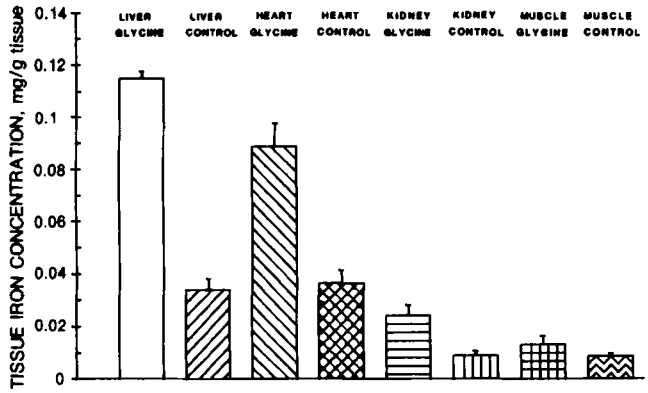

Figure 2—*Iron concentration (±SD) in body tissues 4 h after oral administration of four tablets containing 192.92 mg of ferrous chloride (control) and four tablets containing 197.12 mg of ferrous chloride and 158.12 mg of glycine.*

assumption that the area under the iron concentration–time curve (0–4 h) is an indicator of absorption.

The amount of iron in the different tissues was also consistent with iron levels in the blood. Iron concentration was highest in the tissues of the rabbits that received the glycine–iron tablets (Fig. 2). Iron–glycine tablets generated iron concentrations in tissue that were statistically significantly different from those of control iron tablets ($p < 0.001$ for the tissues of the liver, heart, and kidney; $p < 0.05$ for muscle).

All data were consistent with those from a previous report (7) from our laboratory indicating that glycine increases iron absorption into both blood and tissues. Neutron activation analysis is known to be very useful in analytical problems in which high sensitivity is required (sensitivity can reach 10^{-9} g). It is safe, economical, fast, practical in elemental analysis, and nondestructive (15). Also, thermal neutrons do not activate isotopes with atomic numbers of less than 10; therefore, carbon, hydrogen, oxygen, and nitrogen are not activated.

The data show that neutron activation analysis of final tableted dosage forms of iron can be utilized to investigate the effects of variable formulation factors on the absorption of iron. Further work is needed in the area utilizing multiple dosing, mixed protein hydrolysate, and pure defined amino acid mixtures to optimize tablet formulations for iron absorption. The technique of neutron-activation analysis can be employed for other minerals such as zinc or magnesium and may be used for comparing the rate and extent of absorption of minerals from commercially available vitamin-mineral mixtures (tablets or capsules). This application is currently under investigation in our laboratories.

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Potential Inhibitors of Tyrosine Hydroxylase and Dopamine-β-Hydroxylase

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Received June 28, 1983, from *Howard University, College of Pharmacy and Pharmacal Sciences, Department of Medicinal Chemistry, Washington, DC 20059.* Accepted for publication September 26, 1983.

Abstract □ A series of methyl-substituted 1,2,3,4-tetrahydrocarbazoles was synthesized and screened for *in vitro* activity against tyrosine hydroxylase and dopamine-β-hydroxylase. The most potent compounds were evaluated for inhibition of norepinephrine biosynthesis in rats. The results indicated no significant decrease in norepinephrine levels at three dosage levels.

Keyphrases □ Tyrosine hydroxylase—*in vitro* activity, effect of methyl-substituted 1,2,3,4-tetrahydrocarbazoles □ Dopamine-β-hydroxylase—*in vitro* activity, effect of methyl-substituted 1,2,3,4-tetrahydrocarbazoles □ Methyl-substituted 1,2,3,4-tetrahydrocarbazoles—effect on tyrosine hydroxylase and dopamine-β-hydroxylase, *in vitro*

It has been shown that the 1,2,3,4-tetrahydrocarbazole nucleus is an active pharmacophore. Previous studies have shown that the various carbazoles exhibit anti-inflammatory (1–6), antidepressant (7–12), hypoglycemic (13), analgetic (14), cardiotonic (15), anti-infective (16), and anticancer activities (17). It was of interest to extend the initial work of

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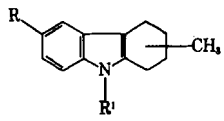
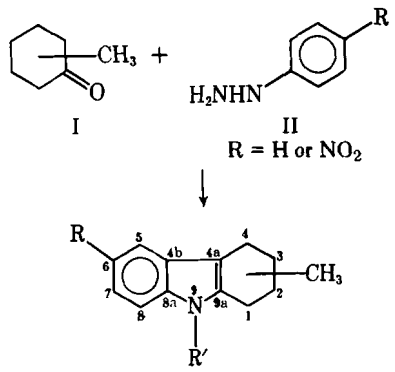
Table I—Physical Constants of Methyl-Substituted 1,2,3,4-Tetrahydrocarbazoles



Compound	Formula[a]	CH_3 Position	R	R^1	Melting Point, °C	Boiling Point, °C (mm Hg)
VIII	$C_{13}H_{15}N$	3	H	H	111.0–111.5[b,c]	130–134 (0.8)
IX	$C_{18}H_{26}N_2$	3	H	$(CH_2)_3N(CH_3)_2$	—	154–155[d] (0.35)
X	$C_{20}H_{28}N_2$	3	H	$(CH_2)_2N(CH_2)_5$[e]	70–71[c]	167–174[f] (0.95)
XI	$C_{16}H_{18}N_2$	3	H	$(CH_2)_2CN$	99–100[g]	188–191 (0.7)
XII	$C_{16}H_{19}NO_2$	3	H	$(CH_2)_2COOH$	104–105[h]	195–197 (0.55)
XIII	$C_{14}H_{17}N$	3	H	CH_3	74–75[c]	—
XIV	$C_{18}H_{25}N_3O_2$	3	NO_2	$(CH_2)_3N(CH_3)_2$	102–103[c,i]	—
XVI	$C_{20}H_{28}ClN_3O_2$	3	NO_2	$(CH_2)_2N(CH_2)_5$[e,j]	253.5–254.0[g]	—
XVII	$C_{16}H_{17}N_3O_2$	3	NO_2	$(CH_2)_2CN$	208.0–209.5[g]	—
XVIII	$C_{16}H_{18}NO_4$	3	NO_2	$(CH_2)_2COOH$	216–217[g]	—
XIX	$C_{18}H_{26}N_2$	2	H	$(CH_2)_3N(CH_3)_2$	—	114–116[k] (1.0)
XX	$C_{20}H_{28}N_2$	2	H	$(CH_2)_2N(CH_2)_5$[e]	—	180–184[l] (0.45)
XXI	$C_{16}H_{18}N_2$	2	H	$(CH_2)_2CN$	96–97[c]	184–185 (0.65)
XXII	$C_{16}H_{19}NO_2$	2	H	$(CH_2)_2COOH$	113–114[g]	190–195 (0.6)
XXIII	$C_{18}H_{26}ClN_3O_2$	2	NO_2	$(CH_2)_3N(CH_3)_2$	219–220[g,j]	—
XXIV	$C_{18}H_{26}N_2$	1	H	$(CH_2)_3N(CH_3)_2$	50–51[g,m]	154–159 (0.4)
XXV	$C_{20}H_{28}N_2$	1	H	$(CH_2)_2N(CH_2)_5$[e]	—	184–185[n] (0.30)
XXVI	$C_{16}H_{18}N_2$	1	H	$(CH_2)_2CN$	68[c] (softens); 83–84	104–105 (0.7)
XXVII	$C_{16}H_{19}NO_2$	1	H	$(CH_2)_2COOH$	133–134[c]	—

[a] Compounds IX–XXVII were analyzed for C, H, and N; in addition XVI and XXIII were analyzed for Cl. All values were within ±0.4% of the theoretical value. [b] Lit. (20) mp 108–111°C. [c] Methanol. [d] Maleate, mp 120–121°C. [e] Piperidinoethyl. [f] Hydrochloride, mp 208–209°C. [g] Ethanol. [h] Benzene–methanol. [i] Hydrochloride, mp 226–227°C. [j] Hydrochloride. [k] Hydrochloride, mp 189.5–190.0°C. [l] Maleate, mp 210.5–211.0°C. [m] Maleate, mp 104–105°C. [n] Maleate, mp 111–112°C.

Mooradian *et al.* (15) and to systematically evaluate the following structural modifications of the 1,2,3,4-tetrahydrocarbazole nucleus: the effect of substitution on the aromatic ring; the effect of removal of the proton on the indole nitrogen; and the effect of the addition of an alkyl group to the tetrahydro ring. Representative analogues were evaluated in two *in vitro* systems, tyrosine hydroxylase (18) and dopamine-β-hydroxylase (19) for potential use as antihypertensive agents. The reaction sequence for the synthesis of the methyl-substituted 1,2,3,4-tetrahydrocarbazoles is shown in Scheme I.



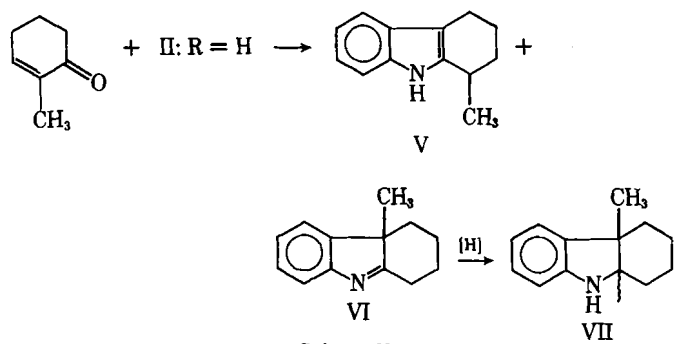
I; II (R = H or NO_2)

III: R′ = H
IV: R′ = CH_3, dialkylaminoalkyl, cycloaminoalkyl, $(CH_2)_2CN$, or $(CH_2)_2COOH$

Scheme I

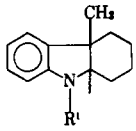
The methyl-substituted cyclohexanone (I) was treated with the appropriate phenylhydrazine (II) (20, 21) using acetic acid (R = H) or acetic acid and hydrochloric acid (R = NO_2) to give the methyl-1,2,3,4-tetrahydrocarbazoles (III). Treatment of III with the appropriate base, followed either by the addition of methyl iodide, a dialkylaminoalkyl chloride, a cycloaminoalkyl chloride, or acrylonitrile, produced the 9-methyl, 9-dialkylaminoalkyl, 9-cycloaminoalkyl, or 9-(3-cyanopropyl) derivative (IV), respectively. The latter compound was hydrolyzed to the propionic acid derivative with either acid or base. It was shown (22) that 2-methylcyclohexanone undergoes the reactions shown in Scheme II.

These products, 1-methyl-1,2,3,4-tetrahydrocarbazole (V) and 2,3,4,4a-tetrahydro-4a-methyl-1*H*-carbazole (VI), were



Scheme II

Table II—Physical Constants of 9-Substituted 2,3,4,4a,9,9a-Hexahydro-4a-methyl-1*H*-carbazoles



Compound	Formula[a]	R^1	Melting Point, °C	Boiling Point, °C (mm Hg)
XXVIII	$C_{13}H_{17}N$	H	52–53[b]	105–112 (0.6)
XXIX	$C_{18}H_{28}N_2$	$(CH_2)_3N(CH_3)_2$	—	146–154[b,c] (0.6)
XXX	$C_{20}H_{30}N_2$	$(CH_2)_2N(CH_2)_5$[d]	—	165–171[e,f] (0.65)
XXXI	$C_{16}H_{20}N_2$	$(CH_2)_2CN$	70–71[b]	178–180 (0.55)
XXXII	$C_{16}H_{21}NO_2$	$(CH_2)COOH$	91–92[e]	—

[a] Compounds XXVIII–XXXII were analyzed for C, H, and N. All values were within ±0.4% of the theoretical value. [b] Methanol. [c] Maleate, mp 140–141°C. [d] Piperidinoethyl. [e] Ethanol. [f] Maleate, mp 137–138°C.

of interest in view of varying reports (22–24) on the yields as well as investigating a new and related series, 2,3,4,4a,9,9a-hexahydro-4a-methyl-1*H*-carbazole (VII), which occurs on reduction of VI.

Selected compounds from each group were tested for *in vitro* activity against tyrosine hydroxylase and dopamine-β-hydroxylase. The most active compound, X, was tested at three dosage levels for inhibition of norepinephrine biosynthesis in rats.

EXPERIMENTAL SECTION

Instruments—Melting points were obtained using a melting point apparatus[1] and are uncorrected. Observed boiling points were also uncorrected. IR spectra were obtained on a spectrophotometer[2] as KBr pellets or neat. NMR spectra were obtained on a 60-MHz spectrometer[3] with tetramethylsilane as the internal reference. Elemental analyses were also performed[4]. Tritium was assayed on a liquid scintillation counter[5].

Reagents—All ketones[6] and phenylhydrazine[7] were distilled before use. *p*-Nitrophenylhydrazine[8] was recrystallized. L-[3,5-^{3}H]Tyrosine, L-[^{3}H]-epinephrine, [^{3}H]tyramine, *S*-[C^{3}H$_3$]adenosyl-L-methionine, and scintillation fluids[9] were used without further purification. 1,4-Dithiothreitol[10], dopamine, norepinephrine, epinephrine, and *S*-adenosyl-L-methionine[11] were used without further purification. Tyrosine hydroxylase was prepared (25), purified (26), and assayed (27); the protein content was also determined (28). Dopamine-β-hydroxylase (EC 1.14.17.1)[12] was assayed (29); phenylethanolamine-*N*-methyltransferase (EC 2.1.1.28)[12] was also assayed (30).

General Procedure for the Preparation of Methyl-Substituted 1,2,3,4-Tetrahydrocarbazoles (III, R = NO$_2$)—The previous method (21) was modified as follows. One-half mole of 4-methylcyclohexanone and *p*-nitrophenylhydrazine were dissolved in 180 g of glacial acetic acid. The mixture was heated at reflux for 4 h, chilled to 5°C, and filtered. The dried hydrazone was dissolved in 1 L of hydrochloric acid and was heated at reflux for 1 h. The product separated as yellow needles (78% yield), mp 166.5–167.5°C [lit. (31) 165–166°C].

General Procedure for Alkylation of Methyl-Substituted 1,2,3,4-Tetrahydrocarbazoles—*Method A* (*IV*, *R′* = *CH$_3$*)—The previous method (17) was employed using 0.05 mol of 3-methyl-1,2,3,4-tetrahydrocarbazole (VIII) and 0.05 mol of methyl iodide with 3 g of sodium hydride (51%, freed from mineral oil) in 25 mL of dimethylformamide. Chromatography on silica gel afforded 9.2 g (92% yield) of 3,9-dimethyl-1,2,3,4-tetrahydrocarbazole (XIII) as white crystals, mp 74–75°C; ^{1}H-NMR (CDCl$_3$): δ 1.90 (3, d, *J* = 4 Hz, CH$_3$), 3.10 (6, m, CH$_2$), 3.40 (1, m, CH), 3.80 (3, s, CH$_3$—N), and 7.30 ppm (4, m, ArH).

Anal.—Calc. for $C_{14}H_{17}N$: C, 84.23; H, 8.61; N, 7.06. Found: C, 84.46; H, 8.63; N, 7.01.

Table III—1,2,3,4-Tetrahydrocarbazoles and 2,3,4,4a,9,9a-Hexahydrocarbazoles as Inhibitors of Tyrosine Hydroxylase[a]

Compound	IC_{50}, M[b]	±*SEM*, × 10^{-4}	*n*
1,2,3,4-Tetrahydrocarbazole[c]	>4 × 10^{-4}	0.3	3
6-Nitro-1,2,3,4-tetrahydrocarbazole[d]	>4 × 10^{-4}	0.4	3
VIII	>4 × 10^{-4}	0.6	3
IX[e]	>4 × 10^{-4}	0.6	3
X[f]	4 × 10^{-4}	0.3	4
XI	>4 × 10^{-4}	0.3	3
XII	4 × 10^{-4}	0.4	4
XIV[f]	2 × 10^{-4}	0.2	6
XVI[f]	2 × 10^{-4}	0.3	6
XVII	4 × 10^{-4}	0.3	4
XVIII	>4 × 10^{-4}	0.6	3
XIX[f]	>4 × 10^{-4}	0.8	3
XX[e]	>4 × 10^{-4}	0.7	4
XXI	4 × 10^{-4}	0.4	4
XXII	>4 × 10^{-4}	0.6	3
XXIII[f]	4 × 10^{-4}	0.6	4
XXIV[e]	>4 × 10^{-4}	0.8	3
XXV[e]	>4 × 10^{-4}	0.9	3
XXVI	>4 × 10^{-4}	0.7	3
XXVII	>4 × 10^{-4}	0.8	3
XXX[e]	>4 × 10^{-4}	0.4	3
DL-α-Methyltyrosine[g]	2 × 10^{-4}	0.2	6

[a] Each compound was preincubated with the reaction mixture for 5 min before addition of L-tyrosine (5 × 10^{-5} M) as substrate. [b] Data represent a range of 45–55% inhibition; where >4 × 10^{-4} M concentrations are indicated, inhibition was <20% with 4 × 10^{-4} M inhibitor. [c] Data from Ref. 20. [d] Data from Ref. 21. [e] Maleate. [f] Hydrochloride. [g] Data from Ref. 18 gives 2.5 × 10^{-5} M.

Table IV—1,2,3,4-Tetrahydrocarbazoles as Inhibitors of Dopamine-β-Hydroxylase

Compound[a]	*In Vitro* Inhibition, % of Control[b]	±*SEM*	*n*
X[c]	44	1.6	4
XII	72	7.4	4
XIV	68	6.8	6
XVI	55	6.3	4
XVII	76	3.6	4
XXI	59	3.8	6
XXIII	94	1.8	3
O-Benzylhydroxylamine[c]	33	2.2	4

[a] For the salt form, see Table III. [b] Each compound (5 × 10^{-4} M) was preincubated for 15 min before addition of tyramine (5 × 10^{-4} M) as substrate. [c] Significantly different from control, $p < 0.05$.

[1] Thomas-Hoover.
[2] Beckman IR-18A.
[3] Varian EM360A.
[4] Performed by the Schwarzkopf Microanalytical Laboratory, Woodside, N.Y.
[5] Beckman LS-230.
[6] Eastman Chemicals.
[7] Fisher.
[8] Aldrich Chemical Co.
[9] Liquifluor and Econofluor; New England Nuclear.
[10] Schwarz/Mann.
[11] Calbiochem-Behring.
[12] Sigma Chemical Co.

Table V—*In Vivo* Assay Results

Assay		Norepinephrine Level: 1 mg/kg of X	10 mg/kg of X	50 mg/kg of X	α-Methyltyrosine (400 mg/kg)	Saline
I[a]	ng/g of heart tissue	588.6	587.3	594.3	472.5	602.2
	±*SEM*	47.5	75.2	70.8	25.1	97.8
	Decrease, %	2.3	2.5	1.3	21.5	—
II[b]	ng/g of heart tissue	182.0	169.0	163.0	156.0	193.0
	±*SEM*	14.0	13.0	13.0	17.0	24.0
	Decrease, %	5.7	12.4	15.6	19.2	—
III[a]	ng/g of heart tissue	519.9	425.0	487.5	351.6	468.8
	±*SEM*	46.7	28.0	29.7	30.6	29.4
	Decrease, %	—	9.3	—	25.0	—

[a] Data from Ref. 35. [b] Data from Ref. 36.

Method B [*IV*, $R' = (CH_2)_3N(CH_3)_2$]—The previous method (17) was employed using 0.05 mol of 3-methyl-1,2,3,4-tetrahydrocarbazole (VIII) and 0.05 mol of 3-dimethylaminopropyl chloride (17) in dimethylformamide with 3 g of the aforementioned sodium hydride to give 16.7 g (75% yield) of IX, bp 154–155°C (0.35 mm Hg), as a light-yellow oil. ^{1}H-NMR (acetone-d_6): δ 1.82 (3, d, J = 4 Hz, CH_3), 2.05–2.55 (m, CH_2), 2.25 (3, s, CH_3—N), 3.20 (6, m, CH_2), 3.45 (1, m, CH), and 7.22 ppm (4, m, ArH).

Anal.—Calc. for $C_{18}H_{26}N_2$: C, 79.88, H, 9.70, N, 10.41. Found: C, 80.00; H, 9.87; N, 10.46.

Method C [*IV*, $R' = (CH_2)_2CN$]—The previous method (17) was employed using 0.05 mol of 3-methyl-1,2,3,4-tetrahydrocarbazole (VIII) and 0.10 mol of acrylonitrile in benzene containing 1.5 mL of trimethylbenzylammonium hydroxide to give 9.59 g (80% yield) of XI as a white amorphous powder, bp 188–191°C (0.7 mm Hg), mp 99–100°C. IR (KBr): 2250 cm^{-1} (s, C≡N); ^{1}H-NMR (acetone-d_6): δ 1.80 (3, d, J = 4 Hz, CH_3), 2.40 (1, d, J = 17 Hz, CHCN), 2.80 (1, d, J = 17 Hz, CHCN), 2.90 (2, m, —CH_2N), 3.05 (6, m, CH_2), 3.50 (1, m, CH), and 7.20 ppm (4, m, ArH).

Anal.—Calc. for $C_{16}H_{18}N_2$: C, 80.62; H, 7.63; N, 11.76. Found: C, 80.59; H, 7.77; N, 11.55.

3-Methyl-1,2,3,4-tetrahydrocarbazole-9-propionic acid (XII)—Compound XI (0.03 mol) was dissolved in 400 mL of 90% ethanol containing 0.50 mol of potassium hydroxide. The mixture was refluxed for 18 h and then the solvent was evaporated under reduced pressure. The residue was dissolved in water, decolorized, and acidified with hydrochloric acid. The product was extracted with ether, and the organic phase was washed with water and dried (Na_2SO_4). Evaporation of the solvent and distillation of the residue afforded XII, as white crystals, bp 195–197°C (0.55 mm Hg), mp 104–105°C. IR (KBr): 1740 cm^{-1} (s, C=O); ^{1}H-NMR (acetone-d_6): δ 1.75 (3, d, J = 4 Hz, CH_3), 3.00 (6, m, CH_2), 3.45 (1, m, CH), 4.45 (3, t, J = 7 Hz, $NCHCH_2C$), 6.95–7.50 (4, m, ArH), and 10.45 ppm (1, s, COOH).

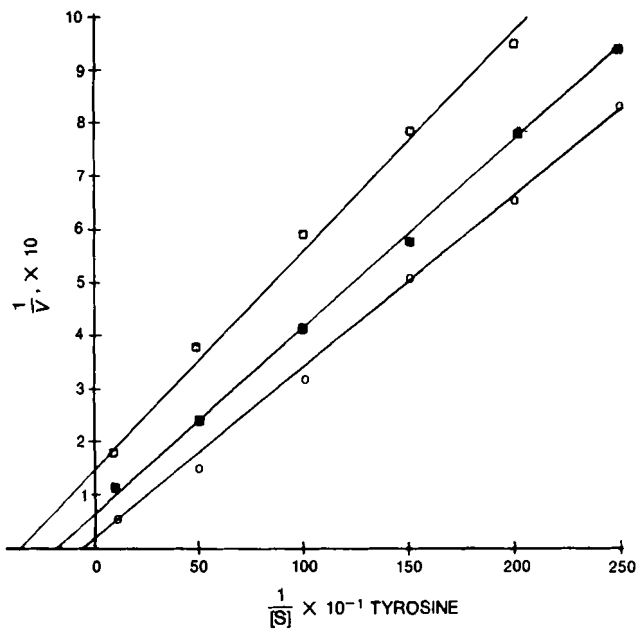


Figure 1—*Effect of X (■) and XIV (□) on the hydroxylation of L-tyrosine. Concentrations of L-tyrosine (substrate) varied from 1 × 10^{-4} to 4 × 10^{-6} M; (○) control.*

Anal.—Calc. for $C_{16}H_{19}NO_2$: C, 74.66; H, 7.46; N, 5.44. Found: C, 74.83; H, 7.62; N, 5.16.

In all alkylation methods when R = NO_2, additional solvent was required.

1-Methyl-1,2,3,4-tetrahydrocarbazole (V)—The previous method for the preparation of III (R = NO_2) was employed. Cyclization was accomplished using dilute sulfuric acid (32). The product was isolated and distilled, bp 121–129°C (0.4 mm Hg), mp 67–68°C as tan crystals [lit. (22) bp 142–146°C (0.3 mm Hg), mp 69°C].

2,3,4,4a-Tetrahydro-4a-methyl-1*H*-carbazole (VI)—The aforementioned hydrazone was cyclized using glacial acetic acid (32) to give a liquid, bp 98–106°C (0.9 mm Hg), picrate 170–171°C as yellow crystals [lit. (33) bp 98–100°C (0.3 mm Hg), picrate 169–171°C]. ^{1}H-NMR ($CDCl_3$): δ 1.26 ppm (3, s, CH_3).

2,3,4,4a,9,9a-Hexahydro-4a-methyl-1*H*-carbazole (VII)—Catalytic hydrogenation of V (0.1 mol) employing platinum oxide in a mixture of hydrochloric acid and alcohol (34) for 8 h gave VII in 82% yield, bp 105–112°C (0.6 mm Hg), mp 52–53°C as white crystals. IR (KBr): 3425 cm^{-1} (s, NH); ^{1}H-NMR ($CDCl_3$): δ 10.2 ppm (1, s, NH).

Anal.—Calc. for $C_{13}H_{17}N$: C, 83.35; H, 9.17; N, 7.48. Found: C, 83.66; H, 9.08; N, 7.48.

Analytical data for the compounds prepared by the above methods are shown in Tables I and II.

Enzyme Inhibition—*Tyrosine Hydroxylase*—Tyrosine hydroxylase inhibitors were screened as previously indicated (27) with the substrate concentration varied from 1 × 10^{-5} to 5 × 10^{-5} M and each potential inhibitor tested at concentrations of 2 × 10^{-4} M and 4 × 10^{-4} M. DL-α-Methyltyrosine[8] was used as a standard inhibitor for this phase. The results are listed in Table III.

Dopamine-β-Hydroxylase—Dopamine-β-hydroxylase inhibitors were screened as previously reported (29). The substrate concentration was 5 × 10^{-4} M. *O*-Benzylhydroxylamine[8] was used as a standard inhibitor in this study. The data are listed in Table IV.

***In Vivo* Determinations**—Thirty male Sprague-Dawley rats, weighing ~200 g, were divided into five groups of six rats each. Each group received an injection of either 1 mg/kg of X, 10 mg/kg of X, or 400 mg/kg of α-methyltyrosine or saline. The intraperitoneal doses were in a volume of 1 mL. After 4 h, the initial dose was repeated and, after an additional 4 h, the animals were sacrificed. The brains, hearts, and blood were taken for analysis (35, 36). Data for each assay are shown in Table V. The experiment was repeated (assay III) with the exceptions that the second dose was repeated after 3 h, the animals were sacrificed 3 h after the last dose, and only the hearts were removed and assayed (35).

RESULTS AND DISCUSSION

From the tyrosine hydroxylase data (Table III), it appears that the active compounds are those that possess a substituent at the 9-position. In the 9-[3-(dimethylamino)propyl] series, the 6-nitro substitution is essential for activity, as IX and XIX were inactive while both XIV and XXIII were active. In the 9-[2-(1-piperidino)ethyl] series, both X (the unsubstituted carbazole) and XVI (the 6-nitro analogue) were active. In the 9-(3-cyanopropyl) series, the initial conclusion reached with the previous analogues held for XI and XVII, but failed with XXI and XXVI due to the extreme insolubility of the latter. In the 9-(3-propionic acid) series, the 6-nitro derivative (XVIII) was inactive, while XII (the unsubstituted analogue) was active. As in the case of the cyanopropyl series, the extreme insolubility of XVIII was a prime factor in the correlation. The hexahydro analogue of X (XXX) was inactive. Inhibition of tyrosine hydroxylase by the tetrahydrocarbazoles was noncompetitive

with tyrosine, as seen in Fig. 1 for X and XIV. Whether this effect is exerted on either tetrahydrofolate or tetrahydropteridine (18) must await further study. All active compounds were assayed for inhibition of dopamine-β-hydroxylase (Table IV). From the data, X was shown to be the most active overall in both systems and was selected for the *in vivo* assay.

In vivo results of X (Table V) are explained on the basis of the assays performed. Assays I and III (35) are paired and show no inhibition. Assay I was repeated due to a high norepinephrine level after α-methyltyrosine treatment. Assay II results indicate significant inhibition; however, the correction for the recovery of norepinephrine from alumina is not included in assay II, while I and III allow for this error[13]. Thus, while X inhibited both tyrosine hydroxylase and dopamine-β-hydroxylase *in vitro*, it was not active *in vivo*. Whether X is metabolized too rapidly and fails to attain the required tissue concentration is not known.

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Direct Preparation of Spherically Agglomerated Salicylic Acid Crystals During Crystallization

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Abstract □ Needle-like salicylic acid crystals were transformed into a spherically shaped dense form during crystallization by the spherical crystallization technique. Agitation of a mixture of ethanol–water–chloroform containing salicylic acid yielded spherically agglomerated salicylic acid crystals. The crystallinity of the agglomerated salicylic acid decreased when the amount of ethanol in the solvent mixture was decreased. The wettability of the agglomerated crystals increased when the amount of ethanol in the solvent mixture was decreased, and this enhanced the dissolution rate of the crystals. The remarkable improvements in the flow and packing of the agglomerated crystals enabled the direct compression of the crystals.

Keyphrases □ Salicylic acid—crystallization of spherically agglomerated crystals □ Crystallization—agglomeration of salicylic acid crystals

A novel agglomeration technique to transform a microcrystalline drug into an agglomerated form during the crystallization process was previously described (1). This technique could enable subsequent processes, such as separation, filtration, drying, etc. to be carried out more efficiently. Furthermore, the resultant agglomerated crystals could be easily

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