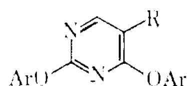
TABLE I
2,4-BIS(ARYLOXY)PYRIMIDINES



Compd	R	Ar	Yield, % (crude)	Reaction[a] Time, min	Reaction[a] Temp, °C	Mp, °C[b]	Solvent of recrystn[d]	Formula[c]
I	H	C_6H_5	95	30	120	111	H	$C_{16}H_{12}N_2O_2$
II	H	p-$CH_3C_6H_4$	95	30	120	105–107	H	$C_{18}H_{16}N_2O_2$
III	H	p-$OCH_3C_6H_4$	98	30	120	116–117	H–E	$C_{18}H_{16}N_2O_4$
IV	H	p-ClC_6H_4	98	60	130	117–118	H	$C_{16}H_{10}Cl_2N_2O_2$
V	H	p-BrC_6H_4	97	60	130	140–141	H–E	$C_{16}H_{10}Br_2N_2O_2$
VI	H	p-$NO_2C_6H_4$	90	60	160	162–163	A	$C_{16}H_{10}N_4O_6$
VII	CH_3	C_6H_5	98	30	120	85–87	H	$C_{17}N_{14}N_2O_2$
VIII	CH_3	p-$CH_3C_6H_4$	90	30	120	107–110	H	$C_{19}H_{18}N_2O_2$
IX	CH_3	p-$OCH_3C_6H_4$	98	30	120	89	H	$C_{19}H_{18}N_2O_4$
X	CH_3	p-ClC_6H_4	90	60	130	115–117	H	$C_{17}H_{12}Cl_2N_2O_2$
XI	CH_3	p-BrC_6H_4	97	60	130	108–110	H–E	$C_{17}H_{12}Br_2N_2O_2$
XII	CH_3	p-$NO_2C_6H_4$	90	60	160	132–135	A	$C_{17}H_{12}N_4O_6$

[a] In the syntheses of compounds VI and XII, 5 ml of toluene was added to the reaction mixture. [b] All melting points were determined in capillary tubes in Gallen-Kumph apparatus and are corrected. [c] All compounds were analyzed for C, H, N. Analytical data were within ±0.4% of the theoretical values. [d] H = hexane, E = Et_2O, A = EtOH.

TABLE II
ANTIMICROBIAL ACTIVITIES OF 2,4-BIS(ARYLOXY)PYRIMIDINES

Compd	Concn for 50% inhib of growth, μg/ml: *S. faecalis*	*S. typhimurium*	*C. albicans*	*E. coli* B
I	18.40	7.20	24.00	8.20
II	5.80	6.40	16.80	6.50
III	16.50	8.21	16.40	7.00
IV	5.60	5.60	14.80	5.00
V	7.60	9.20	23.60	10.40
VI	5.40	6.60	14.16	5.80
VII	20.80	9.00	21.60	8.30
VIII	6.20	7.00	18.00	7.20
IX	11.60	8.50	15.80	7.60
X	5.80	6.60	16.80	5.60
XI	7.80	9.80	22.80	11.00
XII	5.20	8.50	13.20	5.80
2,4-Bis(p-chloroanilino)pyrimidine	0.80	0.36	0.62	0.60
2,4-Bis(p-chloroanilino)-5-methylpyrimidine	1.30	0.85	0.92	1.0
Neomycin	*a*	1.55	1.10	1.30
Chloramphenicol	1.50	0.66	*a*	1.00

[a] Little or no activity.

cooled to room temperature. Finely powdered anhydrous K_2CO_3 (0.025 mole) was added to the reactants and mixed well. The mixture was heated on an oil bath at the optimum reaction temperature until completion of the reaction (see Table I) and cooled, and on addition of 5% KOH (20 ml) an oily substance separated out. The oil was extracted with hexane–ether, washed (dilute KOH, H_2O), and dried (Na_2SO_4). Crystals appeared on evaporating the solvent.

Inhibition of Growth of Microorganisms.—All compounds including two highly active 2,4-bis(arylamino)pyrimidine derivatives[1c,d] and two well-known broad-spectrum antibiotics were tested for antimicrobial activity against *Streptococcus faecalis*, *Salmonella typhimurium*, *Escherichia coli* B, and a pathogenic strain of yeast, *Candida albicans*. The concentrations of these compounds necessary for 50% inhibition of growth were determined turbidimetrically by serial dilution technique in test tubes using liquid growth medium[1b] (see Table II).

Acknowledgment.—The authors wish to thank Dr. D. M. Bose, Bose Institute, and Dr. S. M. Sarkar, Director, Bose Institute, for their interest in this work. Thanks are also due to Dr. A. Sen for helpful suggestions and to Mrs. C. Dutta for microanalyses. One of the authors (M. M.) is a Fellow of the Council of Scientific and Industrial Research, Government of India.

Folic Acid Analogs. I. *p*-{[(2,4-Diamino-5-pyrimidinyl)methyl]amino}benzoyl-L-glutamic Acid and Related Compounds[1,2]

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A number of folic acid antagonists owe their effectiveness to the inhibition of dihydrofolate reductase and thymidylate synthetase. The former enzyme is necessary for the reduction of folic acid (FA) to dihydrofolic acid (FAH_2) and then to tetrahydrofolic acid (FAH_4), and the latter is responsible, together with thymidine kinase, for cellular synthesis of thymidylic acid. In-

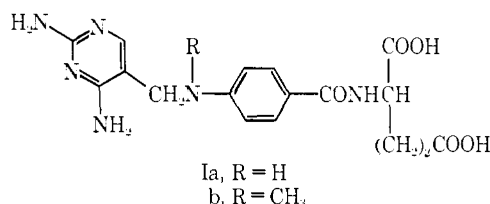
(1) This investigation was supported by the Cancer Chemotherapy National Service Center, National Cancer Institute of the National Institutes of Health, Public Health Service, Contract PH-43-65-94.

(2) Presented in part before the Division of Medicinal Chemistry, 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1968, Abstract N-67.

hibition of these enzymes prevents the utilization of FAH_4 for essential coenzymes, as well as transfer and incorporation of one-carbon units, thereby resulting in the interference with the biosynthesis of purines, pyrimidines, and amino acids. The inhibitory effect is particularly pronounced in cases of rapid cellular growth in bone marrow cells, developing fetus, and placental tissues. Therefore, folic acid antagonists have been shown to be good inhibitory agents against certain leukemias and women's choriocarcinoma.[3]

The two folic acid antagonists in clinical use today are aminopterin and methotrexate. Although they are still the most effective agents in prolonging life in leukemic children and bringing about cures in certain choriocarcinomas, many of these effects are transitory since drug resistance eventually develops. These resistances to chemotherapeutic cure are thought to be due to increased levels of dihydrofolate reductase and changes in the rate of cellular entry of these agents.[4,5]

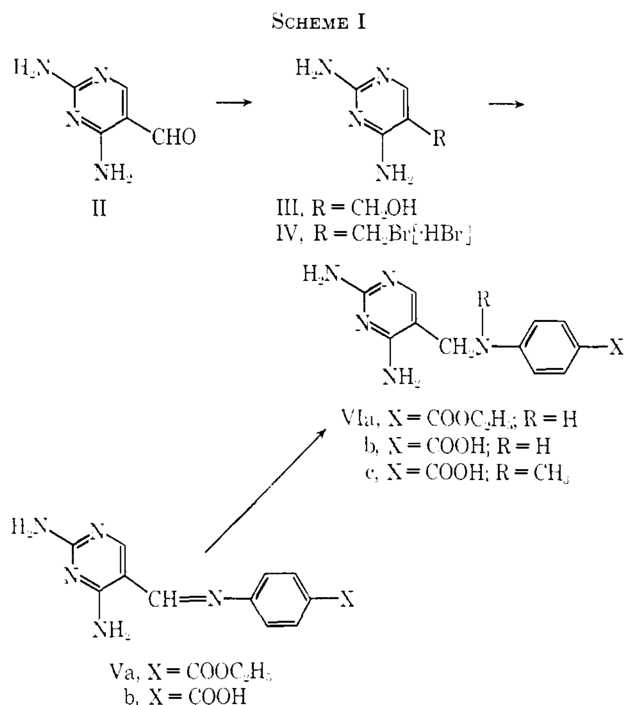
In connection with a program of designing compounds which would maintain similar enzyme-binding characteristics but possess different permeability properties, the 2,4-diamino-5-pyrimidinyl moiety was used in place of the pteridinyl moiety for the synthesis of the following folic acid analogs (I).



The synthesis of compounds of this type was based on the information that (1) a free 2-amino group substituted on the pyrimidine ring is necessary for substrate properties for the enzyme dihydrofolate reductase,[6] (2) the 2-amino group is complexed to thymidylate synthetase,[7] (3) the strongly basic 2,4-diamino heterocycles that are partially protonated at physiological pH are 1500–3000-fold better inhibitors than the corresponding 2-amino-4-hydroxy heterocycles,[7] (4) the carboxy-L-glutamate moiety of folic acid is probably necessary for active transport,[8] (5) the hydrophobic binding characteristics, which affect the dissociation of the enzyme–inhibitor complex, play a leading role in dihydrofolate reductase and thymidylate synthetase inhibition,[7] and (6) the bridge distance between the pyrimidine and the phenyl moieties is variable and does not contribute to hydrophobic binding.[7]

The preparation of 2,4-diamino-5-pyrimidinecarboxaldehyde (II), a key intermediate for the synthesis of I, was reported by Tieckelmann, *et al.*[9] Their method involves hydrogenation of 2,4-diamino-5-cyanopyrimidine[10] in the presence of a W-4 Raney nickel catalyst. In our hands it was found that utilization of the reaction conditions of Árpád, *et al.*,[11] for the conversion of 2-methyl-4-amino-5-cyanopyrimidine to the corresponding aldehyde was more convenient for the preparation of II. Condensation of II with the appropriate aniline derivative and subsequent reduction of the resulting anil V yielded the desired product. As pilot reactions, II was allowed to condense with ethyl *p*-aminobenzoate and *p*-aminobenzoic acid to yield Va and Vb, respectively. These anils were then reduced catalytically to give the corresponding compounds VIa and VIb. Following this procedure, dimethyl *p*-aminobenzoyl-L-glutamate was condensed with II. In this case the intermediate anil was not isolated but was reduced *in situ* and, after hydrolysis, yielded the desired compound Ia. An alternate synthesis of compounds of this type was achieved as follows (Scheme I). 2,4-Diamino-5-(hydroxymethyl)pyrimidine (III),

SCHEME I



obtained by the reduction of IIa, was converted to the corresponding bromo derivative IV. Treatment of the latter with the appropriate *p*-aminobenzoyl derivative afforded the desired product. The second method, in general, gave better yields and, in addition, provided a route for the preparation of N-substituted anilino compounds Ib and VIc.

Compound Ia, which can be regarded as the "depyrazinyl analog" of aminopterin, was found to be considerably more inhibitory than methotrexate against *Escherichia coli* thymidylate synthetase. The compound, on the other hand, is approximately tenfold less active than methotrexate in mouse tumor dihydrofolate reductase. The 50% inhibition values of Ia and

(3) (a) T. H. Jukes and H. P. Broquist in "Metabolic Inhibitors," Vol. I, R. M. Hochster and J. H. Quastel, Ed., Academic Press Inc., New York, N. Y., 1963, p 481; (b) L. F. Larionov, "Cancer Chemotherapy," Pergamon Press, London, 1965, p 209; (c) J. A. Montgomery, *Progr. Drug Res.*, **8**, 431 (1965).

(4) J. R. Bertino, *Cancer Res.*, **25**, 1614 (1965).

(5) W. C. Werkheiser, *ibid.*, **25**, 1608 (1965).

(6) J. R. Bertino, J. P. Perkins, and D. G. Johns, *Biochemistry*, **4**, 839 (1965).

(7) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Enzymic Active-Site," John Wiley and Sons, Inc., New York, N. Y., 1967.

(8) R. C. Wood and G. H. Hitchings, *J. Biol. Chem.*, **234**, 2377, 2381 (1959).

(9) H. Tieckelmann, R. Guthrie, and J. G. Nairn, *J. Org. Chem.*, **25**, 1257 (1960).

(10) W. Huber, *J. Am. Chem. Soc.*, **65**, 2222 (1943).

(11) G. Árpád, F. Ödön, and F. Oszkár, *Magy. Kem. Folyoirat*, **61**, 112 (1955).

methotrexate against three strains of *Streptococcus faecalis* are comparable (see Table I).[12]

TABLE I

COMPARISON OF BIOLOGICAL ASSAYS OF METHOTREXATE AND *p*-{[(2,4-DIAMINO-5-PYRIMIDINYL)METHYL]AMINO}BENZOYL-L-GLUTAMIC ACID (Ia)[12]

Enzyme or organism	Molar concn for 50% inhib	
	Methotrexate	Ia
E. coli thymidylate synthetase	1.0×10^{-4}	6.5×10^{-6}
Mouse tumor dihydrofolate reductase	2.0×10^{-8}	2.6×10^{-7}
S. faecalis (ATCC 8043)	5.5×10^{-10}	2.1×10^{-9}
S. faecalis resistant to tetrahydrohomofolate	4.4×10^{-9}	7.7×10^{-9}
S. faecalis resistant to amethopterin	2.2×10^{-8}	2.6×10^{-8}

Experimental Section[13]

2,4-Diamino-5-pyrimidinecarboxaldehyde (II).—A mixture of 13.5 g (0.1 mole) of 2,4-diamino-5-cyanopyrimidine,[10] 1.35 g of catalyst,[13] and 300 ml of 2 *N* HCl was hydrogenated at 60° (*ca.* 3 hr). The catalyst was removed by filtration and the yellow filtrate was concentrated *in vacuo* to 50 ml. It was then made alkaline and chilled overnight. The resulting bright yellow precipitate was collected by filtration and dried to give 11 g (76% yield) of II, mp 265–268° dec, lit.[9] mp 263–264° dec.

Ethyl *p*-{[(2,4-Diamino-5-pyrimidinyl)methylene]amino}-benzoate (Va).—A mixture of 9.0 g (0.065 mole) of 2,4-diamino-5-pyrimidinecarboxaldehyde (II), 10.7 g (0.065 mole) of ethyl *p*-aminobenzoate, and 10 ml of HCl-saturated CH_3OH in 1 l. of anhydrous MeOH was refluxed for 90 min. The reaction mixture was then cooled and evaporated to dryness *in vacuo*. The resulting yellow residue was recrystallized from EtOH to give 10 g (52% yield) of Va·HCl, mp 232–234° dec. The free base was prepared by stirring the salt in 200 ml of H_2O and then adjusting the pH of the solution to 9 with NH_4OH. The solid was collected by filtration and recrystallized from EtOH to give 9 g (49% yield) of Va, mp 228–230°. *Anal.* ($C_{14}H_{15}N_5O_2$) C, H, N.

2,4-Diamino-5-bromomethylpyrimidine Hydrobromide (IV).—To a hot (90–95°) solution of 6 g (0.043 mole) of 2,4-diamino-5-hydroxymethylpyrimidine[9] (III) in 50 ml of glacial AcOH was added 100 g of AcOH containing 30–32% of HBr. A precipitate formed immediately but slowly dissolved while heating for 2 hr on a steam bath. The solution was then filtered through a sintered-glass funnel and the filtrate was evaporated to dryness under reduced pressure. The residual solid was used immediately for the preparation of VI.

Ethyl *p*-{[(2,4-Diamino-5-pyrimidinyl)methyl]amino}benzoate (VIa). Method A.—A mixture of 10 g (0.035 mole) of Va·HCl, and 1 g of catalyst[13] in 300 ml of DMF was hydrogenated at 50° (*ca.* 15–20 min). The catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The resulting residue was covered with 200 ml of H_2O and the pH was adjusted to 10 with aqueous NaOH. An off-white solid precipitated. It was collected by filtration and dried in an oven to give 4 g of crude material, mp 205–207°. Recrystallization from 150 ml of EtOH gave 2 g (27% yield) of analytically pure product, mp 223–225°. *Anal.* ($C_{14}H_{17}N_5O_2$) C, H, N.

Method B.—A solution of 12.5 g (0.04 mole) of IV and 13.6 g (0.08 mole) of ethyl *p*-aminobenzoate in 300 ml of DMF was allowed to stand for 16 hr with occasional stirring. The solvent was then evaporated under reduced pressure and the resulting yellow solid dissolved in 250 ml of H_2O. The pH of the solution was adjusted to 8–9 by means of aqueous NaOH. The solid which formed was collected by filtration, dried, and recrystallized twice from EtOH to give 6.0 g (53% yield) of product, mp 224–226°.

(12) Test results were obtained by Dr. R. L. Kisliuk, Dr. M. Friedkin, and Miss E. J. Crawford of Tufts University and provided by Dr. H. B. Wood, Jr., of CCNSC. For the method by which the tests have been carried out with different organisms, *cf.* L. T. Plante, E. J. Crawford, and M. Friedkin, *J. Biol. Chem.*, **242**, 1466 (1967), and L. Goodman, *et al.*, *J. Am. Chem. Soc.*, **86**, 308 (1964), and references cited therein.

(13) All melting points (corrected) were taken on a Thomas–Hoover melting point apparatus. The uv spectra were determined with a Beckman DK-2 spectrophotometer. All hydrogenations were carried out on a Parr hydrogenator at temperature and time indicated, using 10% Pd–C at 4.2 kg/cm².

***p*-{[(2,4-Diamino-5-pyrimidinyl)methylene]amino}benzoic Acid (Vb).**—A mixture of 12 g (0.087 mole) of II and 15 ml of methanolic HCl in 1200 ml of anhydrous MeOH was heated to reflux to effect solution. To the solution was added 12 g (0.087 mole) of *p*-aminobenzoic acid in 200 ml of hot anhydrous MeOH and the resulting solution was refluxed for 4 hr. The reaction mixture was evaporated *in vacuo* to dryness to give 26 g of yellow solid, mp 293–295°. This was stirred in 1 l. of boiling MeOH and filtered to give 12 g (47% yield) of Vb·HCl, mp 310–312° dec. *Anal.* ($C_{12}H_{11}N_5O_2$·HCl) C, H, Cl, N.

***p*-{[(2,4-Diamino-5-pyrimidinyl)methyl]amino}benzoic Acid (VIb). Method A.**—A solution of 12 g (0.042 mole) of IV and 12 g (0.087 mole) of *p*-aminobenzoic acid in 200 ml of DMF was stirred at room temperature for 16 hr. The solvent was evaporated *in vacuo* and the residue was covered with 200 ml of H_2O. The resulting mixture was heated to boiling, then filtered. The product, which precipitated from the filtrate on cooling, was collected by filtration to give 8.5 g (62% yield) VIb·HBr, mp 255° dec. *Anal.* ($C_{12}H_{13}N_5O_2$·HBr) C, H, Br, N.

Method B.—A mixture of 7 g (0.024 mole) of Vb·HCl and 2 g of catalyst in 200 ml of DMF was hydrogenated at 60–65° (*ca.* 5 hr). The catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The resulting residue was recrystallized (H_2O) to give 1 g (14% yield) of VIb·HCl, mp 248–251° dec.

***p*-{[(2,4-Diamino-5-pyrimidinyl)methyl]amino}benzoyl-L-glutamic Acid (Ia). Method A.**—A mixture of 9 g (0.065 mole) of II and 19.2 g (0.065 mole) of dimethyl *p*-aminobenzoyl-L-glutamate[14] in 1 l. of anhydrous MeOH was added to 10 ml of methanolic HCl. The mixture was refluxed for 4 hr then evaporated to dryness under reduced pressure. The residual yellow solid was dissolved in 300 ml of DMF and the resulting solution was hydrogenated at room temperature in the presence of 4.5 g of catalyst[13] (*ca.* 11 hr). The catalyst was filtered and the filtrate was evaporated *in vacuo*. The residue was then covered with 200 ml of H_2O. To the mixture was added 8 g of NaOH and the solution was boiled for 10 min. The solution was then cooled and carefully acidified to pH 4.2 with dilute HCl. A gummy substance was obtained. This was stirred for 6 hr in an ice bath and the resulting brown product was collected by filtration. Recrystallization of the crude product from a large amount of H_2O gave, after drying at 135° for 16 hr, 2 g (8% yield) of Ia: mp 197–200° dec (softened at *ca.* 180°); $\lambda_{max}^{pH\ 1}$ 290 mμ (ε 19,600), $\lambda_{sh}^{pH\ 1}$ 228 mμ (ε 10,900), $\lambda_{max}^{pH\ 11}$ 285 mμ (ε 13,600). *Anal.* ($C_{17}H_{20}N_6O_5$) C, H, N.

Method B.—A solution of 12 g (0.042 mole) of IV and 31.4 g (0.086 mole) of a *p*-aminobenzoyl-L-glutamic acid[15] in 200 ml of DMF was stirred at room temperature for 16 hr. The solvent was evaporated *in vacuo* and the residue was dissolved in 250 ml of H_2O. The pH of the solution was adjusted to 4 by the addition of Na_2CO_3. A gummy precipitate was obtained which slowly solidified when stirred in an ice bath for 6 hr. The crude product was collected by filtration and recrystallized from H_2O to give, after being dried at 135° *in vacuo*, 9 g (56% yield) of Ia, mp 198–200° dec. The uv and ir spectra were found to be identical with those of the product prepared by method A.

***p*-{[(2,4-Diamino-5-pyrimidinyl)methyl]-N-methylamino}-benzoic Acid (VIc).**—A mixture of 12 g (0.041 mole) of IV and 13 g (0.086 mole) of *p*-methylaminobenzoic acid in 200 ml of DMF was stirred at room temperature for 16 hr, during which time a white precipitate separated. It was collected by filtration and washed with a small amount of cold DMF to give 12 g (87% yield) of analytically pure VI, mp 247° dec. *Anal.* ($C_{13}H_{15}N_5O_2$·HBr) C, H, Br, N.

***p*-{[(2,4-Diamino-5-pyrimidinyl)methyl]-N-methylamino}benzoyl-L-glutamic Acid (Ib).**—A solution of 8 g (0.028 mole) of of IV and 9 g (0.028 mole) of diethyl N-methyl-*p*-aminobenzoyl-L-glutamate[16] in 250 ml of DMF was stirred at room temperature for 16 hr. The solvent was evaporated *in vacuo* and the residue was dissolved in 200 ml of H_2O. To the solution was added 8 g of NaOH and the resulting mixture was heated on a steam bath until all the ester dissolved and was hydrolyzed to yield the disodio salt. The solution was then heated to boiling, decolorized with charcoal, and filtered. The filtrate was cooled and, after its pH was adjusted to 4.3 with 6 *N* HCl, afforded a gummy solid. The mixture was stirred in an ice bath for 2 hr and then chilled

(14) R. Koehler, L. Goodman, J. DeGraw, and B. R. Baker, *J. Am. Chem. Soc.*, **80**, 5779 (1958).

(15) (a) J. Van Der Scheer and K. Landsteiner, *J. Immunol.*, **29**, 370 (1935); (b) supplied by Frinton Labs., Vineland, N. J.

(16) S.-C. J. Fu and M. Reiner, *J. Org. Chem.*, **30**, 1277 (1965).

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.

Acknowledgment.—The authors thank Mrs. Margaret Rounds and Mr. John R. Gravatt for the analytical and instrumental measurements.

Effect of Monoiodotyrosine Metabolites on Tyrosine Hydroxylase[1a]

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Received June 19, 1968

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_1 = 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 ($K_1 = 3 \times 10^{-3}$) 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

	BuOH–AcOH–H_2O			BuOH–dioxane–NH_4OH		
		Net %			Net %	
Metabolite[a]	R_f	Rat	Rabbit	R_f	Rat	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.

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