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Cholinergic Effects of Molecular Segments of Apomorphine and Dopaminergic Effects of N,N-Dialkylated Dopamines†

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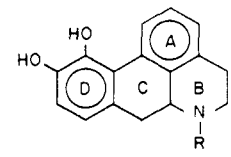
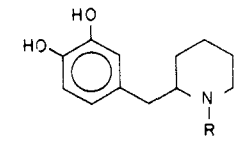
The hydrochlorides of molecular segments of apomorphine [2-(3′,4′-dihydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline, 2-(3′,4′-dihydroxybenzyl)piperidine, and 1,2,3,4-tetrahydroisoquinoline with their respective *N*-methyl and *N*-*n*-propyl homologs] and N,N-dialkylated dopamine compounds were synthesized and studied for (1) LD_{50} in intact mice; (2) stereotypy in intact mice; (3) curving of the body in unilaterally caudectomized mice; (4) rotation in 6-hydroxydopamine-lesioned rats, and (5) activation of adenylate cyclase in homogenates of mouse caudate nuclei. Instead of dopaminergic effects 1-(3′,4′-dihydroxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline and 2-methyl-1,2,3,4-tetrahydroisoquinoline showed cholinergic ones. These effects were blocked in atropine-pretreated animals. Of the N,N-dialkylated dopamine compounds synthesized, the *N*-*n*-propyl-*N*-*n*-butyldopamine ranked in all tests as the strongest dopamine-receptor agonist and *N*-methyl-*N*-*n*-propyldopamine as the weakest. In contrast, *N*,*N*-dimethyldopamine and 1-(3,4-dihydroxyphenylethyl)piperidine showed no dopaminergic effects. The effectiveness of the dopaminergic agonists depended on the length of the *N*-alkyl substituents suggesting interactions with hydrophobic regions of the receptor site.

An attempt to develop drugs effective against Parkinson's disease but having side effects different from those of the dopamine (DA) precursor *l*-Dopa[2a] led to studies of apomorphine **1b** because of some structural similarities between **1b** and DA.[2b] The dopaminergic effects of **1b** seem to be explained by these similarities, but some opposing effects encountered during these studies have been only tentatively explored.[3] The potentiation of the therapeutic effects of oral *l*-Dopa by injected **1b** is compatible with a dopaminergic function, but the diminution of some *l*-Dopa side effects suggests antidopaminergic or even cholinergic properties in **1b**. To clarify these effects we have synthesized molecular segments of **1b** and determined their LD_{50} in mice, and we have studied their effects on unilaterally caudectomized mice,[4] on nigral-lesioned rats,[5] and, when indicated, on the dopamine-activated adenylate cyclase activity of homogenized mouse caudate nuclei.[6,7]

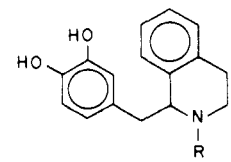
In the present study, elimination of rings A and C from **1b** with retention of the catechol (ring D) and the piperidine (ring B) resulted in loss of recognizable dopaminergic or cholinergic effects (**17a–c**). When the bond joining ring D to ring A was eliminated (**21a–c**), dopaminergic effects disappeared and cholinergic ones appeared so that it was necessary to dissociate the tetrahydroisoquinoline from the piperidine moieties. After synthesizing a series of 1,2,3,4-tetrahydroisoquinolines (**19a–c**) and piperidines we found that some of the former had central cholinergic activities. To preserve dopaminergic while eliminating cholinergic activity we synthesized and investigated *N*,*N*-dialkyl-substituted dopamines (DA) (**9**, **22–25**). When we found that three relatively nontoxic alkyl-substituted DA had dopaminergic activity in lesioned mice and rats, we deemed them worthy of testing on DA-activated adenylate cyclase in homogenized mouse caudate nuclei[6,7] to confirm this activity.



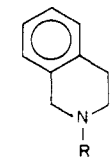
1a,b,c



17a,b,c

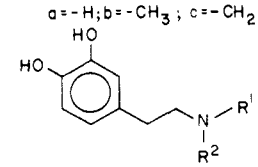


21a,b,c



19a,b,c

a = -H; b = $-CH_3$; c = $-CH_2CH_2CH_3$



22, $R^1 = R^2 = -CH_3$

9, $R^1 = -CH_3$; $R^2 = -CH_2CH_2CH_3$

23, $R^1 = -CH_3$; $R^2 = -CH_2CH_2CH_2CH_3$

24, $R^1 = -CH_2CH_2CH_3$; $R^2 = -CH_2CH_2CH_2CH_3$

25, $R^1, R^2 = -N$⟨⟩

Results and Discussion

Chemistry. 1-Methyl-2-(3′,4′-dihydroxybenzyl)piperidine hydrochloride (**17b**) was synthesized by two different routes as illustrated by Schemes I and II. In Scheme I, hydrogenolysis in step B removed both the *O*-benzyl protective groups and the benzylic hydroxyl,[8] while in Scheme II

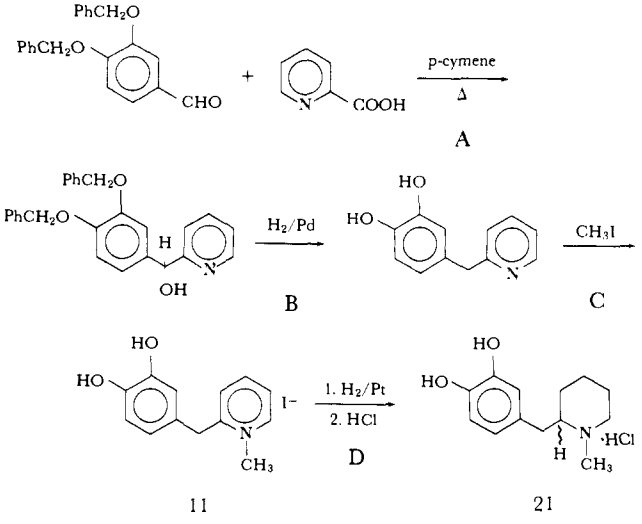
†This work was supported by the National Institutes of Health (Grant No. 11131) and the Energy Research and Development Administration.

Table I. Preparation and Properties of Alkyl Iodide Derivatives

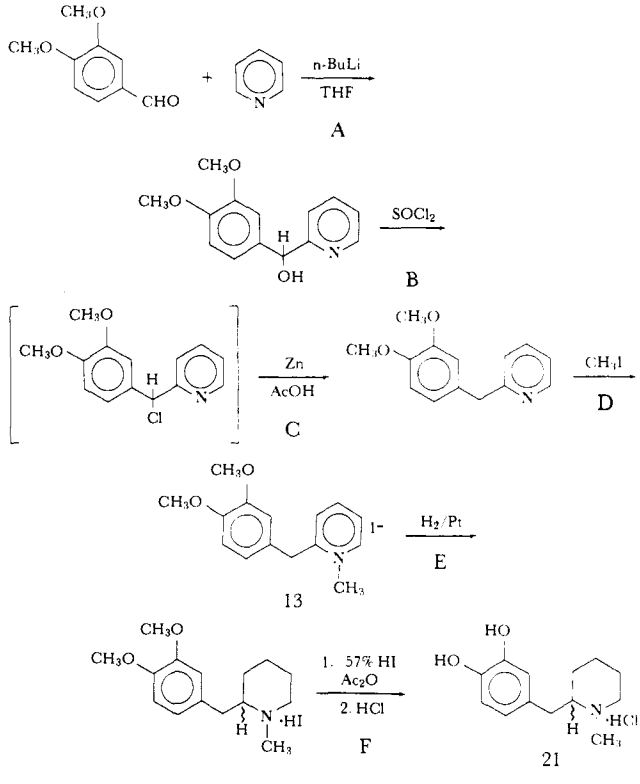
	Compound	Yield, %	Mp (lit.), °C	Recrystn solvent	Formula[a]
12b	1-Methyl-2-(3′,4′-dihydroxybenzyl)-pyridinium iodide	87	185–186	EtOH–EtOAc	$C_{13}H_{14}NO_2I$
12c	1-*n*-Propyl-2-(3′,4′-dihydroxybenzyl)-pyridinium iodide	78	149–150	EtOH–EtOAc	$C_{15}H_{18}INO_2$
13b	1-Methyl-2-(3′,4′-dimethoxybenzyl)-pyridinium iodide	84	166–168	EtOH–EOAc	$C_{15}H_{18}INO_2$
13c	1-Propyl-2-(3′,4′-dimethoxybenzyl)-pyridinium iodide	86	150–152	2-Propanol	$C_{17}H_{22}INO_2$
14b	1-(3′,4′-Dimethoxybenzyl)-2-methyl-isoquinolinium iodide	97	202–203 (139–140[b])	EtOH	$C_{19}H_{20}INO_2$
14c	1-(3′,4′-Dimethoxybenzyl)-2-*n*-propyl-isoquinolinium iodide	91	205–206	EtOH	$C_{21}H_{24}INO_2$
15b	2-Methylisoquinolinium iodide	98	161–162 (161–163[c])	EtOH–ether	
15c	2-*n*-Propylisoquinolinium iodide	98	124–125	EtOH–ether	$C_{12}H_{14}IN$
16c	1-*n*-Propylpyridinium iodide[d]	75	68–72		

[a] All were analyzed for C, H, I, and N and results were within 0.4% of theory. [b] J. L. Neumeyer, M. McCarthy, S. P. Battista, F. J. Rosenberg, and D. G. Teiger, *J. Med. Chem.*, 16, 1227 (1973). [c] v. E. Schlittler and J. Müller, *Helv. Chim. Acta*, 31, 914 (1948). [d] Could not be crystallized (hygroscopic).

Scheme I



Scheme II



the *O*-methyl protective groups were removed in the last step. The overall yield (12%) in Scheme II was twice that obtained by Scheme I (5–6%) (see Tables I–III).

The synthesis of the doubly O-methylated homologs of **21a–c** is described elsewhere.[9] The subsequent hydrogenation of the alkyl iodides and hydrochlorides of the isoquinoline and pyridine derivatives to their corresponding tetrahydroisoquinolines and piperidines gave yields in excess of 90% with two notable exceptions. The hydrogenation of the hydrochlorides of 1-(3′,4′-dimethoxybenzyl)isoquinoline and isoquinoline gave respectively yields of 45 and 66% of the corresponding 1,2,3,4-tetrahydroisoquinolines and 55 and 44% of the 5,6,7,8-tetrahydroisoquinolines. The effect of experimental conditions on the ratio of these two isomers is discussed elsehwere.[9] Compounds **21a–c** (Table IV) were found to be hygroscopic solids, noncrystallizable, with broad melting point ranges and were therefore identified by TLC, ^1H NMR, and elemental analysis after precipitation from ethanol with ether.

Compounds **9** and **22–25** (Table IV) were synthesized according to Scheme III.

Pharmacology. The LD_{50} (μg/g of body weight) was determined for each analog (Table V) so that well-defined, nonlethal dosages could be chosen for screening behavioral effects in animals. The hydrochlorides of **1b**, *N*-*n*-propylnorapomorphine (**1c**), DA, and *N*-methyl-DA were included in the toxicity studies as reference substances. An analysis of variance[10] of the LD_{50} values by computer showed that, except in the case of the piperidine homologs, the *N*-methyl tertiary amines were, in general, the most toxic. Among the piperidine homologs, this analysis showed *N*-*n*-propylpiperidine to be ten times as toxic as *N*-methylpiperidine.

Table II. Preparation and Properties of the Hydrochlorides of 1,2,3,4-Tetrahydroisoquinoline and Piperidine

	Compound	Yield, % (Scheme)	Mp (lit.), °C	Recrystn solvent	Formula[a]
17a	2-(3′,4′-Dihydroxybenzyl)piperidine	86 (I)	212–213	MeOH + EtOAc	$C_{12}H_{18}ClNO_2$
17b	1-Methyl-2-(3′,4′-dihydroxybenzyl)-piperidine	84 (I)	226–227	MeOH–EtOAc	$C_{13}H_{20}ClNO_2$
17c	1-*n*-Propyl-2-(3′,4′-dihydroxybenzyl)-piperidine	92 (I)	230–231	EtOH–EtOAc	$C_{15}H_{24}ClNO_2$
18a	1-(3′,4′-Dimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline	45	227–228	EtOH	$C_{18}H_{22}ClNO_2$
18b	1-(3′,4′-Dimethoxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline	91	233–235 (227–230[b])	EtOH	$C_{19}H_{24}ClNO_2$
18c	1-(3′,4′-Dimethoxybenzyl)-2-*n*-propyl-1,2,3,4-tetrahydroisoquinoline	77	203–204	MeOH–ether	$C_{21}H_{28}ClNO_2$
19a	1,2,3,4-Tetrahydroisoquinoline	66	197–198 (195–197[d])	EtOH	
19b	2-Methyl-1,2,3,4-tetrahydroisoquinoline	96	225–226	EtOH–EtOAc	$C_{10}H_{14}ClN$
19c	2-*n*-Propyl-1,2,3,4-tetrahydroisoquinoline	93	238.5–239.5	EtOH–ether	$C_{12}H_{18}ClN$
20c	1-Propylpiperidine	70	224–226 (225[c])	EtOH–ether	

[a]All were analyzed for C, H, Cl, and N and results were within 0.4% of theory. [b]J. L. Neumeyer, M. McCarthy, S. P. Battista, F. J. Rosenberg, and D. G. Teiger, *J. Med. Chem.*, 16, 1227 (1973). [c]H. W. Magnusson and E. R. Schierz, *Univ. Wyo. Publ.*, 7, 1 (1940). [d]L. Helfer, *Helv. Chim. Acta*, 6, 785 (1923).

Table III. Preparation and Properties of the Hydrochlorides of *N*,*N*-Dialkyl-β-(3,4-dimethoxyphenyl)ethylamine

	Compound	Yield, %	Mp (lit.), °C	Recrystn solvent	Formula[a]
11	*N*,*N*-Dimethyl-β-(3,4-dimethoxyphenyl)ethylamine	88	195–196 (197[b])	EtOH	$C_{12}H_{18}ClNO_2$
8	*N*-Methyl-*N*-*n*-propyl-β-(3,4-dimethoxyphenyl)ethylamine	87	142–143	CH_2Cl_2–EtOAc	$C_{14}H_{24}ClNO_2$
	N-Methyl-*N*-*n*-butyl-β-(3,4-dimethoxyphenyl)ethylamine	88	130.5–131	CH_3OH–Et_2O	$C_{15}H_{26}ClNO_2$
	N-*n*-Propyl-*N*-*n*-butyl-β-(3,4-dimethoxyphenyl)ethylamine	81	76–78 214–215	CH_3OH–Et_2O	$C_{17}H_{30}ClNO_2$
	1-(3,4-Dimethoxyphenylethyl)-piperidine	90	(149–152,[c] 210–211[d])	EtOH–EtOAc	$C_{15}H_{24}ClNO_2$

[a]All were analyzed for C, H, Cl, and N and results were within 0.4% of theory. [b]Reference 20. [c]Z. Arnold and K. Hejno, *Collect. Czech. Chem. Commun.*, **20**, 567 (1955). [d]L. Dúbravková, I. Ježo, P. Šefčovič, and Z. Votický, *Chem. Zvesti*, **9**, 541 (1955).

Two animal tests were used in assessing these drugs for dopaminergic or cholinergic properties. (1) Behavioral effects on caudectomized Sprague–Dawley and Swiss albino mice. These mice had the right caudate nucleus partially ablated[4] and, starting 3 weeks later, reacted to **1b** and other dopaminergic agents by curving the body toward the side with the lesion and to strong cholinergic agents (oxotremorine) by curving opposite to the side with the lesion.[22] (2) Behavioral effects on nigral-lesioned rats (Sprague–Dawley). These rats had 6-hydroxydopamine injected stereotactically in the right substantia nigra by the method of Ungerstedt et al.,[5] and, starting 4 weeks later, injection of **1b** (1 μg/g) caused them to rotate in the direction away from the lesion[11] whereas cholinergic agents caused them to rotate toward the lesion, and the number of turns was measured in rotometers[5] (Table V). Nigral-lesioned rats were far more sensitive than caudectomized mice.

Some of the analogs were screened for DA-stimulated adenylate cyclase activity in mouse caudate homogenates[6,7] (Table VI).

Except for N,N-alkylated DA (**9**, **23**, and **24**) none of the synthesized analogs (Table V) showed dopaminergic activity in either nigral-lesioned rats or caudectomized mice. In contrast, compound **21b** (50 μg/g ip) produced responses in the nigral-lesioned rats that were the opposite of those to dopaminergic agents but like those to oxotremorine[11] and were blocked by atropine pretreatment (0.5 μg/g).

Since others have demonstrated that *N*-ethyltetrahydroisoquinoline derivatives are cholinesterase inhibitors,[12] it was possible that the cholinergic activity of **21b** was contributed by the tetrahydroisoquinoline moiety. Indeed, when **19b** and the tetrahydroisoquinoline and piperidine derivatives (Table V) were tested, only **19b** produced in both caudectomized mice and nigral-lesioned rats responses like that to oxotremorine, which were blocked by atropine pretreatment (5 μg/g).

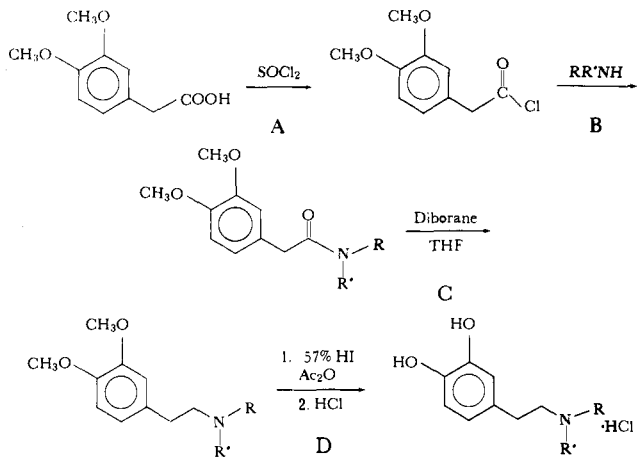
The above findings suggested that **1b** and perhaps its homolog **1c** and even *O*-methyl metabolites of the latter[13,14] have antidopaminergic or cholinergic properties, which might explain some of the antagonistic effects to *l*-Dopa.

Table IV. Preparation of the Hydrochlorides of Catechol Derivatives by Demethylation and Their Properties

	Compound	Yield, % (Scheme)	Mp (lit.), °C	Recrystn solvent	Formula[a]
17b	1-Methyl-2-(3′,4′-dihydroxybenzyl)-piperidine	86 (II)	228–229	MeOH–EtOAc	$C_{13}H_{20}ClNO_2$
21a	1-(3′,4′-Dihydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline	98	Broad range	EtOH–Et_2O[b]	$C_{16}H_{18}ClNO_2$
21b	1-(3′,4′-Dihydroxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline	96	Broad range	EtOH–Et_2O[b]	$C_{17}H_2OClNO_2$
21c	1-(3′,4′-Dihydroxybenzyl)-2-*n*-propyl-1,2,3,4-tetrahydroisoquinoline	94	Broad range	EtOH–Et_2O[b]	$C_{19}H_{27}ClNO_2$
22	*N,N*-Dimethyl-β-(3,4-dihydroxyphenyl)-ethylamine	88	123–125 (127)[c]	EtOH–Et_2O	$C_{10}H_{16}ClNO_2$
9	*N*-Methyl-*N*-*n*-propyl-β-(3,4-dihydroxyphenyl)ethylamine	89	137–138	EtOH–EtOAc	$C_{12}H_{20}ClNO_2$
23	*N*-Methyl-*N*-*n*-butyl-β-(3,4-dihydroxyphenyl)ethylamine	94	146–148	EtOH–EtOAc	$C_{13}H_{22}ClNO_2$
24	*N*-*n*-Propyl-*N*-*n*-butyl-β-(3,4-dihydroxyphenyl)ethylamine	87	136–138	EtOH–Et_2O	$C_{15}H_{26}ClNO_2$
25	1-(3,4-Dihydroxyphenylethyl)piperidine	70	241–242	MeOH–Et_2O	$C_{13}H_{20}ClNO_2$

[a] All were analyzed for C, H, Cl, and N and results were within 0.4%. [b] The compounds were hygroscopic and could not be recrystallized. They were precipitated from ethanol with ether. [c] Reference 20.

Scheme III



This led us to synthesize the *N,N*-dialkyl-DA analogs in which the tetrahydroisoquinoline segment (rings A and B) was eliminated. Of these, **9, 23,** and **24** showed dopaminergic properties in tests with rats and mice but to a far lower degree than **1b.**[11] In the more sensitive nigral-lesioned rats as little as 3 μg/g of **24** produced significant rotation to the left (234 ± 54 turns/30 min) but 5 μg/g of **9** or **23** did not produce rotation significantly greater than that of water-injected controls. For comparison, 1 μg/g of **1b** produced 535 ± 61 turns/30 min.

The greater effectiveness of **24** than of **9** and **23** as a DA-receptor agonist was paralleled by its degree of in vitro stimulation of adenylate cyclase (Table VI). The increase in cAMP production (in excess of nonstimulated production) due to **24** was equal to that due to an equimolar amount of DA, whereas the increases due to **9** and **23** were respectively 24 and 70% of that due to DA under identical conditions. *N*-Methyl-DA, a compound that showed no dopaminergic activity in mice (50–150 μg/g) and only a weak one in nigral-lesioned rats at lethal dosages (25 μg/g), stimulated cAMP as much as did DA under the same conditions. In contrast, **22** showed no dopaminergic effects in any of these tests.

Further studies are required to determine the dependence of dopaminergic effects on the length and the degree of branching of the *N*-alkyl chains. The importance of the stereochemical arrangement of the *N,N*-alkyl substituents with respect to interaction with the receptor site is suggested by the failure of **25,** which (unlike **24**) has its two *N*-alkyl substituents constrained into a piperidine ring, to have an effect in caudectomized mice and nigral-lesioned rats. Although steric effects would be expected to have increasing importance with lengthening of the *N*-alkyl chains, with adverse results, this was not borne out by our findings. Perhaps interaction with the hydrophobic regions of the receptor site[15] is the predominant factor in shaping the "biologically active" molecular conformation.

Our findings that some *N,N*-dialkyl-DA analogs have low toxicity as well as dopaminergic properties suggest that one or more of them may be of potential use in the study of parkinsonism and related diseases. These compounds would offer the following advantages over *l*-Dopa and the other dopaminergic agonists (**1b, 1c,** and bromocriptine[16]).

1. They are easy to synthesize in high yields and in a relatively short time from commercially available starting materials.

2. They have no optical enantiomers necessitating the separation of biologically active from biologically inactive components.

3. Unlike DA, they cross the blood-brain barrier because being tertiary amines they are resistant to deamination by monoamine oxidase (MAO).[17]

Experimental Section

Uncorrected melting points were determined on a Thomas-Hoover apparatus. ^1H NMR spectra were recorded on a T-60 Varian in $CDCl_3$ or Me_2SO-d_6 with $(Me)_4Si$ as the internal standard. Eastman chromagram sheets (6060 silica gel with fluorescent indicator) were used for TLC. The following TLC solvent systems were used: (A) C_6H_6–MeOH, 9:1; (B) 4:1; (C) 3:2; (D) 1-butanol-H_2O-AcOH, 4:1:1; (E) 1-butanol-H_2O-AcOH-pyridine, 15:12:3:10; (F) cyclohexane–EtOAc, 1:1; (G) 1:4; (H) 4:1; (I) CH_3Cl-MeOH-AcOH, 17:2:1; (J) $CHCl_3$–MeOH, 99:5; (K) *n*-PrOH-H_2O, 89:28. Infrared spectra were obtained on a Perkin-Elmer 337 grating spectrometer, and elemental analyses were done by Galbraith Lab., Inc., and Schwarzkopf Microanalytical Lab.

Materials. 3,4-Dimethoxybenzaldehyde, 3,4-dibenzyloxybenz-

Table V. Behavioral Effects on Caudectomized Mice and Nigral-Lesioned Rats and LD_{50} on Normal Mice[a]

Compound	Mice conjugate curvature[b] (eff dose, μg/g)	Nigral-lesioned rats[c] Dose, μg/g	Nigral-lesioned rats[c] Direction (turns ± SEM/min)	LD_{50} (95% confidence limits), μg/g	Manner of death
17a[d]	0			283 (271–295)	C
17b[d]	0		0	126 (120–132)	R and C
17c[d]	0			189 (110–324)	C
19a	0			303 (292–314	G
19b	← (66)	19	→ (14 ± 3.5/139)	131 (128–131)	C
19c	0			132 (129–136)	G
21a[d]	0			500 (433–577)	G
21b[d]	0	37	→ (14.3 ± 4.2/90)	133 (125–142)	C
21c[d]	0			162 (155–168)	C
Piperidine	0			416 (389–445)	C
N-Methylpiperidine	0			465 (439–474)	C
20c	0			54 (50–58	C
Dopamine	0			1978 (1810–2162)	C
N-Methyldopamine	0	10[e]	0	212 (178–253)	C
22	0	10[e]	0	240 (226–255)	C
9	→ (120)	25	← (475 ± 108/70)	295 (284–307)	C
9		10	(161 ± 48/30)		
23	→ (90)	25	(732 ± 83/70)	219 (209–230)	C
23		10	(295 ± 50/30)		
23		5	0		
24	→ (30)	25	← (875 ± 35/70)[f]	129 (123–136)	C
24		5	(266 ± 24/30)[f]		
24		3	(234 ± 54/30)		
25	0				C
1a, (−)-norapomorphine	0			213 (200–221)	Q
1b, (−)-apomorphine	→ (1)	1	← (964 ± 132/70)	145 (138–150)	C
1c, *N*-*n*-propyl-(−)-norapomorphine	→ (0.25)			323 (309–338)	Q
Oxotremorine[g]	← (0.3)	0.3	→ (10.3 ± 3.4/61)		C

[a] All chemicals were given ip as the hydrochloride salts with the exception of oxotremorine. [b] A total of eight mice tested for each compound. For method of testing and determining dose, see text: Tests with Caudectomized Mice. [c] Each compound tested in five rats. For method of testing, see text: Tests with Nigral-Lesioned Rats. [d] Racemic mixtures. [e] Higher doses than 10 μg/g produced acute toxic effects followed often by death. No turns noted. [f] Accompanied by intense gnawing, tail biting, and licking. [g] Potent cholinergic agent. → = conjugate curving and turning toward side of lesion; ← = conjugate curving and turning opposite to side of lesion; C, convulsion; R, rearing; G, gasping; Q, quietly.

aldehyde, 3,4-dimethoxyphenylacetic acid, picolinic acid, piperidine, *N*-methylpropylamine, *N*-methylpiperidine, isoquinoline, and *p*-cymene were obtained from Aldrich Chemical Co.; dimethylamine, *N*-methyl-*N*-*n*-butylamine, 1-iodopropane, and iodomethane from Eastman Organic Chemicals; (−)-apomorphine hydrochloride from Merck & Co.; dopamine hydrochloride from Calbiochem; *N*-*n*-propyl-(−)-norapomorphine hydrochloride from Sterling Winthrop Research Institute; *N*-methyldopamine hydrobromide and (−)-norapomorphine hydrochloride were gifts from Hoffmann-La Roche Inc.; oxotremorine from Nutritional Biochemical Corp.; *N*-*n*-propyl-*N*-*n*-butylamine from ICN-K&K Labs, Inc.

3,4-Dibenzyloxyphenyl(2-pyridyl)carbinol Hydrochloride (2). 2 was prepared as described elsewhere[8] with the following modifications. N_2 was bubbled through the reaction mixture. In the extraction with aqueous 2 *N* HCl, the lowest two layers were combined and basified with NH_4OH, and the free base, a dark amber oil, was extracted with $CHCl_3$. The breaking of the ensuing emulsion was facilitated by warming of the separatory funnel. After removal of the $CHCl_3$ and pyridine under vacuum, the dark oily residue was taken up in ether and treated with HCl-saturated ether. The semisolid salt was triturated repeatedly with ether until it solidified completely. The crude product was dissolved in 40 ml of MeOH and diluted with 90 ml of ether and was allowed to crystallize out at room temperature and then overnight at 4° following the addition of more solvent. The white needles were collected on a filter: wt 21.7 g (32%); mp 127-128°. The ^1H NMR (Me_2SO-d_6) spectrum was consistent with the structure of **2**. Anal. ($C_{26}H_{24}NO_3Cl$) C, H, Cl, N.

3,4-Dihydroxyphenyl(2-pyridyl)methane hydrochloride (3) was prepared as described by others[8] but recrystallized twice from EtOH-EtOAc before a mp of 149-151° (lit. 152-154°) was attained. TLC (B, D, I) yielded one spot visualized with uv and I_2 vapors. The spot darkened on standing. Its ^1H NMR spectrum was consistent with its structure.

3,4-Dimethoxyphenyl(2-pyridyl)carbinol (4). 4 was prepared essentially as described by Sankey et al.[8] The BuLi used was obtained from a commercial source. The crude product was recrystallized from 16 ml of acetone and 30 ml of heptane: mp 92-93° (lit.[8] 93-94°).

3,4-Dimethoxyphenyl(2-pyridyl)methane Hydrochloride (5). 5 was prepared from 4 by the method of Sperber et al.[18] 5 was

Table VI. In Vitro Stimulation of Adenylate Cyclase of Mouse Caudate Nucleus by Dopamine and N-Alkyl Derivatives Thereof[a]

Compound	Net cyclic AMP,[b] pmol (mean ± SEM)	p value[d]
Dopamine (7)[c]	21.0 ± 2.8	<0.001
N-Methyl-DA (6)[c]	24.3 ± 2.4	<0.001
9, N-methyl-N-n-propyl-DA (6)[c]	5.1 ± 1.4	<0.036
23, N-methyl-N-n-butyl-DA (6)[c]	14.4 ± 1.7[e]	<0.001
24, N-n-propyl-N-n-butyl-DA (6)[c]	18.8 ± 2.6[e]	<0.001
25, 1-(3,4-dihydroxyphenyl)-piperidine (6)[c]	0	

[a]Each compound tested at a concentration of 10 μM. [b]Net cAMP was obtained by subtracting the mean number of picamoles of cAMP produced by the nonstimulated controls from each mean of measurements of stimulated production of cAMP. [c]Number of determinations per assay. [d]Comparison between total cAMP assayed and nonstimulated cAMP. [e]p value (23 vs. 24) < 0.05.

purified by distillation [bp 128-132° (0.05-0.07 mmHg)] (42%). Its ^{1}H NMR spectrum was consistent with its structure.

3,4-Dimethoxyphenylacetyl Chloride (6). 6 was prepared as described by Lindenmann.[19] In addition, expulsion of the HCl generated during the heating period was accomplished with a steady stream of N_2. The crude, red oil product was distilled at 124-126° (0.7 mmHg). The distillate (90%) was light orange and crystallized on cooling.

N-Methyl-N-n-propyl-β-(3,4-dimethoxyphenyl)acetamide (7). To a solution of 35 ml of $CHCl_3$ and 11.24 g (0.19 m) of N-methyl-N-n-propylamine was added dropwise with agitation a solution of 35 ml of $CHCl_3$ and 13.6 g (0.063 mol) of 6 at 0°. The reaction was completed as shown by TLC (C, F, G, H) after 1 hr of heating at 50-55°. The $CHCl_3$ solution was washed successively with 0.1 N HCl, 10% aqueous NaOH, and water. The organic layer, after drying over anhydrous $MgSO_4$, yielded upon removal of the solvent 15.3 g (97%) of 7, a yellow oil. This was purified by high vacuum distillation. The fraction obtained at 138-140° (0.09-0.1 mmHg) (14.1 g, 88%) was used in the next synthetic step. TLC showed one spot visualized with uv and I_2 vapors: ir (film) 1650 cm^{-1} (C=O stretch, tertiary amide), OH, NH, and COCl stretching bands completely absent. The ^{1}H NMR spectrum ($CDCl_3$) was consistent with its structure.

N-Methyl-N-n-propyl-β-(3,4-dimethoxyphenyl)ethylamine Hydrochloride (8). A solution of 150 ml of tetrahydrofuran (THF) and 14.0 g (0.0557 mol) of 7 was stirred slowly into 99.7 ml (0.95 M) of diborane at −5-0° under anhydrous conditions and under a N_2 atmosphere over a period of 15-20 min. The temperature was allowed to rise to 20°C. TLC (D, I, K) showed little reaction. After refluxing for 0.75 hr, TLC showed that the reaction was complete. After cooling to room temperature, 6.0 N HCl (25 ml) was added carefully to the reaction solution because of the considerable foaming caused by the evolution of H_2. The total volume was reduced to about 40 ml by distillation at atmospheric pressure. The remaining aqueous phase was diluted with 40 ml of water, basified with 50% aqueous NaOH, and extracted with ether (4 × 50 ml). The ether extracts were combined, washed with water to a neutral pH, dried over anhydrous $MgSO_4$, and filtered. The ether was reduced in volume to 50 ml and treated with HCl-saturated anhydrous ether. The oily precipitate crystallized out slowly under refrigeration to a white solid which was filtered, washed with ether, and dried to a constant weight: 10.5 g (69%); mp 138-141°. An ir (film) and a ^{1}H NMR scanning of the colorless viscous oil (3.98 g) remaining after evaporation of the mother liquor indicated that most of it consisted of the amine complexed with diborane. This was dissolved in 10 ml of THF and refluxed with aqueous 6 N HCl, and the above extractive procedure was repeated, yielding an additional 2.76 g of 8 (mp 140-142°), which brought the total yield to 13.3 g (87%). The two crops were combined and recrystallized from 40 ml of CH_2Cl_2 diluted with 120 ml of EtOAc: mp 141.5-142.5°. The ^{1}H NMR spectrum ($CDCl_3$) was consistent with its structure.

N-Methyl-N-n-propyl-β-(3,4-dihydroxyphenyl)ethylamine Hydrochloride (9). A solution of 13.1 ml of 57% HI, 6.5 ml of Ac_2O, and 2.25 g (8.25 mmol) of 8 was refluxed for 0.75 hr under N_2 and then the solvent was removed under reduced pressure. The viscous residue was redissolved in 7 ml of absolute EtOH and evaporated again under high vacuum. The pale yellow residue was redissolved in 7 ml of absolute EtOH, diluted with 25 ml of EtOAc, and induced to crystallize by scratching. The crystalline product was filtered off, dissolved in 30 ml of water, and basified with $NaHCO_3$, and the free amine was extracted exhaustively with EtOAc. The solid residue obtained after removal of the solvent was dissolved in 8 ml of absolute EtOH and treated with HCl-saturated absolute ether. This yielded a colorless viscous oil as a precipitate, which was centrifuged off and triturated with ether until it changed into a sticky solid. This was recrystallized from 5 ml of warm EtOH diluted with 65 ml of EtOAc: 1.39 g (89%); mp 137-138°. Recrystallization from the same solvent system did not improve the melting point. TLC (F, G, H) yielded one spot visualized with uv and I_2 vapors. The ^{1}H NMR ($CDCl_3$) spectrum was consistent with the structure of 9.

N,N-Dimethyl-β-(3,4-dimethoxyphenyl)ethylacetamide (10). 10 was prepared in the same way as 7. The crude product, an oil, weighed 14.2 g (96.4%). TLC (F, G, H) showed only one spot visualized with uv and I_2 vapors, and the ir (film) spectrum showed a strong band at 1648 cm^{-1} (C=O stretch, tertiary amide), OH, NH, and COCl stretching bands were completely absent. The colorless fraction distilled at 148-151° (0.17-0.18 mmHg), which weighed 12.9 g (91%), was used for the preparation of 11.

N,N-Dimethyl-β-(3,4-dimethoxyphenyl)ethylamine Hydrochloride (11). 11 was prepared in the same manner as 8: yield 88%; mp 195-196° (lit.[20] 197°); TLC (D, I, K) showed only one spot.

The compounds N-methyl-N-n-butyl-β-(3,4-dihydroxyphenyl)ethylamine hydrochloride (**23**), N-n-propyl-N-n-butyl-β-(3,4-dihydroxyphenyl)ethylamine hydrochloride (**24**), and 1-(3,4-dihydroxyphenylethyl)piperidine hydrochloride (**25**) were prepared according to Scheme III. Experimental conditions and procedures were essentially the same as for the preparation of **9** and **22**. All of the amide intermediates were viscous liquids, distillable under high vacuum (0.1-0.5 mmHg). The structures of all intermediates and final products were consistent with their ir and ^{1}H NMR spectra.

Preparation of Methyl and n-Propyl Iodides of Isoquinoline and Pyridine Derivatives. Nitromethane was used as a solvent in the reaction of the free amine bases with an excess of alkyl iodides (five- to tenfold). The reaction temperature varied from room temperature to 100°, and an inert atmosphere of N_2 was used whenever one of the reactants was a catechol derivative with free phenolic OH. The progress and completion of the reaction were monitored by TLC, and the iodide salt either crystallized out on cooling or was precipitated with ether or EtOAc. The products were recrystallized, in general, from absolute EtOH or absolute EtOH plus ether or EtOAc and were characterized by TLC, melting points, elemental analyses, and ^{1}H NMR spectra (Table I).

Reduction of Alkyl Iodides and Hydrogen Chlorides of Isoquinoline and Pyridine Derivatives to Their Corresponding 1,2,3,4-Tetrahydroisoquinoline and Piperidine Derivatives. Reduction was accomplished by hydrogenation over PtO_2 in absolute MeOH at atmospheric pressure with vigorous agitation in a baffled hydrogenation vessel. The reaction was monitored by the amount of H_2 absorbed and was discontinued when this reached the theoretical equivalent; the reaction then either ceased or became sluggish. The molar ratio of substrate to PtO_2 used was 1:5-7. The reaction time varied from 0.5 to 2.0 hr. Finally, the catalyst was removed by filtration, the solvent was evaporated under reduced pressure, and the HI salts were converted to the HCl salts by treating an absolute ether solution of the free base with HCl-saturated absolute ether. In general, the HCl salts were recrystallized and characterized in the same manner as their precursors (Table II).

Demethylation of the O-Methyl Derivatives. The method used was essentially that of Neumeyer et al.[21] TLC (D, I, K) showed that the demethylation was complete after 0.75 hr of refluxing. The HI salt, obtained by removing the solvent under high vacuum with N_2 bleeding through, was neutralized with aqueous $NaHCO_3$, and the free base was extracted with $CHCl_3$ or ether and then treated with HCl-saturated ether. The HCl salt was centrifuged or filtered off and recrystallized from ethanol plus ether or EtOAc. (Compound **9**, above, provides an illustration of preparation and characterization.)

Toxicity. LD_{50} was determined by injecting the chemical (dissolved in water, vol 0.2-1.0 ml) intraperitoneally (ip) into Hale-Stoner male Swiss albino mice (6-8 weeks old, wt 22.5-27.5 g) and

recording the number of deaths within 24 hr. For each LD_{50} value, six to eight groups, each consisting of 12-18 mice, were used. The behavior and the manner of death were noted. A computer-programmed probit analysis yielded the LD_{50} value with their 95% confidence limits (Table V) expressed in μg/g. The LD_{50} for APO had previously been found similar for male mice of two strains, Hale-Stoner and Sprague-Dawley.

Tests with Caudectomized Mice (Table V). The compound being tested was injected into pairs of animals successively every 15 min in amounts corresponding to 4, 6, 10, and 20% of the LD_{50} (total cummulative dose, 40% of the LD_{50}). To be certain of absence of behavioral effects a different pair of animals was injected with 40% and 15 min later with 50% of the LD_{50}, and another two pairs were injected with only 40% of the LD_{50}. The dose to which the animals responded positively was confirmed by further testing in six additional animals. Three control compounds were used, **1b**, oxotremorine, and water. Since the LD_{50} of oxotremorine was not available, 0.3 μg/g was given on the basis of previous experience with this drug.

Tests and Nigral-Lesioned Rats. Single ip injections of compounds **19b** (19 μg/g) and **21b** (37.0 μg/g) were given respectively to each member of groups of six and five nigral-lesioned rats, each in an automatic rotometer.[5] Later (4-10 min), a slow but sustained rotation to the left (similar to that caused by oxotremorine[11]) was induced, accompanied by tremor, salivation, urination, and ptosis. These effects were absent in six animals pretreated with atropine (0.5 μg/g) 30 min before injection of **21b.** The effects of compound **19b** were blocked in three out of five animals pretreated with 3 μg/g of atropine and in four out of five pretreated with 5 μg/g. At 3-4-day intervals, compounds **9** and **22-24** were administered ip (25 μg/g) to the same group of five nigral-lesioned rats, which had been selected for their ready response to **1b** (1 μg/g ip). In each test a nigral-lesioned rat injected ip with water, to serve as a control, showed intermittent, random turning. When 25 μg/g of compounds **9, 23,** and **24** were administered ip to nigral-lesioned rats, turns were induced for periods ranging from 70 to 90 min. Intensities of rotational effects were compared by recording the number of turns induced by each compound for 70 min (Table V). When the same compounds were given at 10 μg/g or less, the rotational behavior, whenever induced, lasted from 30 to 50 min, and the rotational intensities were compared by recording the number of turns observed for 30 min (Table V).

Measurement of Adenylate Cyclase Activity. Mouse caudate nuclei were removed, homogenized, and incubated as described by Kebabian et al.,[6] and the cAMP produced from ATP was assayed according to Gilman.[7]

Each assay was performed on the pooled caudate nuclei of two mice. Each compound was tested at a concentration of μM, and a total of six to seven pairs of mice were used to obtain a mean value and a SEM for cAMP produced. From this mean value, the net mean value for each compound (Table VI) was obtained by subtracting the mean value for nonstimulated cAMP production (determined in homogenates from ten pairs of mice).

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