40 (v/v) NaH_2PO_4 (0.05 M) in CH_3CN containing OSA (5×10^{-3} M), pH = 3.00]; flow rate (1.5 ml/min); 40°C; detection (uv at 254 nm). A linear gradient over 20 min from 75% A/25% B to 40% A/60% B was used. After completion the mixture was returned to the initial gradient in 1 min, followed by 9 min stabilization before the next injection; retention times [**1a** (7.65 min), **2a** (19.3 min), **1b** (6.25 min), **2b** (17.8 min)].

Analysis of L-Dopa. Column as before; mobile phase [83/17 (v/v) mixture of NaH_2PO_4 (0.1 M) in CH_3OH containing OSA (5.5×10^{-3} M), pH = 3.27]; flow rate (1 ml/min); 28.5°C; detection [uv at 280 nm and electrochemical (0.7 V) in series]; retention times [L-Dopa (5.7 min), dopamine (14.7 min)].

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