3-Alkyltyrosines.[1a] Potential Antihypertensive Agents

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Received October 12, 1970

The synthesis of 3-methyl- and 3-isopropyltyrosine from the corresponding alkylanisoles by modified Gatterman and Erlenmeyer syntheses is described. The compounds were tested *in vitro* as tyrosine hydroxylase inhibitors and *in vivo* by the tyramine pressor test and for their ability to alter the levels of heart and brain biogenic amines. The *in vivo* activity of the 3-alkyltyrosines appeared to be related to their ability to inhibit tyrosine hydroxylase *in vitro* and to their resistance to biodegradation.

Tyrosine hydroxylase is the rate-limiting enzyme in the biosynthesis of the sympathetic neurotransmitter norepinephrine (NE)[1c] and inhibition of this enzyme provides an approach to altering sympathetic function. One of the most potent inhibitors of tyrosine hydroxylase *in vitro* is 3-iodo-L-tyrosine; however, it has little activity *in vivo*, presumably due to its rapid disappearance from tissue[2] and deactivation by tissue dehalogenases. α-Methyl-*p*-tyrosine (α-MPT) is a less potent inhibitor *in vitro*, but possesses *in vivo* activity, apparently because its side chain is resistant to metabolic alteration.[3]

Alkyl groups have been successfully used to replace I atoms in the 3′ position of triiodothyronine,[4] and because alkyl groups would not be expected to be metabolized as readily as I atoms, 3-methyl-DL-tyrosine (MT) and 3-isopropyl-DL-tyrosine (IpT) were prepared for evaluation to ascertain whether any pharmacologic advantage could be derived from the metabolic stability of the 3-alkyl substituent.

Chemistry.—The preparation of 3-methyltyrosine has been described by Jorgensen and Wiley;[5] 3-isopropyltyrosine has likewise been prepared by Jorgensen's group,[6] but details have not been reported. Their chloromethylation procedure was, in our hands, moderately successful for the preparation of MT but unsuccessful in the preparation of IpT.

Synthesis of both MT and IpT in desirable quantities was achieved by the modified methods of Erlenmeyer[7] and Harington.[8] The appropriate benzaldehyde was obtained using a modification of the method of Gatterman.[9] Condensation of the aldehyde with hippuric acid to the azlactone was followed by base-catalyzed hydrolysis of the azlactone; the substituted acrylic acid was reduced with red P–HI to produce the desired 3-alkyl amino acid. IpT was purified by double resin ion-exchange chromatography and isolated as the very water-soluble monohydrate of the amino acid.

Biological Activity.—The *in vitro* activity of the 3-alkyltyrosines was assessed by tyrosine hydroxylase inhibition studies and their *in vivo* activity by evaluating the effect of 3-alkyltyrosines on biogenic amine levels. The response of iv tyramine in anesthetized rats was used to evaluate the effect of IpT on sympathetic activity.

A comparison of the degree of tyrosine hydroxylase inhibition produced by the 3-alkyltyrosines with that of the other 3-substituted tyrosines (Table I) indicates that the inhibitory effect of 3-iodotyrosine cannot be attributed to molecular size *per se*. McGeer and McGeer[10] have found that 3-nitro-L-tyrosine and 3-amino-L-tyrosine are able to inhibit tyrosine hydroxylase to an identical extent (65% at 10^{-4} *M*); therefore, it is doubtful whether inhibitory activity can be attributed to the electronic properties of the 3 substituent. The relative K_i values listed in Table I are consistent with the $[1/S]_{50}$ data obtained by Udenfriend[3] for 3-halotyrosines and 3-halo-α-methyltyrosines.

TABLE I

K_i VALUES FOR SOME 3-ALKYL AND SOME OTHER 3-SUBSTITUTED TYROSINES[a]

Derivative of tyrosine	Isomer	K_i
3-I	L	4.5×10^{-7}
3-Cl	L	6.6×10^{-6}
3-Me	DL	2.3×10^{-4}
3-MeO	DL	3.8×10^{-4}
3-*i*-Pr	DL	3.9×10^{-4}
3-NH_2	L	6.3×10^{-4}
3-F	DL	8.4×10^{-4}

[a] Values for 3-alkyltyrosines were obtained by the method of Lineweaver and Burk (see Figure 1) those for other 3-substituted tyrosines by the method of Dixon (see Figure 2).

The pressor response to iv tyramine in anesthetized rats was used to evaluate the effect of IpT on functional sympathetic activity (Table II). A 2-hr ip pretreatment of rats with 2.1 mmoles/kg of IpT reduced the pressor activity of tyramine to half that found in saline-pretreated controls. Pretreatment with an equiv dose of α-MMT, a compound known to release NE from its storage sites, produced similar results. A 2.1-mmoles/kg dose of α-MMT has been shown to cause almost complete depletion of NE in rats.[11] The pressor response of ad-

(1) (a) Supported by Research Fellowship 5-FL-GM-28, 269 and National Institutes of Health Grant AM-06480. (b) Most of these data are taken from the thesis submitted by Dr. Caplan in partial fulfillment of the requirements for the Ph.D. degree in Medicinal Chemistry and were presented at the 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969. (c) Abbreviations used in the text are: MT, 3-methyltyrosine; IpT, 3-isopropyltyrosine; α-MPT, α-methyl-*p*-tyrosine; α-MMT, α-methyl-*m*-tyrosine; NE, norepinephrine.

(2) S. Spector, R. O. Mata, A. Sjoerdsma, and S. Udenfriend, *Life Sci.*, **4**, 1307 (1965).

(3) S. Udenfriend, P. Saltzman-Nirenberg, and T. Nagatsu, *Biochem. Pharmacol.*, **14**, 837 (1965).

(4) C. M. Greenberg, B. Blank, F. R. Pfeiffer, and J. F. Pauls, *Amer. J. Physiol.*, **6**, 554 (1963).

(5) E. C. Jorgensen and R. A. Wiley, *J. Pharm. Sci.*, **52**, 122 (1963).

(6) E. C. Jorgensen, personal communication, 1967.

(7) E. Erlenmeyer, *Justus Liebigs Ann. Chem.*, **275**, 1 (1893).

(8) C. R. Harington and W. McCartney, *Biochem. J.*, **21**, 852 (1927).

(9) L. Gatterman, *Ber.*, **31**, 1149 (1898).

(10) E. G. McGeer and P. L. McGeer, *Can. J. Biochem.*, **45**, 115 (1967).

(11) S. Spector, A. Sjoerdsma, and S. Udenfriend, *J. Pharmacol. Exp. Ther.*, **147**, 86 (1965).

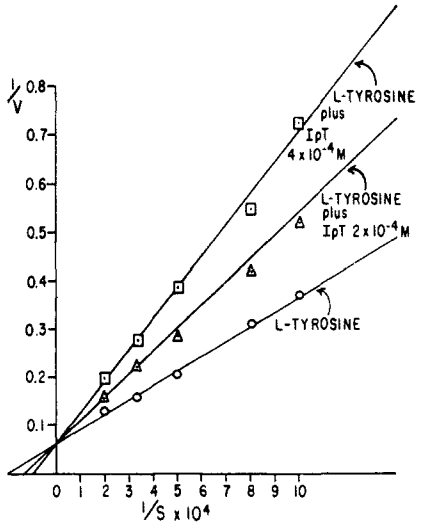

Figure 1.—3-Isopropyl-DL-tyrosine monohydrate (IpT) inhibition of tyrosine hydroxylase by the method of Lineweaver and Burk. Activity was measured in the standard incubation mixture with the substrate concentration ranging from 1×10^{-5} M to 5×10^{-5} M. V = nanomoles/mg of enzyme protein per 15 min. Curves are corrected by the method of least squares.

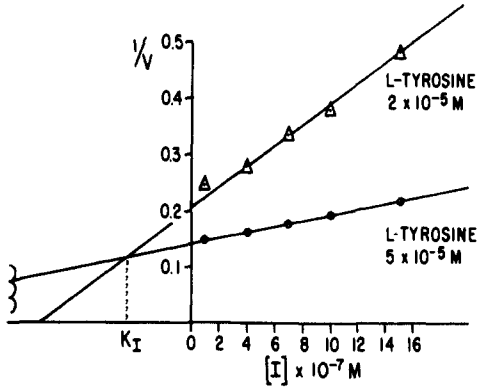

Figure 2.—3-Iodo-L-tyrosine inhibition of tyrosine hydroxylase by the method of Dixon. Activity was measured in the standard incubation mixture with inhibitor concentrations ranging from 1×10^{-7} M to 1.5×10^{-6} M. V = nanomoles/mg of enzyme protein per 15 min. $K_I = 4.5 \times 10^{-7}$ M. Curves are corrected by the method of least squares.

ministered NE was not significantly affected by pretreatment with these inhibitors, which suggests that α-MMT and IpT do not reduce the activity of tyramine by adrenergic blockade. These results along with the large increase in tyramine pressor response observed after NE treatment in all animals suggest that IpT reduces the amount of endogenous NE available to be released by tyramine. However, from these studies it is not possible to indicate whether the decrease in endogenous NE is due to a releasing action, the indicated mechanism for α-MMT, or to an inhibition of biosynthesis, the indicated mechanism for α-methyl-p-tyrosine (α-MPT).[11]

If the assumption is made that 3-alkyltyrosines act solely by inhibition of NE biosynthesis, then the alkyltyrosines ($K_i = 2 \times 10^{-4}$ for IpT; $K_i = 4 \times 10^{-4}$ for MT) would be expected to be less active than α-MPT ($K_i = 1.7 \times 10^{-5}$) in lowering brain biogenic amines. If, on the other hand, the ability of IpT to inhibit the tyramine pressor response is the result of a peripheral release of NE, then its effect should be of the same magnitude as that of α-MMT. The data of Table III, which present analyses of biogenic amine levels 3 hr after ip injection, suggest that *in vivo* tyrosine hydroxylase inhibition is mainly responsible for the moderate activity of 3-alkyltyrosines which was observed. The response of IpT when compared with the reported activity of α-MPT[11] would suggest possible use of IpT as an antihypertensive agent.

TABLE II

PRESSOR RESPONSE (PER CENT CHANGE)[a] IN RATS AFTER A 2-HR PRETREATMENT WITH α-METHYL-m-TYROSINE (α-MMT) AND 3-ISOPROPYL-DL-TYROSINE (IpT)

Compound administered	Tyramine[b]	NE[c]	Tyramine[d]
Saline (control)	43.9 (47.3, 40.4)	112.9 (92.2, 133.5)	66.3 (47.0, 85.5)
α-MMT	21.4 (19.6, 23.1)	106.2 (75.4, 137.0)	71.4 (71.4)
IpT	22.6 (21.7, 23.5)	84.9 (30.2, 89.5)	87.0 (88.3, 85.6)

[a] Values represent the mean value, generally of 2 animals (numbers in parentheses), of the per cent change in systolic blood pressure from the resting value after administration of tyramine or NE. [b] Values are the average of a series of 3–6 repeated tyramine injections, 300 μg/kg. [c] Values represent the average response to the 2 repeated injections of NE (0.2 ml, 1:25,000). [d] Values represent the response to the first dose of tyramine after NE treatment since a rapid tachyphylaxis was observed on subsequent tyramine injections.

Experimental Section

Synthetic Methods.—Ir spectra were obtained from Model 137 Perkin-Elmer infracord spectrophotometer or from Model 257 Perkin-Elmer grating infrared spectrophotometer. Visible and uv spectra were prepared on a Beckman DB spectrophotometer or on a Model 350 Perkin-Elmer spectrophotometer. Melting points are uncorrected. Elemental analyses were performed by Dr. G. Weiler and Dr. F. B. Strauss, Microanalytical Laboratory in Oxford, England. Eastman Chromagram sheets, silica gel adsorbent with fluorescent indicator (No. 6060), and alumina adsorbent with fluorescent indicator (No. 6063) were used for tlc and were not activated.

3-Methyl-4-methoxybenzaldehyde (Ia).—To a mixt of 2-methylanisole (46.3 g, 0.38 mole) and dry C_6H_6 (75 ml) was added $Zn(CN)_2$ (52 g, 0.44 mole). Dry HCl was passed rapidly through the ice-cooled mixt for 60 min, after which anhyd $AlCl_3$ (49 g, 0.34 mole) was added slowly with further cooling and stirring. A slow stream of dry HCl was then passed through the stirred, heated (40–45°) mixt for 4 hr. The mixt was added slowly to an excess of 3 N HCl and heated under reflux for 30 min. The org layer was sepd and steam distd, removing first the C_6H_6 and then Ia. The aldehyde fraction was shaken with 40% $NaHSO_3$ (100 ml), the bisulfite adduct was sepd and washed with Et_2O, and the aldehyde was regenerated with 20% Na_2CO_3. After dissolving the aldehyde in C_6H_6 and drying (Na_2SO_4), the soln was distd producing 25 g (0.17 mole, 45%) of colorless Ia: bp 100° (2 mm) [lit. bp 135° (13 mm), 251°][12,13] $n^{36}D$ 1.5656.

The compd was homogenous by tlc ($CHCl_3$, C_6H_6) and glpc. *Anal.* (for the 2,4-dinitrophenylhydrazone, $C_{15}H_{14}N_4O_5$, mp 232–234°) C, H, N.

Azlactone of α-Benzoylamino-β-(3-methyl-4-methoxyphenyl)-acrylic Acid (IIa).—A mixt of Ia (10 g, 0.066 mole), powdered, dry hippuric acid (12.8 g, 0.071 mole), powdered, freshly fused NaOAc (5.4 g, 0.068 mole), and reagent grade Ac_2O (20 g, 0.2 mole) were heated with constant shaking on a hot plate. The almost solid mixt was allowed to liquefy gradually and heated on the steam bath for 2 hr. While the flask was cooled, abs EtOH

(12) J. R. A. Pollock and R. Stevens, Ed., "Dictionary of Organic Compounds," Vol. I, 4th ed, Oxford University Press, New York, N. Y., 1965, p 1863.

(13) W. E. Truce, *Org. React.*, **9**, 55 (1957).

TABLE III

BRAIN BIOGENIC AMINE AND HEART NOREPINEPHRINE LEVELS AFTER THE INJECTION OF 3-ALKYLTYROSINES AND OF REFERENCE COMPOUNDS[a]

Compound administered	Brain serotonin, μg/g ± SEM	Brain norepinephrine, μg/g ± SEM	Brain dopamine, μg/g ± SEM	Heart norepinephrine, % control ± SEM[b]
Saline (control)	0.53 ± 0.01 (4)	0.31 ± 0.01 (4)	0.64 ± 0.02 (4)	100 ± 5 (4)
α-Methyl-*p*-tyrosine	0.57 ± 0.01 (3)	0.14 ± 0.02 (3)	0.25 ± 0.03 (3)	84 ± 6 (3)
	$P < 0.05$	$P < 0.01$	$P < 0.01$	$P < 0.1$
α-Methyl-*m*-tyrosine				46 ± 1 (4)
				$P < 0.01$
3-Methyltyrosine	0.47 ± 0.03 (4)	0.24 ± 0.02 (4)	0.44 ± 0.03 (4)	83 ± 8 (6)
	NS	$P < 0.05$	$P < 0.01$	$P < 0.1$
3-Isopropyltyrosine	0.51 ± 0.04 (4)	0.24 ± 0.02 (4)	0.40 ± 0.01 (4)	78 ± 4 (6)
	NS	$P < 0.05$	$P < 0.01$	$P < 0.01$

[a] Compds were injected ip to male Wistar rats at a dose of 1 mmole/kg. The animals were sacrificed 3 hr after the injection. The number of animals in each group is indicated in parentheses. [b] Data represent summation of 2 experiments. Control values were 0.66 μg/g and 0.71 μg/g in the respective experiments.

(27 ml) was added, and the mixt was allowed to stand at room temp overnight. The crude yellow product was collected and washed twice on the filter with portions of boiling H_2O (15 ml). Recrystn from C_6H_6 produced 14.7 g (0.05 mole, 76%) of IIa, mp 149–151°. *Anal.* ($C_{18}H_{15}NO_3$): C, H, N.

α-Benzoylamino-β-(3-methyl-4-methoxyphenyl)acrylic Acid (IIIa).—IIa (5 g, 17 mmoles) was dissolved in a soln of NaOH (1.75 g) in 50% aq EtOH (75 ml) and heated under reflux for 20 min. After addition of hot H_2O (150 ml), acidification with 2 *N* HCl caused the product to ppt. Crystn from 95% EtOH yielded 4.9 g (15.8 mmoles, 92%) of white to yellow crystals of IIIa; mp 241–243° dec. *Anal.* ($C_{18}H_{17}NO_4$): C, H, N.

3-Methyl-DL-tyrosine (IVa).—To a cooled mixt of IIIa (1.5 g, 4.8 mmoles), purified red P (1 g), and Ac_2O (9 ml), 47% HI (9 ml) was added dropwise, and the mixt was heated under reflux for 1.5 hr. Upon cooling, the unreacted P was removed by filtration and washed twice with AcOH (2 ml). The combined filtrate was evapd to dryness under reduced pressure. H_2O (30 ml) and Et_2O (30 ml) were added to the dry powder, and the mixt was shaken until soln was complete. The Et_2O layer was removed, the aq layer was washed with Et_2O (3 × 20 ml), and the Et_2O washings were discarded. Decolorizing C (0.5 g) and a trace of Na_2SO_3 were added to the aq layer, and the mixt was filtered after all Et_2O had been removed by heating on the steam bath. The filtrate was neutralized to pH 5 with 15% NH_4OH, and pptn was induced by partial removal of solvent. Recrystn from EtOH–H_2O–Et_2O produced a 39% yield of flaky IVa: mp 268° dec (lit. mp 285°, 276°);[5,14] homogenous by tlc (BuOH–AcOH–H_2O). *Anal.* ($C_{10}H_{13}NO_3$): C, H, N.

2-Isopropylanisole (V) was prepd in 94% yield from 2-isopropylphenol and Me_2SO_4 by standard methods.

3-Isopropyl-4-methoxybenzaldehyde (Ib) was prepd in 29.6% yield according to the procedure described for Ia, bp 85° (0.25 mm). The compound was homogenous by tlc ($CHCl_3$, C_6H_6) and glpc. The spectra of Ib were consistent with a reference spectrum of 3,4-dimethoxybenzaldehyde.[15] *Anal.* (for the 2,4-dinitrophenylhydrazone, $C_{17}H_{18}N_4O_5$): C, H, N.

Azlactone of α-benzoylamino-β-(3-isopropyl-4-methoxyphenyl)acrylic acid (IIb) was prepd in 66.4% yield according to the procedure described for IIa. *Anal.* ($C_{20}H_{19}NO_3$): C, H, N.

α-Benzoylamino-β-(3-isopropyl-4-methoxyphenyl)acrylic acid (IIIb) was prepd in 95% yield by the procedure listed under IIIa, mp 232–234° dec. *Anal.* ($C_{20}H_{21}NO_4$): C, H, N.

3-Isopropyl-DL-tyrosine monohydrate (IVb) was prepd in 38.1% yield in the same manner as IVa. When it appeared impossible to purify IVb by crystn or isoelectric pptn, the compd was dried by lyophilization and redissolved in H_2O, and the soln was adjusted to pH 6 and added to cation-exchange resin (Dowex 50-W-X8, H^+ form, 200–400 mesh). The column was washed with H_2O until neutral, the compd was eluted with 1.5 *N* NH_4OH, and the eluate was lyophilized. The lyophilized material was dissolved in H_2O, and the soln was adjusted to pH 2.5 and added to an anion-exchange resin (Dowex 1-X8, OH^- form, 200–400 mesh). The column was washed with H_2O until neutral and eluted with 2 *N* AcOH. The acidic eluate was lyophilized, and the resulting powder twice redissolved in H_2O and relyophilized to produce IVb: mp (range) 195–220°; homogenous by tlc (BuOH–AcOH–H_2O). *Anal.* (as the monohydrate $C_{12}H_{18}NO_4$) C, H, N. The H_2O of hydration could not be removed at 78° (1 mm) over P_2O_5 for 24 hr.

Biological Methods.—L-Tyrosine and 3-iodo-L-tyrosine were obtained from Nutritional Biochemicals Corp. 3-Fluoro-DL-tyrosine was purchased from Aldrich Chemical Co. and 3-chloro-L-tyrosine from Pierce Chemicals. 2-Amino-4-hydroxy-6,7-dimethyltetrahydropteridine ($DMPH_4$) was purchased from Calbiochem. 3-Hydroxy-4-bromobenzyloxamine dihydrogen phosphate was kindly supplied by Dr. Sidney Udenfriend of the National Institutes of Health. [3,5-^{3}H]L-Tyrosine, POP, and POPOP were obtained from New England Nuclear Corp. All other chemicals were reagent grade. Tyramine·HCl, norepinephrine (free base), and dopamine were obtained from Nutritional Biochemicals Inc., the last compd was purified by pptn from a mixt of EtOH, AcCl, EtOAc, and Et_2O. 5-Hydroxytryptamine was obtained from Calbiochem, and the norepinephrine soln used in the tyramine pressor test was prepared by diln from ampoules of Levophed bitartrate (Winthrop Laboratories) which contained the equiv of 1 mg of base per ml. Heparin Na (1 g = 141,000 units) and atropine sulfate were obtained from Fisher Chemicals; other chemicals and solvents used in the fluorometric assay of biogenic amines were reagent or spectral quality. Fluorometric glassware was cleaned by 2-hr boiling in 1–2% HNO_3 in MeOH and rinsing with tap and distd H_2O. Fluorescence measurements were made on an Aminco-Bowman spectrophotofluorometer.

Enzyme-Inhibition Studies. Tyrosine hydroxylase was prepared as described by Nagatsu[16] and modified by Lutsky and Zenker,[17] purified by a modification of Ikeda's procedure,[18] and assayed according to the procedure of Nagatsu, *et al.*[19] Protein was assayed by the method of Lowry.[20] $(NH_4)_2SO_4$ precipitations were carried out using conversion tables corrected to 0°.

The tyrosine hydroxylase purification procedure described by Ikeda, *et al.*,[18] was modified as follows. The crude enzyme preparation was suspended in 150 m*M* phosphate buffer and dialyzed against 10 m*M* phosphate buffer for 3 hr at 0°. Fractionation was achieved at 0° with a satd soln of $(NH_4)_2SO_4$, pH 7. The 25–35% $(NH_4)_2SO_4$ fraction was resuspended in 10 m*M* phosphate buffer and lyophilized.

Tyrosine hydroxylase inhibitors were screened by a modification of the method of Udenfriend, *et al.*,[3] and the kinetics of inhibition of the enzyme by MT and IpT were determined by the method of Lineweaver and Burke[21] with the substrate concentration varied from 1×10^{-5} *M* to 5×10^{-5} *M* and each inhibitor tested at concentrations of 2×10^{-4} *M* and 4×10^{-4} *M* (Figure 1).

Since comparison of the 3-alkyl with the other 3-substituted tyrosines was desired, K_i values for 3-fluoro-, 3-chloro-, 3-iodo-,

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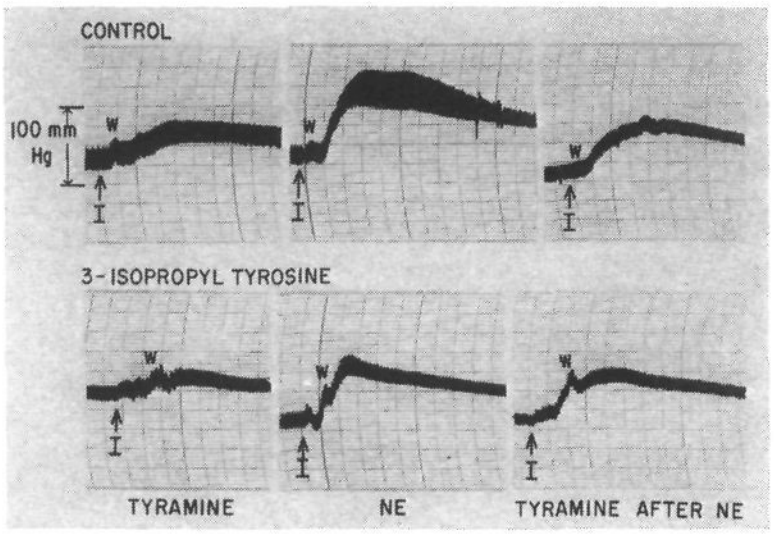

Figure 3.—Tyramine pressor response in pretreated rats. Responses shown are those of one control animal and one animal pretreated 2 hr earlier with 3-isopropyl-DL-tyrosine (monohydrate). I = point of injection; W = saline flush. Systolic blood pressure is indicated by the upper limit of the recording and diastolic blood pressure by the lower limit.

3-methoxy-, and 3-aminotyrosine were determined for the analysis of the series in terms of substituent constants. Since 3-substituted tyrosines other than 3-alkyltyrosines have been shown to be competitive inhibitors of tyrosine,[3] the more convenient method of Dixon[22] (Figure 2) was used to obtain inhibition constants for these analogs. These values are presented in Table I.

Tyramine Pressor Test.—The tyramine pressor response in rats (260–280 g female, Sprague Dawley) was determined 2 and 24 hr after the ip injection of α-methyl-*m*-tyrosine (α-MMT) and IpT (2.1 mmoles/kg) in physiological saline (2.15 mmoles/ml); control animals received saline only. Each animal was injected with atropine sulfate (0.5 mg) 15 min prior to the assay to block compensatory cholinergic reflexes.

The rats were anesthetized with Et_2O, a laparatomy was performed, and an iv catheter was inserted into a large mesenteric vein for injections. A cannula (PE-90 tubing), inserted into the abdominal aorta, was connected to a Statham pressure transducer (No P23AC), and the blood pressure was recorded on a Model 5D Grass polygraph. Transducer and tubing were filled with physiological saline containing 2 units/ml of heparin. The transducer was calibrated against an anaeroid manometer and the polygraph chart speed set at 1 mm/sec.

After the blood pressure had equilibrated, and a resting value had been recorded, a series of responses to 300 μg/kg of tyramine in 0.5 ml of saline, to several 0.2-ml doses of NE (1:25,000 in physiological saline), and finally to 300 μg/kg of tyramine were recorded.

Each injection was followed immediately by a 0.2-ml saline flush of the cannula. Pressor response was calcd as per cent change in systolic blood pressure from preinjection value to peak of response. A typical response to tyramine before and after norepinephrine is reproduced in Figure 3 and the complete results of this study are shown in Table II.

Biogenic Amine Analysis.—Biogenic amine analyses were performed 3 hr after the ip injection of MT or IpT (1 mmole/kg) and the results compared with those of saline, α-MPT, and α-MMT (1 mmole/kg) treated groups. Brain and heart from decapitated (guillotine) male Wistar rats (200 g) were placed on Dry Ice immediately after excision and stored at −15° until assayed. At that time the tissues were homogenized in acidified *n*-BuOH, serotonin was measured fluorometrically as the *o*-phthaldialdehyde condensation product according to a modification of the method of Maickel.[23] Dopamine and NE were assayed by measuring fluorescence after I_2 oxidn by modification of the method of Chang.[24] Table III lists these results.

(22) J. L. Webb, "Enzyme and Metabolic Inhibitors," Vol. I, Academic Press, New York, N. Y., 1963, p 153.

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Synthesis and Biological Activity of Some 5-(1-Adamantyl)pyrimidines. 2[1,2]

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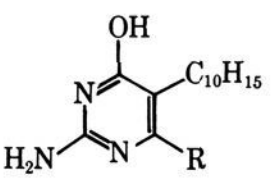
Received September 4, 1970

The synthesis and biological activity of eleven new pyrimidines with lipophilic substituents at the 5 position are described. Two compounds, 5-(1-adamantyl)-2,4-diaminopyrimidine (**3**) and 5-(1-adamantyl)-2,4-diamino-6-methylpyrimidine (**4**), show exceptionally high activity toward mouse mammary adenocarcinoma cells (TA3) in culture; the latter inhibits cell growth as effectively as methotrexate. An attempt is made to correlate the structure of the compounds with their activity.

A previous publication from this laboratory[3] described the synthesis and biological activity of some 5-(1-adamantyl)-2-amino-4-hydroxypyrimidines. 5-(1-Adamantyl)-2-amino-4-hydroxypyrimidine (**1a**) and the 6-Me analog **1b** were found to be moderate growth inhibitors of mouse sarcoma 180 cells in culture. In addition, **1b** was a good growth inhibitor of the cultured mouse mamary adenocarcinoma cells (TA3). Neither compound inhibited folate reductase isolated from a strain of sarcoma 180 cells grown *in vitro*, indicating that the mode of action of these compounds may not be related to the inhibition of this enzyme. In continuation of this work, seven 2,4-diaminopyrimidines (**3–9**), three 4-hydroxy-2-mercaptopyrimidines (**10–12**), and one 2-amino-4-hydroxypyrimidine (**13**) were prepared, having highly lipophilic substituents in position 5 of the pyrimidine ring.



1a, R = H
1b, R = CH_3

Synthesis.—Four basic synthetic approaches were used. The diaminopyrimidines **3** and **4** (Table I) were prepared from the corresponding 2-amino-4-hydroxy compounds[3] by replacement of OH by Cl followed by

(1) Presented in part before the Division of Medicinal Chemistry, 160th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1970.

(2) The synthetic work was supported in part by Grant CA-02906 and biological testing by CA-11047 from the National Cancer Institute of the U. S. Public Health Service.

(3) J. P. Jonak, S. F. Zakrzewski, L. H. Mead, and M. T. Hakala, *J. Med. Chem.*, **13**, 1170 (1970).