TABLE II
INHIBITION OF TYROSINE TRANSAMINASE BY TYROSINE ANALOGS[a]

Analog	Manometric assay Concn, mM	Manometric assay % inhib	Enol-borate method Concn, mM	Enol-borate method % inhib	Lit.[b]
dl-m-Tyrosine	2	66	1	88	..
α-Methyl-*m*-tyrosine	2	0	1	5	..
dl-DOPA	2	70	1	72	86
α-Methyl-DOPA	2	0	1	17	11
Dopamine	2	60	1	93	89
α-Methyldopamine	2	34	1	72	..
Norepinephrine	1	74	0.5	60	91
α-Methylnorepinephrine	1	79	0.5	42	..
Dopamine	1	74	0.5	29	89

[a] The tyrosine concentration was 2 m*M* in the manometric assay and 1 m*M* in the enol–borate assay. [b] Results obtained by G. A. Jacoby and B. La Du [*J. Biol. Chem.*, **239**, 419 (1964)] at a tyrosine and inhibitor concentration of 3 m*M*.

DOPA; dopamine inhibits tyrosine degradation to a higher degree than α-methyldopamine, but α-methylnorepinephrine is as effective an inhibitor of tyrosine degradation as norepinephrine itself. When the effect of tyrosine analogs on tyrosine transaminase is assessed by the more specific enol-borate method (Table II), the inhibitory effect of *meta*-hydroxylated amino acids is seen to be markedly decreased by α methylation, while that of *m*-hydroxyphenethylamines is moderately decreased by α methylation, but increased by β hydroxylation.

Discussion

The presence of an α-methyl group in tyrosine analogs results in a large amount of steric hindrance to Schiff base formation; it explains the lack of biochemical reactivity of members of this group.

The presence of an α-methyl group in phenethylamines provides a definite but moderate amount of steric hindrance to Schiff base formation. That a similar steric relation holds for rat liver mitochondrial monoamine oxidase, possibly a pyridoxal P enzyme,[12] has been shown by Fuller and Walters.[13] Fuller and Walters state that rat monoamine oxidase was more inhibited by substrate amines than by α-methylated analogs; thus 0.1 m*M* phenethylamine was able to prevent 74% of kynurenamine oxidation, whereas, at the same concentration, α-methylphenethylamine was 31% and α,α-dimethylphenethylamine (which is sterically similar to an α-methylamino acid) was only 8% effective.

No attempt has been made in the present study to explain the increased affinity of β-hydroxylated phenethylamines for pyridoxal P.

Acknowledgment.—The author is indebted to Miss Eileen Ferrin for technical assistance.

(12) S. R. Guha, *Biochem. Pharmacol.*, **14**, 159 (1965).
(13) R. W. Fuller and C. P. Walters, *ibid.*, **14**, 159 (1965).

2-Aminotyrosine, an Analog of Tyrosine[1,2]

ALVIE L. DAVIS, JOHN W. ZAUN,[3] PERRY C. REEVES, ROBERT L. HANCE, AND TOMMY J. MCCORD

Department of Chemistry, Abilene Christian College, Abilene, Texas

Received June 10, 1966

A new tyrosine analog, 2-aminotyrosine, and several related compounds were prepared and tested for growth inhibition of *Escherichia coli* 9723 and *Leuconostoc dextranicum* 8086. Growth inhibition of *E. coli* by 2-aminotyrosine was shown to be reversed in a competitive and specific manner by tyrosine over a 100-fold range of increasing concentrations with an inhibition index of approximately 300. Ethyl 2-acetamido-2-(4-methoxy-2-nitrobenzyl)malonate, resulting from the condensation of 4-methoxy-2-nitrobenzyl bromide with the sodium salt of ethyl acetamidomalonate, was hydrolyzed with hydrobromic and hydrochloric acid to give 2-nitrotyrosine and 4-methoxy-2-nitrophenylalanine, respectively. Catalytic hydrogenation of the free bases of 2-nitrotyrosine and 4-methoxy-2-nitrophenylalanine gave 2-aminotyrosine and 2-amino-4-methoxyphenylalanine, respectively.

Among the previously reported ring-substituted analogs of the aromatic amino acids, some rather interesting biologically antagonistic activities have been observed with the aminophenylalanines in growth inhibition studies. Although no appreciable inhibitory activity was detected using *Leuconostoc dextranicum* as the test organism,[4] *p*-aminophenylalanine caused a growth inhibition of *Escherichia coli* which was reversed by either phenylalanine or tyrosine.[5] *m*-Aminophenylalanine, which is a competitive antagonist of phenylalanine in both *L. dextranicum*[4] and *E. coli*,[6] has also been reported to be a competitive antagonist of lysine in *Saccharomyces cerevisiae*.[6] More recently, *o*-aminophenylalanine has been prepared and found to antagonize specifically and competitively the utilization of phenylalanine for the growth of *E. coli*.[7]

Because of the interesting biological activities of the aminophenylalanines, the synthesis of comparable analogs which are structurally related to other natural amino acids was desirable. Accordingly, in the present investigation, 2-aminotyrosine and several related compounds were prepared, and their biological properties were studied in *E. coli* and *L. dextranicum* as herein presented.

2-Aminotyrosine and related compounds were synthesized through the usual acetamidomalonic ester

(1) The support of this work in part by a research grant (R-085) from the Robert A. Welch Foundation, Houston, Texas, and in part by an undergraduate research participation grant (GY-217) from the National Science Foundation is gratefully acknowledged.

(2) Presented in part at the 20th Southwest Regional Meeting of the American Chemical Society, Shreveport, La., Dec 1964.

(3) Taken in part from the M.S. Thesis of John W. Zaun, Abilene Christian College, Aug 1965.

(4) A. L. Davis, J. M. Ravel, C. G. Skinner, and W. Shive, *Arch. Biochem. Biophys.*, **76**, 139 (1958).

(5) E. D. Bergmann, S. Sicher, and B. E. Volcani, *Biochem. J.*, **54**, 1 (1953).

(6) K. Dittmer, Abstracts of Papers, 131st National Meeting of the American Chemical Society, Miami, Fla., April 1957, p 37C.

(7) A. L. Davis, R. Lloyd, J. Fletcher, L. Bayliss, and T. J. McCord, *Arch. Biochem. Biophys.*, **102**, 48 (1963).

synthesis, in which the interaction of 4-methoxy-2-nitrobenzyl bromide with the sodium salt of ethyl acetamidomalonate yielded ethyl 2-acetamido-2-(4-methoxy-2-nitrobenzyl)malonate. The latter compound was hydrolyzed with hydrobromic and hydrochloric acids to give 2-nitrotyrosine and 4-methoxy-2-nitrophenylalanine, respectively. Catalytic hydrogenation of these nitro amino acids gave 2-aminotyrosine and 2-amino-4-methoxyphenylalanine, respectively.

The effects of 2-aminotyrosine, 2-amino-4-methoxyphenylalanine, 2-nitrotyrosine, and 4-methoxy-2-nitrophenylalanine upon the growth of *E. coli* 9723 and *L. dextranicum* 8086 are compared to previously reported *o*-aminophenylalanine[7] as indicated in Table I. 2-Aminotyrosine is completely inhibitory to the growth of *E. coli* and *L. dextranicum* at concentration levels of about 2 and 6 μg/ml, respectively. In contrast, *o*-aminophenylalanine[7] inhibits the growth of *E. coli* at 0.6 μg/ml, but is not inhibitory to the growth of *L. dextranicum* at a concentration level of 600 μg/ml. Of the other amino acids tested only 2-amino-4-methoxyphenylalanine showed appreciable biological activity and inhibited the growth of *L. dextranicum* at a concentration of 60 μg/ml.

TABLE I

SUMMARY OF INHIBITORY ACTIVITY OF 2-AMINOTYROSINE AND RELATED COMPOUNDS ON MICROORGANISMS

Compd	Amt (μg/ml) of analog necessary for complete bacterial growth inhib *E. coli*	*L. dextranicum*
o-Aminophenylalanine	0.6[a]	>600
2-Aminotyrosine	2[b]	6[b]
2-Amino-4-methoxyphenylalanine	>200	60
2-Nitrotyrosine	>200	>200
4-Methoxy-2-nitrophenylalanine	>200	>200

[a] Reversed over a broad range of inhibitor concentrations by phenylalanine. [b] Reversed over a broad range of inhibitor concentrations by tyrosine.

A more extensive biological study was made of the reversal of 2-aminotyrosine inhibition by DL-tyrosine in *E. coli* as indicated in Table II. Growth inhibition by 2-aminotyrosine is reversed in a competitive manner over a 100-fold range of increasing concentrations by tyrosine with an inhibition index (ratio of the concentration of 2-aminotyrosine to the concentration of tyrosine required for complete inhibition of growth) of approximately 300. In contrast, 2 μg/ml of phenylalanine does not reverse the inhibition of 2-aminotyrosine.

TABLE II

REVERSAL OF 2-AMINOTYROSINE INHIBITION BY DL-TYROSINE IN *E. coli* 9723[a]

2-Aminotyrosine, μg/ml	Absorbance readings[b] — DL-Tyrosine, μg/ml 0	0.06	0.20	0.60	2.0	6.0
0	0.80	0.84	0.80	0.82	0.79	0.80
0.2	0.85					
0.6	0.75	0.75				
2	0.12	158	0.79			
6	0	0	0.48	0.59		
20			0.21	0.41	0.66	
60			0	0.32	0.61	0.74
200				0.14	0.58	0.72
600					0.27	0.66
2000						0.26

[a] Incubated 15 hr at 37°. [b] A measure of culture turbidity in which absorbance readings of 0.52, 0.30, 0.16, and 0.05 are equivalent to 0.76, 0.43, 0.215, and 0.06 mg of dry weight of cells/ml of culture, respectively.

Substitution of a primary amino group for a hydrogen atom at the 2 position on the benzene ring of tyrosine has been successful in producing an analog which is a specific and competitive antagonist of tyrosine. These results demonstrate the availability of an additional inhibitory analog, 2-aminotyrosine, which may be used for the study of tyrosine metabolism.

Experimental Section[8]

4-Methyl-3-nitroanisole.—To a boiling solution of 238 ml of 6 *N* HCl was added 91 g (0.5 mole) of 2-methoxy-5-methyl-4-nitroaniline. After the amine was dissolved, 125 ml of concentrated HCl was added, and the solution was cooled to a temperature of −5 to 0°. A solution of 41.3 g of $NaNO_2$ in 88 ml of water was added to the cold solution from a dropping funnel over a period of approximately 1 hr. To the diazonium salt solution was added dropwise 260 ml of 50% aqueous hypophosphorous acid (precooled to 0°) over a period of about 30 min, and the solution was stirred at −5 to 0° for 1 additional hr. The solution was extracted with approximately 2 l. of ether, and the ether was removed by distillation. Fractional distillation under reduced pressure of the resulting liquid gave 54.5 g (71%) of product, bp 150° (20 mm) [lit.[9] bp 138° (11–14 mm) *via* a different synthetical route].

Ethyl 2-Acetamido-2-(4-methoxy-2-nitrobenzyl)malonate.—To a solution of 11.5 g of ethyl acetamidomalonate in 100 ml of Mg-dried ethanol containing 1.22 g of sodium was added 13.0 g of 4-methoxy-2-nitrobenzyl bromide.[10] The reaction mixture was refluxed for 2.5 hr, after which NaBr was removed by filtration. Then, the filtrate was cooled overnight in the refrigerator to yield 16.5 g of crude material. Recrystallization from ethanol yielded 14.2 g (70%) of product, mp 108–109°.

Anal. Calcd for $C_{17}H_{22}N_2O_8$: C, 53.39; H, 5.79; N, 7.32. Found: C, 53.33; H, 5.93; N, 7.42.

2-Nitrotyrosine Hydrobromide.—A sample of 5.5 g of ethyl 2-acetamido-2-(4-methoxy-2-nitrobenzyl)malonate was hydrolyzed in the presence of 50 ml of 48% HBr acid for 4 hr. After cooling the reaction mixture in the refrigerator for 1 hr, there was obtained 4.0 g (94%) of crystalline product, mp 225–231° dec. Paper chromatograms of the product in 1-butanol–acetic acid–water (4:1:1), and 65% pyridine showed one spot with R_f values of 0.39 and 0.76, respectively.

Anal. Calcd for $C_9H_{10}N_2O_5 \cdot HBr$: C, 36.13; H, 3.63; N, 9.08. Found: C, 36.13; H, 3.64; N, 8.93.

2-Aminotyrosine.—A sample of 1.4 g of 2-nitrotyrosine hydrobromide was dissolved in a minimum amount of water, and NH_4OH was added dropwise to pH 7.0 to yield 1.05 g (97%) of 2-nitrotyrosine, mp 238–240° dec. A sample of 0.5 g of 2-nitrotyrosine dissolved in 75% methanol was hydrogenated under 3.52 kg/cm² of hydrogen pressure in the presence of 100 mg of palladium black for 3 hr. The catalyst was removed by filtration, and the resulting solution was concentrated under reduced pressure. After chilling in the refrigerator, there was obtained 0.4 g (92%) of crude product, which was recrystallized from water, mp 179–181° (subl), 240° dec. Paper chromatograms of the product in 1-butanol–acetic acid–water (4:1:1) and 65% pyridine showed R_f values of 0.36 and 0.59, respectively.

Anal. Calcd for $C_9H_{12}N_2O_5 \cdot H_2O$: C, 50.46; H, 6.60; N, 13.08. Found: C, 50.37; H, 6.54; N, 12.89.

(8) All melting points were determined by the capillary technique and are corrected. The paper chromatograms were determined by the ascending techniques using the solvents indicated, and the spots were developed with ninhydrin reagent. The elemental analyses were determined by International Chemical and Nuclear Corporation, City of Industry, Calif. The Authors are indebted to Mrs. D. Howell and Mrs. J. Zaun for the microbiological assays.

(9) N. N. Suvorov, M. V. Fedotova, O. B. Ogareva, and E. G. Balasheva, *Zh. Obshch. Khim.*, **30**, 3118 (1960).

(10) F. Kuffner, G. Lenneis, and H. Bauer, *Monatsh. Chem.*, **91**, 1152 (1960).

4-Methoxy-2-nitrophenylalanine.—A solution of 5.0 g of ethyl 2-acetamido-2-(4-methoxy-2-nitrobenzyl)malonate in 50 ml of concentrated HCl was heated under reflux for 3 hr. The reaction mixture was placed in the refrigerator for 1 hr to effect crystallization. The resulting crystals were removed by filtration to give 2.70 g (74%) of product, mp 212–214°. The hydrochloride salt (1 g) was dissolved in a minimum amount of water, and dilute NH_4OH was added dropwise to pH 7.0 to precipitate 0.7 g (81%) of the base, mp 212–214° dec. Paper chromatograms of the product in 1-butanol–acetic acid–water (4:1:1) and 65% pyridine showed one spot, R_f 0.48 and 0.74, respectively.

Anal. Calcd for $C_{10}H_{12}N_2O_5$: C, 50.00; H, 5.03; N, 11.66. Found: C, 50.13; H, 4.94; N, 11.40.

2-Amino-4-methoxyphenylalanine.—A solution of 0.7 g of 4-methoxy-2-nitrophenylalanine in 100 ml of 75% methanol was hydrogenated under 3.52 kg/cm² of hydrogen pressure using palladium black as a catalyst for 3 hr. After removing the catalyst by filtration, the filtrate was concentrated. The resulting solution was cooled in the refrigerator to yield 0.55 g (89%) of desired product. Following recrystallization from water, the product melted at 184.5–186.5°. Paper chromatograms of the product in 1-butanol–acetic acid–water (4:1:1) and 65% pyridine showed one spot, R_f 0.52 and 0.82, respectively.

Anal. Calcd for $C_{10}H_{14}N_2O_3 \cdot H_2O$: C, 52.62; H, 7.04; N, 12.23. Found: C, 52.53; H, 7.03; N, 12.18.

Microbiological Assays.—For *E. coli* 9723, a previously described inorganic salts medium[11] was employed, and the organism was incubated at 37° for about 16 hr. For *L. dextranicum* 8086, the same assay procedure was employed as previously reported.[5] In all assays the amount of growth was determined photometrically at 625 mμ with a Bausch and Lomb Spectronic 20 spectrophotometer, in terms of absorbance readings of the turbid culture medium against a blank of uninoculated medium set at 0 absorbance. For *E. coli* the data in Table II are recorded as absorbance readings which are related to the milligrams of dry cells calculated from a standard curve of mg of dry cells/ml *vs.* absorbance readings.

(11) E. H. Anderson, *Proc. Natl. Acad. Sci. U. S.*, **30**, 120 (1916).

N-Methyl-N-2-propynyl-1-indanamine. A Potent Monoamine Oxidase Inhibitor

C. F. HUEBNER, ELLEN M. DONOGHUE, A. J. PLUMMER, AND P. A. FURNESS

Research Department, CIBA Pharmaceutical Company, Division of CIBA Corporation, Summit, New Jersey

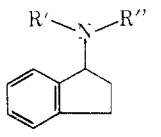
Received May 24, 1966

The synthesis of a series of tertiary indanamines and related compounds containing an N-2-propynyl substituent is described. Among this series are two (**1** and **10**, Table I) which are among the most potent monoamine oxidase inhibitors yet reported. Some activity–structure correlations have been made.

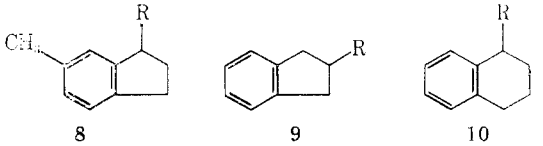
Our continuing interest in incorporating the indane grouping in molecules of potential pharmacological value, an activity which has already led to antihistamines,[1] analgesics, and monoamine oxidase inhibitors,[2] prompted us to synthesize a propynylamine containing this moiety. This followed the report of the monoamine oxidase inhibitory activity of N-methyl-N-2-propynylbenzylamine (pargyline).[3] The first compound prepared, N-methyl-N-2-propynyl-1-indanamine (**1**),[4] showed approximately 20 times the activity of pargyline and is indeed in certain tests the most potent, irreversible monoamine oxidase inhibitor known. We also wish to report on a few congeners of **1** prepared to explore activity–structure relationships (Table I).

The synthesis of these tertiary amines was straightforward. The requisite primary amines were formylated or acylated, reduced to the secondary amine with lithium aluminum hydride, and finally alkylated with propargyl bromide in the presence of sodium carbonate. For the preparation of large quantities of **1** a more economical procedure was developed by Dr. W. Rosen in which the intermediate N-methylindanamine was prepared from 1-chloroindane and methylamine.

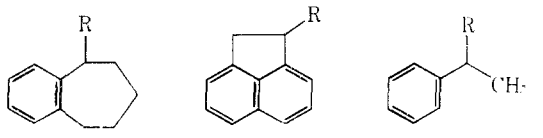
Changing the N-methyl substituent of **1** to hydrogen (**2**), ethyl (**3**), or 2-propynyl (**4**) lessened activity. Exchanging the N-2-propynyl substituent of **1** for



1, R′ = CH_3; R″ = $CH_2C{\equiv}CH$
2, R′ = H; R″ = $CH_2C{\equiv}CH$
3, R′ = C_2H_5; R″ = $CH_2C{\equiv}CH$
4, R′ = $CH_2C{\equiv}CH$; R″ = $CH_2C{\equiv}CH$
5, R′ = CH_3; R″ = $CH_2C{=}CH_2$
6, R′ = H; R″ = $C(CH_3)_2C{\equiv}CH$
7, R′ = CH_3; R″ = $C(CH_3)_2C{\equiv}CH$



8 9 10



11 12 13

R = $N(CH_3)(CH_2C{\equiv}CH)$

allyl (**5**) destroyed activity. Where the substituent was 1,1-dimethylpropynyl (**6** and **7**), activity was also lessened. Nuclear methyl substitution in **1** (**8**) or substitution of a 2- for a 1-indanyl residue (**9**) resulted in compounds about one-fourth as active as the parent. Ring enlargement of the five-membered, alicyclic ring of indane (**10**) gave the most active compound in the series which showed a 50% increase in activity over that of **1**. Enlarging the alicyclic ring to seven members (**11**), however, again lessened activity.

(1) C. F. Huebner, E. M. Donoghue, P. Wenk, E. Sury, and J. A. Nelson, *J. Am. Chem. Soc.*, **82**, 2077 (1960).

(2) C. F. Huebner, E. M. Donoghue, P. L. Strachan, P. Beak, and E. Wenkert, *J. Org. Chem.*, **27**, 4465 (1962).

(3) L. R. Sweet, W. B. Martin, J. D. Taylor, G. M. Everett, A. A. Wykes, and Y. C. Gladish, *Ann. N. Y. Acad. Sci.*, **107**, 89 (1963).

(4) The authors would be pleased to fill any requests for this potent monoamine oxidase inhibitor from interested biochemical and pharmacological investigators for animal use.