overnight to yield a yellow solid. The compound was purified by recrystallization of the crude product twice from 50% aqueous CH_3OH and once from H_2O. After being dried at room temperature for 4 hr and then at 135° for an additional 16 hr the product weighed 2.5 g (22% yield); mp 203–205° dec (sintered at 185°); $\lambda_{max}^{pH\ 1}$ 300 mμ (ε 14,200), $\lambda_{sh}^{pH\ 1}$ 280 mμ (ε 12,900), $\lambda_{max}^{pH\ 11}$ 290 mμ (ε 24,000). *Anal.* ($C_{18}H_{22}N_6O_5$) C, H, N.

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Effect of Monoiodotyrosine Metabolites on Tyrosine Hydroxylase[1a]

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The largest and most effective class of tyrosine hydroxylase inhibitors is made up of tyrosine analogs. Monoiodotyrosine (MIT), the most potent monohalogenated tyrosine analog *in vitro*, is 100 times as active as α-methyltyrosine, the most active nonhalogenated tyrosine analog. *In vivo*, however, α-methyltyrosine is considerably more effective than MIT in its ability to block the synthesis of catecholamines and to produce pharmacological effects ascribed to the inhibition of norepinephrine synthesis.

In this study, an appraisal of the inhibitory effect of MIT metabolites on tyrosine hydroxylase was made to indicate the significance of certain metabolic steps on the inactivation of MIT as a tyrosine hydroxylase inhibitor. Further, in order to bring such inactivation into perspective, a quantitative estimate of such metabolites was made by radioautographic studies on rat and on rabbit liver tissue slices.

When ^{14}C-MIT was incubated with rat and rabbit liver slices it was converted in each instance into several iodinated and noniodinated metabolites. The location of identified metabolites and the net percentage of each is given in Table I.

Both deiodination[2a] and transamination[2b] have been suggested to be the main path of monoiodotyrosine metabolism. While the results of Table I point to deiodination to tyrosine as the main pathway of MIT degradation in rat and rabbit tissue slices, the presence of 3-iodo-4-hydroxyphenylpyruvic acid (MIP) and of 3-iodo-4-hydroxyphenylacetic acid (MIA) indicate the extent to which transamination occurs; the accumulation of MIA as an end product of the transamination pathway is to be expected as MIP is unable to serve as substrate of *p*-hydroxyphenylpyruvic oxidase.[3]

The effect of some MIT metabolites on tyrosine hydroxylase activity is listed in Table II. MIT, a powerful inhibitor of tyrosine hydroxylase (K_i = 9.2×10^{-7}, lit.[4] 3.9×10^{-7}), was included as a standard at the concentrations listed. A study of the effect of 3-iodo-4-hydroxyphenylpyruvate on tyrosine hydroxylase suggests this metabolite as a weak ($K_i = 3 \times 10^{-3}$) noncompetitive inhibitor of the substrate tyrosine. No explanation is offered for the apparent lack of correlation between inhibitor concentration and enzyme inhibition.

The weak inhibition of adrenal tyrosine hydroxylase by the metabolites listed tend to substantiate the conclusion of McGeer and McGeer[5] that, in a brain tyrosine hydroxylase preparation, a free amino group is required for inhibition of the enzyme. The present study shows that the weak *in vivo* activity of MIT may well be due to its rapid conversion into inactive metabolites. The results suggest also that molecular modifications of MIT in which metabolic degradation of the α-amino group or of the 3-iodo substituent could be prevented would be a potent inhibitor of the enzyme.

TABLE I

MONOIODOTYROSINE METABOLITES AS DETERMINED FROM RADIOAUTOGRAPHS

Metabolite[a]	BuOH–AcOH–H_2O R_f	BuOH–AcOH–H_2O Net % Rat	BuOH–AcOH–H_2O Net % Rabbit	BuOH–dioxane–NH_4OH R_f	BuOH–dioxane–NH_4OH Net % Rat	BuOH–dioxane–NH_4OH Net % Rabbit
MIT, % metabolized	0.65	53.2	39.1	0.43	44.0	36.9
Metabolites						
Tyrosine	0.47	10.0	22.0	0.22	15.9	33.9
MIP[b] and PHPP	0.91	1.2	1.8	0.99	0.6	0.8
MIA	0.97	4.3	1.1	0.91	4.1	0.7

[a] The following abbreviations were used: MIP, 3-iodo-4-hydroxyphenylpyruvic acid; PHPP, 4-hydroxyphenylpyruvic acid; and MIA, 3-iodo-4-hydroxyphenylacetic acid. [b] The two pyruvic acids (identified by the R_f values obtained for authentic compounds, uv light, and 2,4-dinitrophenylhydrazine spray) could not be separated consistently by the first solvent system and could not be separated by the second; they are therefore listed together.

TABLE II

MONOIODOTYROSINE METABOLITES AS INHIBITORS OF TYROSINE HYDROXYLASE

Metabolite[a]	Metabolite concn[b]	% inhib[c]
PHPP	10^{-4}	5.2
	10^{-3}	20.0
MIP	10^{-5}	19.3
	10^{-3}	20.5
PHPA	10^{-5}	18.1
	10^{-3}	17.6
MIA	10^{-5}	15.9
	10^{-3}	16.9
PHPL	10^{-4}	22.0
DIPL	10^{-4}	24.1
MIT	10^{-4}	100
	5×10^{-7}	67

[a] The following abbreviations were used; PHPP, 4-hydroxyphenylpyruvic acid; MIP, 3-iodo-4-hydroxyphenylpyruvic acid; PHPA, 4-hydroxyphenylacetic acid; MIA, 3-iodo-4-hydroxyphenylacetic acid; PHPL, 4-hydroxyphenyllactic acid; DIPL, 3,5-diiodo-4-hydroxyphenylactic acid; MIT, 3-iodotyrosine. [b] Concentrations are in moles/l. [c] All figures represent the average of at least two incubations, each done in triplicate at the time of assay. Each compound was preincubated with the reaction mixture for 5 min before the addition of L-tyrosine-3,5-^{3}H (5×10^{-5} *M*) as substrate.

(1) (a) Supported by Grant AM-06480 from the National Institutes of Health, U. S. Public Health Service. (b) In partial fulfillment of the requirements for the degree of Master of Science, University of Maryland, Aug 1967.

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Experimental Section

L-Tyrosine, 3-iodo-L-tyrosine, and 4-hydroxyphenylpyruvic acid were used as obtained from the Nutritional Biochemicals Corp. 2-Amino-4-hydroxy-6,7-dimethyltetrahydropteridine ($DMPH_4$) and α-ketoglutaric acid were supplied from Calbiochem. 3-Iodo-4-hydroxyphenylpyruvic acid was obtained from the Cyclo Chemical Corp. Pyridoxal phosphate and 4-hydroxyphenyl-DL-lactic acid were obtained from the Sigma Chemical Corp.; the compound was recrystallized (Et_2O) before use. 3,5-^{3}H-L-Tyrosine and ^{14}C-L-tyrosine, uniformly labeled, were obtained from the New England Nuclear Corp. *p*-Bromo-*m*-hydroxybenzoxyamine, a powerful dopa decarboxylase inhibitor, was supplied generously by Dr. Sidney Udenfriend of N. I. H. Elemental analyses were performed by G. Weiler and F. B. Strauss, Oxford, England.

3-Iodo-4-hydroxyphenylacetic Acid.—The iodination of 4-hydroxyphenylacetic acid was carried out in an ice–salt bath according to the procedure of Nakano and Danowski.[6] The white crystalline material obtained after one recrystallization (hot H_2O) was dried at 79° *in vacuo* over P_2O_5; mp 182–186°. *Anal.* ($C_8H_7IO_3 \cdot H_2O$) C, H, I.

3-Iodo-^{14}C-L-tyrosine.—Uniformly labeled 3-iodo-^{14}C-L-tyrosine was prepared from labeled ^{14}C-L-tyrosine according to the method of Pitt-Rivers,[7] adapted for microsynthesis in the following manner. Uniformly labeled ^{14}C-L-tyrosine (0.5 mCi, 1.08 μM, in 1.0 ml of 1 *N* HCl) was carefully dried under N_2 and the residue dissolved in 320 μl of 1 *N* NH_4OH. The solution was cooled to near freezing, then iodinated with 212 μl of 0.01 *N* I_2 over 30 min. The iodinated mixture was dried under N_2 and redissolved in 200 μl of 1 *N* NH_4OH. The solution was chromatographed in the BuOH–AcOH–H_2O system described below and radioautographed for 10 min. The band corresponding to monoiodotyrosine was cut from the paper and eluted slowly with 20 ml of 2 *N* HCl, and the eluate was dried under N_2. All but 3% of the radioactivity could be recovered in this manner. The residue was taken up in 6 ml of 0.005 *N* NaOH, the amount of monoiodotyrosine was determined from the absorbance measurement at 300 mμ, and the radioactivity of the product was determined from a planchet count of a 20-μl aliquot. The specific activity of the monoiodotyrosine was found to be 0.46 mCi/μmole; the yield of MIT was 79%. The NaOH solution was dried and the residue was dissolved in 2 ml of Krebs–Ringer phosphate buffer without Ca^{2+}; the final concentration was 426 nmoles/ml.

Paper Chromatography.—Ascending paper chromatography was carried out on Whatman No. 1 paper at room temperature using *n*-BuOH–AcOH–H_2O (120:35:50) and *n*-BuOH–dioxane–2 *N* NH_4OH (4:1:2).[8] Approximately 50 μg of MIT and of authentic metabolites were chromatographed with alkalized samples from slice and homogenate experiments. After development the location of the authentic compounds was established with uv light and with the following spray reagents: ninhydrin for amino acids, 2,4-dinitrophenylhydrazine for α-keto acids, and fast blue salt B followed by NaOH for phenols.[9] Bands of concentrated radioactivity identified by radioautography and intervening areas were counted on a low-background planchet counting system.

Tyrosine Hydroxylase.—A tyrosine hydroxylase preparation was obtained using the directions of Nagatsu, *et al.*[10] The enzyme was precipitated from the 105,000*g* supernatant of a bovine medullary tissue homogenate by the slow addition of solid $(NH_4)_2SO_4$ to 40% saturation. The precipitate was centrifuged at 14,000*g*, suspended in a minimum of 0.15 *M* phosphate buffer, and lyophilized under 1 mm for 3 hr; the dry powder was collected and stored in a dessicator over silica gel at 0° until ready for use.

Tyrosine hydrolyase was assayed according to the method of Udenfriend, *et al.*,[9] but $DMPH_4$ was used instead of tetrahydrofolate and Fe^{2+}; L-dititiotyrosine was diluted to yield a solution containing 5×10^{-5} mmole and an activity of 1.0 μCi in the 20-μl aliquot used in the assay.

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Fluorinated α,α-Dialkylphenethylamines

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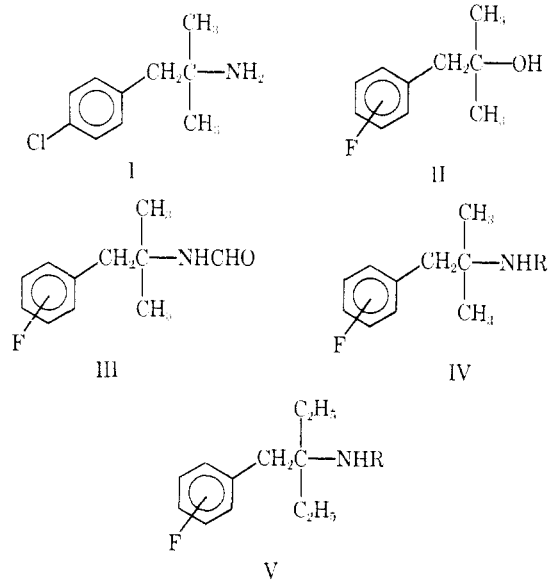
Holms, *et al.*,[1] have shown that the anorexigenic activity of phenethylamines can be dissociated from their effect on the central nervous system; halogenation in the nucleus and α,α-dimethylation produce compounds which are only anorexigenic;[2–4] groups bulkier than methyl in the α position and N substitution also give active anorexigenic compounds.[5] The most important of them is chlorophentermine (α,α-dimethyl-4-chlorophenethylamine, I), and it is known that the chloro compounds are more active than the bromo analogs. It thus seemed of interest to prepare the analogous fluorine compounds.

The condensation of 3- and 4-fluorobenzylmagnesium chloride with acetone gave the tertiary alcohols II, which, when subjected to the Ritter reaction[6,7] with NaCN in the presence of AcOH and H_2SO_4, led to the fluorinated α,α-dimethyl-*N*-formylphenethylamines (III). Their acid hydrolysis afforded the desired com-



pounds IV (R = H),[8] while their reduction ($LiAlH_4$) led to the corresponding N-methyl derivatives (IV, R = CH_3). When diethyl ketone was used instead of acetone, compounds of type V were obtained.

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