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References and Notes

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New Dopaminergic and Potential Anti-Parkinson Compounds, N,N-Disubstituted β-(3,4-Dihydroxyphenyl)ethylamines

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Of the dopamine analogues synthesized and tested for dopaminergic agonism, the *N*-*n*-propyl-N-substituted homologues induced strong postural asymmetric behavior indicative of dopaminergic action in caudectomized mice when injected intraperitoneally. *N*-*n*-Propyl-*N*-phenylethyl-, *N*-*n*-propyl-*n*-pentyl- (HI salt), and *N*-*n*-propyl-*N*-*n*-butyl-β-(3,4-dihydroxyphenyl)ethylamine hydrochloride (**2**) (0.087 μmol/g of body weight) ranked in decreasing order with respect to long-lasting effects in nigra-lesioned rats. In contrast, neither the N-monosubstituted nor the *N*,*N*-dialkyl analogues possessing identical *N*-alkyl groups showed dopaminergic effects while the *N*-methyl-N-substituted analogues demonstrated little or no effect. Analogues with branching *N*-alkyl substituents (*N*-methyl-*N*-isobutyl and *N*-*n*-propyl-*N*-isobutyl) showed also dopaminergic effects. In contrast to **2**, *N*-*n*-propyl-*N*-*n*-butyl-β-(3,4-methylenedioxyphenyl)ethylamine hydrochloride failed to elicit any behavioral effects when tested similarly. The results suggest that the nitrogen substituents may play also an important role in binding to the receptor site by possibly interacting with its hydrophobic regions. Furthermore, in contrast to currently used dopamine agonists in the treatment of parkinsonism, N,N-disubstituted dopamine analogues can be easily and inexpensively synthesized with a spectrum ranging from short to prolonged dopaminergic effects. In accordance with current trends, one or more of these agonists could be used either alone or in combination with L-Dopa, as required by each patient, to optimize the treatment of Parkinson's disease.

The two aporphines, apomorphine (**1a**) and *N*-*n*-propylnorapormorphine (**1b**), alleviate the symptoms of parkinsonism when given alone and potentiate the therapeutic effects of L-Dopa when either is given in combination with L-Dopa.[1] These latter synergistic effects have been ascribed to the molecular similarities between the aporphines and dopamine (DA), the effective metabolite

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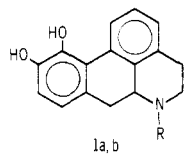


1a,b

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

of L-Dopa.[1] Both aporphines, however, also antagonize the L-Dopa-dependent dyskinesias,[1] possibly because their structure includes a tetrahydroisoquinoline moiety possessing antidopaminergic properties[2] or because, in sufficiently high concentrations, these compounds rather inhibit than stimulate the dopamine-activated adenylate cyclase in homogenates of mouse caudate nuclei.[3]

In our earlier efforts to dissociate the dopaminergic from the antidopaminergic effects in **1a** and **1b**, we synthesized a series of N-mono- and N,N-dialkylated DA analogues, some of which had DA agonist properties. Among the latter, *N*-*n*-propyl-*n*-butyldopamine (**2**) was the most potent DA receptor agonist[2b] and was further studied in animals as a potential anti-Parkinson drug.[4] The present study delineates further the dependence of dopaminergic effects on the structure of the N-substituents. Furthermore, the discovery that several of these N,N-disubstituted DA analogues possess dopaminergic activity suggests that one or several of these, given alone or in combination, may surpass the therapeutic value of presently available drugs. That combinations of drugs for the treatment of parkinsonism may be efficacious was demonstrated originally by the clinical use of **1a** or **1b** together with L-Dopa.[1] This new trend is being followed by others through the use of the ergot alkaloids, bromocriptine[5] and lergotrile,[6] also in combination with L-Dopa. Our approach promises several advantages over the currently available drug regimens as discussed below.

Results and Discussion

Chemistry. Scheme I illustrates the stepwise synthesis of *N*-methyl-N-substituted and N,N-disubstituted DA analogues. The *O*-methyl protective groups were removed in the last step (H or F). Steps A, B, and C show the stepwise synthesis of the N-monosubstituted β-(3,4-dimethoxyphenyl)ethylamines which were then converted to their respective *N*-methyl homologues by catalytic formylation (steps G and H) or to their corresponding tertiary amine analogues (steps D and E).

Scheme II (steps C, D, and E), two reaction steps less than Scheme I, is the preferred route for the synthesis of *N*,*N*-dialkyldopamine analogues where the appropriate *N*,*N*-dialkylamine is commercially available. Scheme II is also an alternate route for the synthesis of N,N-disubstituted DA analogues. One may prepare the N,N-disubstituted amine first, purify it by fractional distillation, and then use it as in step C (Tables I–III).

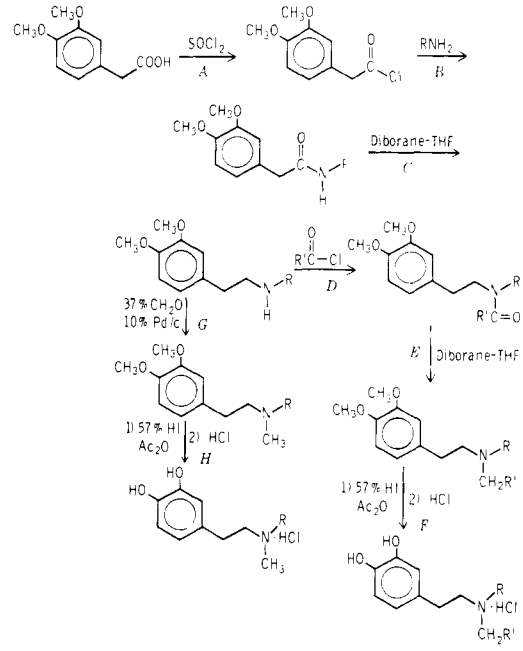
Because the HCl salt of **20** could not be prepared as a solid, the HI salt was prepared instead. The effect of iodide ions on the induction of asymmetric posture in lesioned animals is unknown. Therefore, the HI salt of **2** was prepared and its effects in lesioned animals were compared with those of the HCl salt of **2**. No significant difference was detected (Table IV).

Compound **14** was prepared according to Scheme II using, however, as the initial reactant the commercially available 3,4-methylenedioxyphenylacetic acid instead of the 3,4-dimethoxyphenylacetic acid.

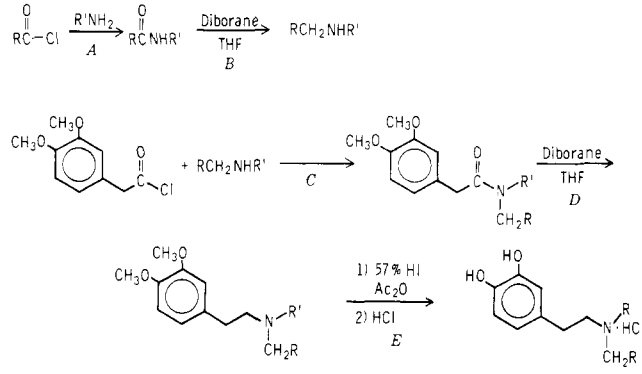
All compounds were characterized by TLC, ^1H NMR, infrared spectra, and elemental analysis.

Pharmacology. Two animal tests were used to demonstrate dopaminergic properties. (1) Postural effects on

Scheme I



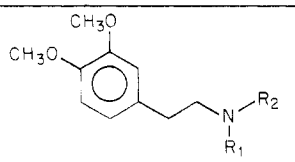
Scheme II



unilaterally caudectomized Swiss albino, Hale–Stoner male mice.[7] These mice had a right caudectomy by suction; the operation was considered successful if 3 weeks after caudectomy the mice always reacted to **1a** (1.0 μg/g) by curving their bodies toward the side with the lesion and extending their contralateral limbs. (2) Rotatory behavior of nigra-lesioned rats (Charles River, Sprague–Dawley male rats). These rats were injected with 6-hydroxydopamine stereotaxically in the right substantia nigra by the method of Ungerstedt et al.[8] The operation was considered successful if 4 weeks following the lesion, injection of **1a** (1.0 μg/g) caused them to rotate in the direction away from the lesion. The number of turns was measured in automatic rotometers.[8] All compounds that showed in caudectomized mice dopaminergic activity equal to or greater than **2** were tested also in nigra-lesioned rats (Table IV).

Of the compounds synthesized and tested, the *N*-*n*-propyl-N-substituted homologues were, in general, the most effective in inducing asymmetric postures in caudectomized mice. **27**, **20**, and **2** ranked in decreasing order with respect to duration of effects in nigra-lesioned rats (Figure 1, Table IV). In contrast, the *N*-methyl-N-substituted homologues showed little or no effects, and none of the N-monosubstituted DA analogues induced asymmetric postures. Moreover, the *N*,*N*-dialkyldopamine analogues with identical *N*-alkyl groups failed also to show DA agonism. These results confirm our previous findings.[2a] Analogues with branching *N*-alkyl substituents, **23** and **24**,

Table I. Preparation and the Properties of the Hydrochlorides of N-Monosubstituted and N,N-Disubstituted β-(3,4-Dimethoxyphenyl)ethylamines[a,b]



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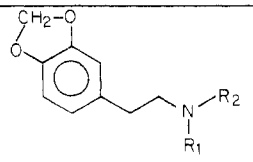


14

Compd	R_1	R_2	Yield, % (scheme, step)	Lit. mp, °C	Recrystn solvent	Formula[c]
3	n-$CH_3(CH_2)_3$	n-$CH_3(CH_2)_3$	79 (II, D)	90-91	EtOAc + ether	$C_{18}H_{32}ClNO_2$
4	H	n-$CH_3(CH_2)_4$	85 (I, C)	197-198	EtOH + ether	$C_{15}H_{26}ClNO_2$
5	CH_3	n-$CH_3(CH_2)_4$	89 (I, G)	109-111	CH_2Cl_2 + ether	$C_{16}H_{28}ClNO_2$
6	n-$CH_3(CH_2)_2$	n-$CH_3(CH_2)_4$	81 (I, E)	74-77	CH_2Cl_2 + ether	$C_{18}H_{32}ClNO_2$
7	n-$CH_3(CH_2)_4$	n-$CH_3(CH_2)_4$	58 (II, D)	86-87	EtOAc + ether	$C_{20}H_{36}ClNO_2$
8	H	$(CH_3)_2CHCH_2$	95 (I, C)	184-185	EtOH + ether	$C_{14}H_{24}ClNO_2$
9	CH_3	$(CH_3)_2CHCH_2$	94 (I, G)	124-126	EtOH + ether	$C_{15}H_{26}ClNO_2$
10	n-$CH_3(CH_2)_2$	$(CH_3)_2CHCH_2$	70 (I, E)	Oil[a]		$C_{17}H_{29}NO_2$
11	H	$C_6H_5CH_2CH_2$	95 (I, C)	187-188	EtOH + ether	$C_{18}H_{24}ClNO_2$
12	CH_3	$C_6H_5CH_2CH_2$	92 (I, G)	124-127	EtOH + ether	$C_{19}H_{26}ClNO_2$
13	n-$CH_3(CH_2)_2$	$C_6H_5CH_2CH_2$	75 (II, E)	Oil[b]		
14	n-$CH_3(CH_2)_2$	n-$CH_3(CH_2)_3$	54 (II, D)	80-82	CH_2Cl_2 + ether	$C_{16}H_{26}ClNO_2$

[a] The free base of **10** was fractionated by short-path distillation at 90-98 °C (0.30-0.35 mmHg) and used in the next reaction step. [b] The HCl salt of **15** was a viscous oil that could not be crystallized. The free amine was used in the next reaction step. [c] All, with the exception of **15**, were analyzed for C, H, Cl, and N and results were within 0.4% of theory.

Table II. Preparation of *N,N*-Dialkylamines and Their Properties

No.	Compd	Yield, %	Bp, °C (mmHg) [lit.]
15	*N*-*n*-Propyl-*n*-pentylamine	87	72-76 (39-41)
16	*N*-*n*-Propyl-*N*-isobutylamine[a]	81	50 (3.2-3.5) [83-84 (200[a])]

[a] K. N. Campbell, A. H. Sommers, and B. K. Campbell, *J. Am. Chem. Soc.*, **66**, 82 (1944).

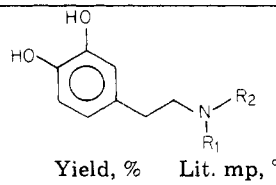
showed also dopaminergic effects. In addition to rotation, compounds **2**, **20**, and **27** induced in nigra-lesioned rats other behavioral effects including the following intense but intermittent stereotypy: rearing, followed by twisting of the body to the left, and pawing of the lower jaw or left side with the left forelimb. Less frequent and intense were sniffing, gnawing, and licking.

In order to determine whether the postural and rotational effects induced by **17** in lesioned animals could be prolonged by blocking the two catechol hydroxyl groups with a methylenedioxyphenyl bridge, **14** was synthesized and injected (105.8 μg/g, ip) in caudectomized and nigra-lesioned rats. It was expected that **14** would gradually become metabolized to the DA agonist **2** in a manner similar to that proposed for Piribedil (ET 495).[9] No behavioral effects were evident, however, 1 h after injection (Table IV).

Our findings indicate that structural modifications of N,N-disubstituted DA analogues can lead to prolongation or shortening of the dopaminergic effects as desired in sharp contrast to current agonists (*N*-*n*-propylnorapormorphine,[1b] bromocriptine,[5] lergotrile[6]) used in combination with L-Dopa in the treatment of parkinsonism. These DA agonists yielded qualitatively similar results as our N,N-disubstituted DA analogues.[10] It is therefore probable that one or more of these new compounds can be given along with L-Dopa to optimize the treatment of parkinsonism as required by each patient. These N,N-disubstituted DA analogues have the following advantages over the dopaminergic drugs currently used in Parkinson's disease.

(1) They are easy to synthesize and modify in high yields in a short time from relatively inexpensive, commercially available materials and have no optical enantiomers.

Table III. Preparation of the Hydrochlorides and the Properties of N-Monosubstituted and N,N-Disubstituted β-(3,4-Dihydroxyphenyl)ethylamine by Demethylation[a,b]



Compd	R_1	R_2	Yield, %	Lit. mp, °C	Recrystn solvent	Formula[b]
17	n-$CH_3(CH_2)_3$	n-$CH_3(CH_2)_3$	87	108-109	EtOH + ether	$C_{16}H_{28}ClNO_2$
18	H	n-$CH_3(CH_2)_4$	60	141-142	EtOH + ether	$C_{13}H_{22}ClNO_2$
19	CH_3	n-$CH_3(CH_2)_4$	93	71-73	EtOH + ether	$C_{14}H_{24}ClNO_2$
20	n-$CH_3(CH_2)_2$	n-$CH_3(CH_2)_4$	75	112-113[a]	EtOH + ether	$C_{16}H_{28}INO_2$
21	n-$CH_3(CH_2)_4$	n-$CH_3(CH_2)_4$	95	90-91	EtOH + ether	$C_{18}H_{32}ClNO_2$
22	H	$(CH_3)_2CHCH_2$	55	191-192	EtOH + ether	$C_{12}H_{20}ClNO_2$
23	CH_3	$(CH_3)_2CHCH_2$	82	135-136	EtOH + ether	$C_{13}H_{22}ClNO_2$
24	n-$CH_3(CH_2)_2$	$(CH_3)_2CHCH_2$	70	94-96	EtOH + ether	$C_{15}H_{26}ClNO_2$
25	H	$C_6H_5CH_2CH_2$	50	75-80[c]	Ether[d]	$C_{16}H_{20}ClNO_2$
26	CH_3	$C_6H_5CH_2CH_2$	75	80-85	Ether[d]	$C_{17}H_{22}ClNO_2$
27	n-$CH_3(CH_2)_2$	$C_6H_5CH_2CH_2$	91	50-55	EtOH + ether	$C_{19}H_{26}ClNO_2$

[a] **20** was prepared as the HI salt. [b] All were analyzed for C, H, Cl, and N; the results were within 0.4% of theory. [c] Softens at 50 °C. [d] Solidifies as an amorphous solid on trituration with ether.

Table IV. Behavioral Effects on Caudectomized Mice and Nigra-Lesioned Rats[a]

Compd	Dose, μg/g (μmol/g)	Mice conjugate curvature[b] Onset, min ± SE	Mice conjugate curvature[b] Duration, min ± SE	Nigra-lesioned rats[c] turns ± SE/min ± SE
17	104.8 (0.348)		0	
	131.0 (0.435)		0	
18	90.8 (0.348)		0	
19	23.8 (0.087)		0	
	47.6 (0.174)	3.8 ± 0.4	2.5 ± 1.1	
	95.3 (0.348)	14.6 ± 1.8	20.6 ± 4.5	
20	34.2 (0.087)	4.2 ± 2.1	14.0 ± 2.6[f]	628 ± 75/79 ± 1[k]
21	100.5 (0.305)		0[g]	
22	85.5 (0.348)		0	
23	22.6 (0.087)		0	
	45.2 (0.174)	4.0 ± 0.6	2.8 ± 1.3	
	90.4 (0.348)	3.6 ± 0.9	31.8 ± 4.9	
24	25.0 (0.087)	3.9 ± 0.6	10.0 ± 1.7	
25	102.2 (0.348)		0	
26	26.8 (0.087)		0	
	107.2 (0.348)	6.7 ± 1.1	21.2 ± 5.0	
27	29.2 (0.087)	5.0 ± 0.5	15.4 ± 2.6	1065 ± 246/98 ± 9[k]
14	105.8 (0.348)		0	0
1a[h]	1.0 (0.003)	5.0 ± 0.5	14.2 ± 2.1[i]	828 ± 184/83 ± 4
2[h]	33.0 (0.087)[d]	3.5 ± 1.2	6.7 ± 0.7[f,i]	457 ± 57/48 ± 6[j]
	25.0 (0.087)	4.6 ± 1.8	6.5 ± 1.8	552 ± 76/45 ± 4[j]

[a] All chemicals were given ip as the hydrochloride salts unless otherwise stated. [b] A total of six mice tested for each compound unless otherwise stated. The mean time and SE of the onset and the duration of the behavioral effect (curvature) were recorded. [c] Each compound tested in six rats. For method of testing see text (Tests with Nigra-Lesioned Rats). [d] Tested as the HI salt. [e] One death 7 min following injection. [f] p value (2 vs. 20) < 0.02. [g] A total of eight mice tested of which three died during the testing with convulsions. [h] Used as reference compound. [i] p value (2 vs. 1a) < 0.017. [j] p value (HCl of 2 vs. HI salt of 2) < 0.35. [k] p value (20 vs. 27) < 0.06. All p values were obtained by Student's t test.

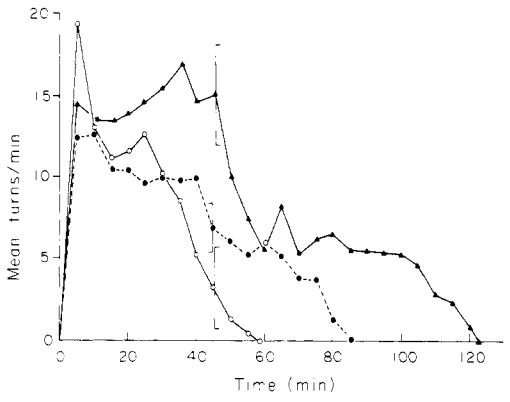


Figure 1. Rates of rotation of nigra-lesioned rats as a function of time induced by compounds **27** (-▲-, 29.2 μg/g, 0.087 mol, ip), **2** (-○-, 25 μg/g, 0.087 mol, ip), and **20** (-●-, 34.2 μg/g, 0.087 mol, ip). The number of turns induced was measured in six rats in automatic recording rotometers. The means and the SE of their turns were recorded and plotted as turns per minute vs. time in minutes.

(2) Unlike DA they cross the blood–brain barrier because as tertiary amines they are resistant to deamination by monoamine oxidase (MAO).[11]

(3) Since they are direct DA receptor agonists, their action is not dependent on the activity of Dopa decarboxylase which is necessary for the conversion of L-Dopa to DA but deficient in the brains of patients with parkinsonism.[5]

Moreover, the availability of such congeners of dopamine analogues may facilitate the study of mechanisms underlying the effects of chronically administered L-Dopa in mice on longevity and fertility[12] and the induction of hypersentitivity[13] of the striatal dopamine-dependent adenylate cyclase. The enhancement by pharmacological means of the sensitivity of the adenylate cyclase in the striatum assumes a more general significance in view of the high correlation observed between such sensitivity and the propensity for spontaneous development of breast cancer in various strains of female mice.[14,15] Such a correlation suggests a possible CNS involvement in immune mechanism controls via its dopaminergic apparatus.

Experimental Section

Uncorrected melting points were determined on a Thomas-Hoover apparatus. ^{1}H NMR spectra were recorded on a Varian T-60 in $CDCl_3$ 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) cyclohexane–EtOAc, 1:1; (B) 1:4; (C) 4:1; (D) $CHCl_3$–MeOH–AcOH, 17:2:1; (E) $CHCl_3$–EtOAc–AcOH, 85:15:1; (F) n-BuOH–H_2O–AcOH, 4:1:1. Visualization was done with UV and/or I_2 vapors.

Materials. 3,4-Dimethoxyphenylacetic acid (practical), N,N-di-n-pentylamine, and phenylethylamine were obtained from Eastman Organic Chemicals; N-isobutylamine (99%), N-n-pentylamine (99%), N,N-di-n-butylamine (99%), propionyl chloride, and isobutyryl chloride from Aldrich Chemical Co.; 3,4-methylenedioxyphenylacetic acid from ICN-K&K Labs, Inc.; hydriodic acid (55–57%) and thionyl chloride from Fisher Scientific Co.; diborane (1 M) from Alpha Products-Ventron.

Dimethylformamide (DMF) was fractionally distilled under reduced pressure and under anhydrous conditions after drying over KOH pellets. After discarding the forerun, the major fraction distilling within a range of 4 °C was collected and stored over molecular sieve 4A in a tightly sealed brown bottle and kept under refrigeration. Tetrahydrofuran (THF) was distilled over $LiAlH_4$ and stored over molecular sieve 4A under refrigeration. Pyridine used was reagent grade and stored over molecular sieve 4A.

^{1}H NMR spectra were obtained routinely for each of the preparations described below, and, unless otherwise stated, they were consistent with the structures of the compounds synthesized.

The following preparations are described as representative illustrations of synthetic Schemes I and II.

3,4-Dimethoxyphenylacetyl Chloride (28). **28** was prepared as described elsewhere.[2]

***N*-Isobutylpropionamide (29).** To a stirred solution of 50 mL of DMF, 20.5 g (0.26 mol) of pyridine, and 20.0 g (0.22 mol) of isobutylamine was added dropwise under anhydrous conditions 16.4 g (0.26 mol) of freshly distilled propionyl chloride; the temperature was kept below 55 °C. The reaction solution was stirred for 1 h at 55 °C and then cooled to ice–water temperature.

The pyridine hydrochloride salt was removed by filtration and the filtrate transferred to a separatory funnel with water, followed by extraction with ether (6 × 40 mL). The ether extracts were combined and washed successively with water, aqueous 1 N HCl, aqueous 1 N NaOH, and finally with water to a neutral pH. The organic layer, after drying over anhydrous $MgSO_4$, yielded upon removal of the solvent 18.4 g (66%) of an oil: TLC (A, B, and C) showed only one spot; IR (film) showed a strong band at 1640 cm^{-1} (C=O stretch, secondary amide), the COCl stretching band was completely absent. The crude product was fractionated by a short-path distillation apparatus as a colorless liquid at bp 70–76 °C (0.20–0.50 mmHg).

***n*-Propylisobutylamine (16).** To a stirred solution of 134 mL of diborane–THF (1.0 M) was carefully added, at below 20 °C and under anhydrous conditions, 10.0 g (0.078 mol) of **29** dissolved in 130 mL of THF. The reaction was carried out under nitrogen. After refluxing for 1.5 h, the major portion of the solvent was removed under reduced pressure and the oily residue refluxed with 50 mL of 6 N methanolic HCl for 0.5 h. The solvent was removed and the acid residue was extracted with ether several times. The ether extracts were combined and evaporated, and the oily residue was treated again as above with methanolic HCl. This aqueous phase was combined with the one obtained from the first extraction and made basic with aqueous NaOH, followed by extraction of the oily precipitate with ether (4 × 40 mL). The combined ether extracts were washed with small amounts of water to a neutral pH and dried over anhydrous $MgSO_4$, followed by evaporation of the solvent under reduced pressure. The oily residue was dried to constant weight under high vacuum: 7.3 g (81%); TLC (A, B, and C) showed one spot; IR (film) showed the complete absence of the C=O stretching band, and the fractional distillation of **16** yielded a colorless liquid at bp 50 °C (3.2–3.5 mmHg).

***N*-*n*-Propyl-*N*-isobutyl(3,4-dimethoxyphenyl)acetamide (30).** To a stirred solution of 5.34 g (0.0464 mol) of **16**, 5.0 g (0.063 mol) of pyridine, and 40 mL of DMF was added slowly 9.0 g (0.042 mol) of **28** under anhydrous conditions with temperature maintained below 50 °C. After heating the reaction mixture at 60 °C for 0.5 h, it was diluted with 50 mL of water and extracted several times with ether. The combined ether extracts were washed successively with water, aqueous 1 N HCl, aqueous 1 N NaOH, and finally with water to a neutral pH. Removal of the ether under reduced pressure, after drying over anhydrous $MgSO_4$, yielded 9.8 g (80%) of **30**: TLC (A, B, and C) showed only one spot; IR (film) 1635 cm^{-1} (C=O stretch, tertiary amide), NH and COCl stretching bands were completely absent.

***N*-*n*-Propyl-*N*-isobutyl-β-(3,4-dimethoxyphenyl)ethylamine (10).** **10** was prepared in the same way as **16**. The crude product, an oil, weighed 6.7 g (70%): TLC (A, B, and C) showed one spot; IR (film) C=O stretching band absent.

***N*-*n*-Pentyl-3,4-dimethoxyphenylacetamide (31).** **31** was prepared in the same way as **30** with one modification. Instead of pyridine, an excess of *n*-pentylamine was used to neutralize the evolving HCl. The product crystallized from ether on refrigeration yielding colorless crystals weighing 12.3 g (92%): mp 66–67 °C; TLC (A, B, and C) showed only one spot: IR (KBr) 1640 cm^{-1} (C=O stretch, secondary amide), the COCl stretching band was absent.

***N*-*n*-Pentyl-β-(3,4-dimethoxyphenyl)ethylamine Hydrochloride (4).** **4** was prepared in the same way as **16**. The crude amine was converted to its HCl salt in ether saturated with gaseous HCl. The yield was 9.8 g (87%): mp 196–198 °C. It was recrystallized from EtOAc and ether: mp 197–198 °C; TLC (D, E, and F) showed only one spot; IR (KBr) no amide carbonyl band was detectable.

***N*-Methyl-*N*-*n*-pentyl-β-(3,4-dimethoxyphenyl)ethylamine Hydrochloride (5).** A solution of 4.79 g (0.0191 mol) of the free amine of **4**, 9.3 g of 37% formaldehyde, and 50 mL of absolute ethanol was hydrogenated over 0.3 g of 10% Pd/C in a Parr shaker hydrogenator at an initial pressure of 55 psi.[16] After 0.5 h, the calculated amount of H_2 was absorbed. The reaction mixture was filtered and the volatile material removed by evaporation under reduced pressure. The residue oil was made basic with 5% KOH and extracted exhaustively with ether (3 × 50 mL). The combined ether extracts were evaporated under reduced pressure. The oily residue was dissolved in 10% aqueous HCl and extracted with ether, and the ether layer was then discarded. The aqueous phase was made basic with aqueous NaOH solution and the free amine product extracted with ether (3 × 40 mL). The combined ether extracts were filtered after drying over anhydrous $MgSO_4$ and then evaporated to a constant weight, oily residue: 4.6 g (97%); TLC (A, B, and D) showed only one spot. The free amine was converted to the HCl salt with HCl–saturated ether: 5.1 g (88%); mp 109–110 °C. Crystallization from ethanol–ether yielded a crystalline product: 5.0 g; mp 110–111 °C; TLC (D, E, and F) showed only one spot.

Demethylation of the *O*-Methyl Derivatives. The method is described elsewhere[2] (Table III).

Tests with Caudectomized Mice (Table IV). A total of 18 animals was used for each compound tested with 5–6-day intervals elapsing before the animals were retested. Animals were selected and injected ip at random in groups of six by one observer, while the second observer, without knowledge of the compound being tested, observed and recorded the onset of the asymmetric posture (or its absence) and its duration. The mean and SE of the onset and duration of the asymmetric posture induced by each compound in total of six animals were calculated. To facilitate comparison, dosages used were multiples of 0.087 μmol of each compound tested. The maximum injected dosage was 0.348 μmol. All compounds tested were dissolved in normal saline–water (0.9%) with volumes of injection equal to 0.4–0.6 mL. In addition to **1a**, **2** was selected also as a reference compound because it was shown to be the most potent and long-lasting DA agonist among the DA analogues that had been synthesized and studied previously by us.[2]

Tests with Nigra-Lesioned Rats (Table IV). At 5-day intervals, compounds **1a**, **2**, **20**, and **27** were administered ip (0.087 μmol/g) to the same group of six nigra-lesioned rats (each in an automatic rotometer[8]) which had been selected for their ready response to **1a** (1 μg/g, ip). In each test, a nigra-lesioned rat injected with normal saline–water (0.9%), to serve as a control, showed intermittent, random turning. The mean and SE of the total number of turns and duration induced by each compound in six rats were calculated (Table IV). In Figure 1 are shown the mean and SE of the number of turns per minute, calculated every 5 min, and duration of the rotational behavior for compounds **2**, **20**, and **27**.

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Study of the Structural Requirements for Dopa Potentiation and Oxotremorine Antagonism by L-Prolyl-L-leucylglycinamide

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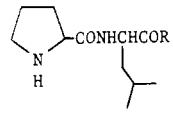
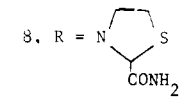
A number of analogues of the tripeptide L-prolyl-L-leucylglycinamide (1) were synthesized and evaluated in the Dopa potentiation and oxotremorine antagonism tests. The replacement of the glycinamide residue with either the glycine methylamide, glycine, aminoacetonitrile, amino-2-propanone, semicarbazide, or β-alaninamide residues resulted in a loss of activity in both tests. A 1:1 mixture of L-prolyl-L-leucyl-(−)-thiazolidine-2-carboxamide (8) and L-prolyl-L-leucyl-(+)-thiazolidine-2-carboxamide (9) showed marked activity in the Dopa potentiation test but was unable to antagonize the tremors induced by oxotremorine. L-Prolyl-L-leucyl-L-prolinamide (11), on the other hand, was active in the oxotremorine antagonism test but inactive in the Dopa potentiation test. The replacement of the pyrrolidine ring of 1 with either a thiazolidine or cyclopentane ring system caused a loss of activity. The cyclopentanecarboxylic acid analogue 13, however, was found to have moderate activity in the serotonin potentiation test.

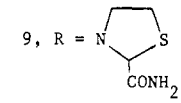
Several of the hypothalamic releasing and release-inhibiting hormones have recently been shown to have effects on the central nervous system which are independent of their endocrine effects.[2] Among these polypeptides is L-prolyl-L-leucylglycinamide (1). This tripeptide has been



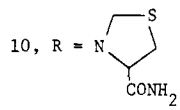
1, R = $NHCH_2CONH_2$
2, R = $NHCH_2CONHCH_3$
3, R = $NHCH_2COOH$
4, R = $NHCH_2CN$
5, R = $NHCH_2COCH_3$
6, R = $NHNHCONH_2$
7, R = $NHCH_2CH_2CONH_2$



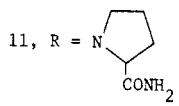
8, R = (−)-isomer



9, R = (+)-isomer



10, R =



11, R =

postulated to be the hypothalamic hormone that inhibits the release of melanocyte-stimulating hormone from the anterior pituitary gland.[3-6] The extra-endocrine effects of 1 were first demonstrated by Plotnikoff et al.[7] with Everett's Dopa potentiation test.[8] In this test 1 was found to potentiate the behavioral effects of L-3,4-dihydroxyphenylalanine (L-Dopa) in both normal and hypophysectomized mice. Subsequent studies with 1 have also shown that this tripeptide antagonizes the central and peripheral effects of oxotremorine,[9,10] reverses the sedative effects of deserpidine,[11] potentiates the behavioral effects of apomorphine,[12] attenuates puromycin-induced amnesia,[13] and facilitates the development of morphine dependence.[14] Moreover, a number of preliminary clinical studies have indicated that 1 may be of potential value in the treatment of Parkinson's disease.[15,16]

In an effort to determine the structural features of 1 that enable this tripeptide to potentiate the behavioral effects of L-Dopa and antagonize the tremors induced by oxotremorine, we have synthesized several analogues of 1 in which the prolyl and glycinamide residues have been modified. In order to determine the importance of the primary carboxamide moiety, the glycinamide residue has been replaced with the glycine methylamide, glycine, aminoacetonitrile, and amino-2-propanone residues, analogues **2–5**, respectively. In the case of analogue **6** an imino group has been substituted for the methylene group while in compound **7** an additional methylene group has been inserted into the peptide chain. In addition to these modifications, several analogues (8–11) have been synthesized where either a thiazolidine or pyrrolidine ring system has been incorporated into the glycinamide portion of the molecule.

The prolyl residue has been modified in an attempt to determine the importance of the pyrrolidine ring system. Castensson et al.[10] have reported that the replacement of the prolyl residue with a pyroglutamyl residue provides a compound (14) which is an even better antagonist of oxotremorine-induced tremors than 1. We have thus synthesized compounds **12** and **13** whereby the pyrrolidine ring has been replaced by a thiazolidine and cyclopentane ring system, respectively.

Results and Discussion

Chemistry. The protected dipeptide Z-Pro-Leu-OH (**15**), which was prepared in a manner similar to that previously described by Cash,[17] served as the key intermediate in the synthesis of **2–11**. Aminoacetonitrile, semicarbazide, β-alaninamide, L-prolinamide, and methyl

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