# Articles

## A Potential Anti-Parkinson Drug, *N-n*-Propyl-*N-n*-butyl-β-(3,4-dihydroxyphenyl)ethylamine Hydrochloride, and Its Effects in Intact and Nigra-Lesioned Rats[1a]

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Despite marked structural differences, both *N-n*-propyl-*N-n*-butyl-β-(3,4-dihydroxyphenyl)ethylamine hydrochloride (2), a potential anti-Parkinson dopaminergic drug, and apomorphine hydrochloride (3) produced identical stereotypy in intact rats and contralateral rotation in 6-OHDA nigra-lesioned rats when administered intraperitoneally. Dose–response studies in intact rats suggested that 3, although more potent than 2, may be a partial agonist. Following either 2 or 3, both the onset and peak of stereotypy in intact rats appeared later than and disappeared sooner than rotation induced in nigra-lesioned rats. 2 was measured in rat blood by means of O-methylation. *S*-Adenosyl-L-methionine-*methyl*-$^{14}C$ served to donate a labeled methyl group with rat liver catechol *O*-methyltransferase as the catalyst. A high correlation coefficient (0.96) was obtained between the concentration of 2 in blood of intact rats and induced rotatory behavior in nigra-lesioned rats. By contrast, scores of stereotypy in intact rats showed a low correlation coefficient (0.59) with concentration of 2 in rat blood. This is consistent with the hypothesis that rotatory and stereotyped behavior may result from stimulation of two functionally different dopaminergic receptors. Moreover, the rapid and almost complete metabolic O-methylation of 2 in contrast to 3 may explain why a higher dose of 2 was necessary to achieve comparable behavioral effects induced by 3. Consistent with the in vitro findings, the catechol *O*-methyltransferase inhibitor, pyrogallol, prolonged the stereotyped behavior induced by 2. The two *O*-methyl isomers of 2 synthesized and used for the identification of its in vitro O-methylated products lacked dopaminergic effects when injected in nigra-lesioned rats.

Our effort to generate new dopaminergic drugs for the treatment of parkinsonism led us to synthesize and test N,N-disubstituted dopamine (DA) analogues.[2] We found that some of these tertiary amine DA analogues have dopaminergic properties, as well as low toxicity.[2b] This suggested that several of these compounds alone or in combination with L-Dopa might surpass the therapeutic value of presently available drugs. For example, *N-n*-propylnorapomorphine (1) when administered together with L-Dopa resulted in fewer adverse effects than with L-Dopa alone.[3] Combination therapy with L-Dopa is now being tried with the ergot alkaloids, bromocriptine[4] and lergotrile.[5]

The N,N-disubstituted DA agonists offer the following advantages over L-Dopa and the aforementioned DA agonists: they are easy to synthesize in high yields and in a relatively short time from commercially available starting materials and, therefore, are cheaper; unlike DA they cross the blood–brain barrier because as tertiary amines they are resistant to deamination by monoamine oxidase (MAO);[6] 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.[4]

Such dopamine agonists as 2 may be of more general importance in view of the effects that chronic administration of L-Dopa, a precursor of dopamine, has been shown to have in mice, specifically on longevity and fertility,[7] on the induction of hypersensitivity of the dopamine-dependent adenylate cyclase, and on its implications with respect to immune mechanisms and propensity to breast cancer.[8–11]

Of the tertiary DA agonists now being investigated for the treatment of Parkinson's disease, the most potent DA agonist among them,[2b] *N-n*-propyl-*N-n*-butyl-β-(3,4-dihydroxyphenyl)ethylamine (2), was chosen as a prototype for study. The experiments compared the effects of 2 with those of apomorphine (3). The rotatory behavior of 6-OHDA nigra-lesioned rats and the stereotyped behavior induced in intact rats were used for quantitative comparisons of 2 and 3. In order to study the correlation between the concentration of 2 in rat blood and its behavioral effects on nigra-lesioned and intact rats, we developed a radioassay based on catechol O-methylation.[12] We also synthesized the two likely *O*-methyl metabolites of 2, *N-n*-propyl-*N-n*-butyl-β-(3-methoxy-4-hydroxyphenyl)- (4) and *N-n*-propyl-*N-n*-butyl-β-(3-hydroxy-4-methoxyphenyl)ethylamine (5), to aid in the identification of its in vitro O-methylated products. Both of the putative metabolites were tested in animals for possible dopaminergic effects.

### Results and Discussion

**Chemistry. Synthesis and Separation of 4 and 5.** 4 and 5 were synthesized according to Scheme I. The starting material for 5 was 3-hydroxy-4-methoxyphenylacetic acid, prepared from 3-hydroxy-4-methoxybenzaldehyde,[13] and for 4 it was 3-methoxy-4-hydroxyphenylacetic acid. To protect their phenolic hydroxyl groups, these reagents were converted to the respective benzyl esters of 3-methoxy-4-benzyloxy- and 3-benzyloxy-4-methoxyphenylacetates with subsequent hydrolysis to the acids (Scheme I, step 2). The conditions for steps 3–5 were similar to those for the preparation of 2.[2b] The benzyl groups were removed in the last step by catalytic hydrogenolysis. *N-n*-Propyl-*N-n*-butyl-β-(3-benzyloxy-4-methoxyphenyl)ethylamine hydrochloride was found by TLC and $^{1}H$ NMR to be partially debenzylated during refluxing with methanolic HCl (Scheme I, step 5). This yielded a mixture of two compounds which could not be separated by crystallization. Subsequent hydrogenolysis

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Scheme I

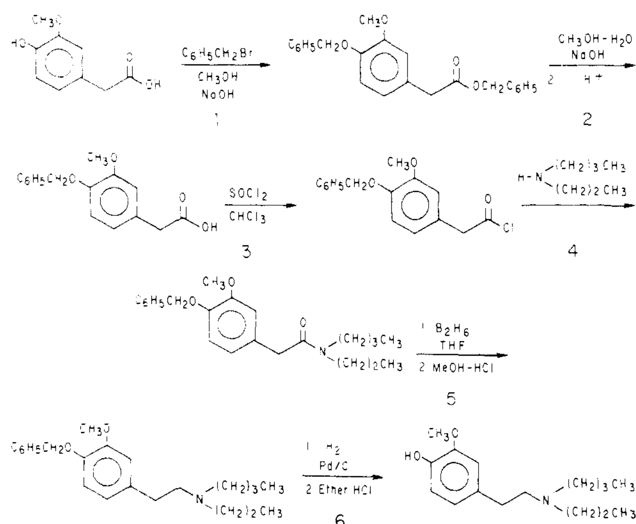

Table I. Effect of Antioxidant on Blank Size

| Antioxidant | β-Mercaptoethanol | $Na_2S_2O_5$ | No $Na_2S_2O_5$ |
|---|---|---|---|
| cpm[a] | 15400-16200 | 197-220 | 1030-1280 |

[a] Range of mean values of duplicate and triplicate determinations obtained on different occasions.

of the mixture yielded **5**. All products were characterized by IR, $^1$H NMR, and TLC.

**4** and **5** could not be separated by means of TLC on silica gel or on alumina oxide plates. Thus mixtures of the two isomers yielded only one spot when applied on silica gel with $R_f$ 0.61 for 1-butanol–water–AcOH (4:1:1) and 0.76 for 1-propanol–water (89:28). Autoradiography on chromatograms developed with the same solvent systems revealed also only one spot for the in vitro O-methylated isomers of **2**. $R_f$ values were identical with those for the synthetic ones. In contrast, gas chromatography (see the Experimental Section) was effective in separating the two compounds and showed that the ratio of **5** to **4** in an incubation mixture was equal to 0.72. This accords with previous findings that increasing the hydrophobic nature of the substituted nitrogen results in significant increase in para O-methylation.[14]

**Radioenzymatic Assay.** Because **2** as well as its congeners lacks sufficient fluorescence and because the spectrophotofluorometric techniques for measuring **3**[15] in biological fluids proved inadequate in our hands, we developed instead a radioenzymatic assay based on catechol O-methylation with *S*-adenosyl-L-methionine-*methyl*-$^{14}C$ as the methyl donor. This method was used by others for measuring a variety of catecholamines in animal plasma and tissue.[12] It entails methylating **2** with a labeled [$^{14}$C]methyl group, extracting the product from water or whole blood with an organic solvent, and measuring its radioactivity in a liquid scintillation counter. The incubation mixture used was similar to that used by Missala et al.[12a] with one modification. Mercaptoethanol was replaced with sodium metabisulfite which yielded considerably lower blanks (Table I). These were further reduced by evaporating the extractive medium with its radioactive volatiles. Subsequently, the residue was redissolved in 1 mL of the same extractive medium and assayed for radioactivity.

The rate of O-methylation of **2** was compared with that of catechol, an excellent substrate for COMT and with the highest relative activity among many catechol derivatives and several catecholamines.[16] The rate of O-methylation of **2** was almost twice that of the catechol (Figure 1)

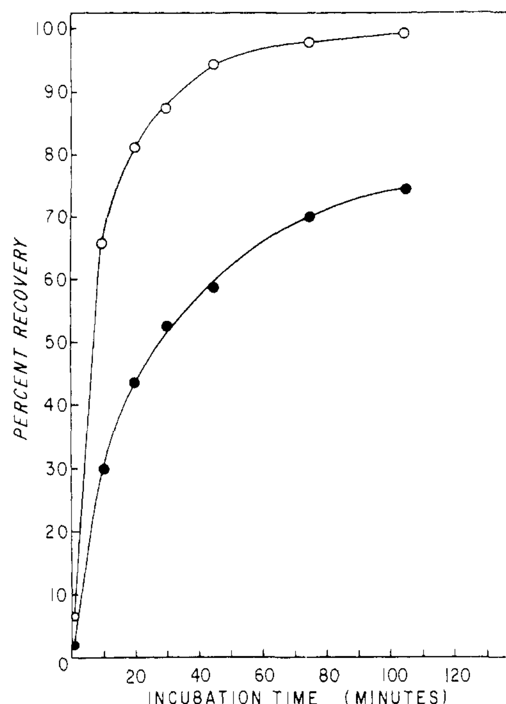


**Figure 1.** Percent recovery of $^{14}$C O-methylated products as a function of time: O-methylated **2** (-o-); O-methylated catechol (-●-).

Table II. Percent Recovery of O-Methylated Products (O-Methylation in Water)

| Substrate (0.004 mol) | % recovery |
|---|---|
| 2[a] | 93-98[b] |
| 3[a] | 0.65-1.0[b] |
| 1[a] | 0.45-0.85[b] |
| Catechol[a] | 69-75[b] |
| 4[c] | 0.11 |
| 5[c] | 0 |

[a] Incubation period: 90 min. [b] Range of mean values of duplicate and triplicate determinations obtained on different occasions. [c] Incubation period: 150 min.

suggesting that O-methylation of **2** may be an important if not a major metabolic pathway in vivo. In contrast, the major metabolic pathway of **3** is glucuronidation.[17] By comparison, **3** and **1** were poor substrates for COMT (Table II). Consequently, only blood levels of **2** were measured in rats. Table II shows also that once **2** is O-methylated in either position, no further O-methylation occurs. This agrees with previous findings.[16]

**O-Methylation of 2 in Rat Blood (in Vitro).** To establish whether there is a correlation between blood levels of **2** in rats and rotatory and stereotyped behavior in corresponding lesioned and intact rats, two male intact rats were injected intraperitoneally (ip) with 25 μg/g of **2** and blood was sampled at various intervals over 1 h and analyzed for **2**. Samples were corrected for an 82.4% recovery determined as shown in the Experimental Section. Because it has been shown that COMT is present in red blood cells,[18] a 105-min incubation of **2** (0.0033 M) was carried out in rat blood without the addition of COMT and was shown that only 0.26% of **2** was O-methylated. Figure 2 (a) shows the rate of disappearance of **2** in blood expressed for micrograms per milliliter of blood in rats 1 and 2. Peak concentrations in both animals were attained approximately within 5–6 min after ip injection (25 μg/g) and thereafter diminished rapidly over a period of 70–80 min with $t_{1/2}$ of 18.5 min for rat 1 and 14.5 min for rat 2. Half-lives were calculated from straight lines obtained by

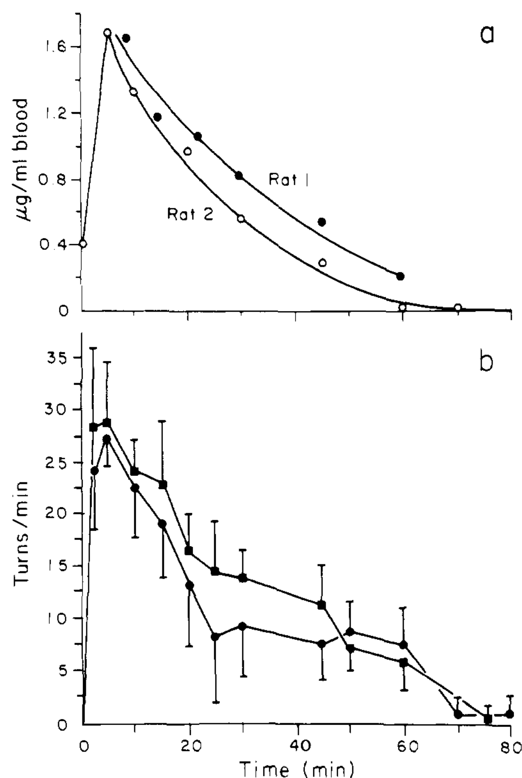


**Figure 2.** (a) Blood levels of **2** determined in intact rats (25 μg/g of body wt, ip) as a function of time: rat 1 (-●-); rat 2 (-○-). (b) Rates of rotation of 6-OHDA nigra-lesioned rats receiving **2** (-●-, 25 μg/g of body wt, ip) or **3** (-■-, 1 μg/g of body wt, ip) as a function of time. Each point represents the mean of six animals. Vertical bars represent the SEM.

plotting the log of the concentration of **2** in rat blood against time. The peak concentrations determined in the blood of either rat were between 1.6 and 1.7 μg/mL. The sensitivity of the present method is such that samples containing as little as 2 ng of **2** can be measured with a yield of $^{14}$C O-methylated **2** counts, twice that of the assay blanks. By using a 5-mL instead of a 2-mL aliquot of the organic extractive medium for counting radioactivity, the sensitivity could be increased by a factor of 2.5.

**Stereotyped Behavior in Intact Rats.** Although **2** and **3** are significantly different structurally, both compounds produced similar stereotypy when given ip to both intact and nigra-lesioned rats. Such stereotypy is believed to be mediated by the DA precursor L-Dopa,[19] the indirect DA agonist amphetamine,[20] or by direct DA agonists, such as **3**.[21] In intact rats, the behavior consists of sniffing, licking, gnawing and/or biting, or rearing and/or locomotor activity in a repetitive compulsive manner.[12a,21,22] To these we have also included ptosis as a part of stereotyped behavior in accord with Missala et al.[12a]

The rating scale used to evaluate and compare the stereotypy (see the Experimental Section) regularly showed increasing scores with increasing ip doses of **3** (0.25–2.0 μg/g) and **2** (5–60 μg/g) (Figure 3). Figure 3 shows the following. (1) The latency to onset for both drugs was less than 5 min after injection. (2) A peak score was attained for both drugs, usually 10 min following injections. (3) The parallel log dose–response plots for Figure 4 suggest that both drugs act on the same type receptor.[23] (4) The time–effect curves for **2** and **3** show that the effects of **2** diminish more rapidly. (5) The mean peak stereotypy score achieved with **3** did not exceed 7.1. By contrast, the mean peak stereotypy score of 9.0 with **2** was significantly higher ($p < 0.002$). This seems to be in accordance with the fact that **3** is a partial DA agonist in the renal

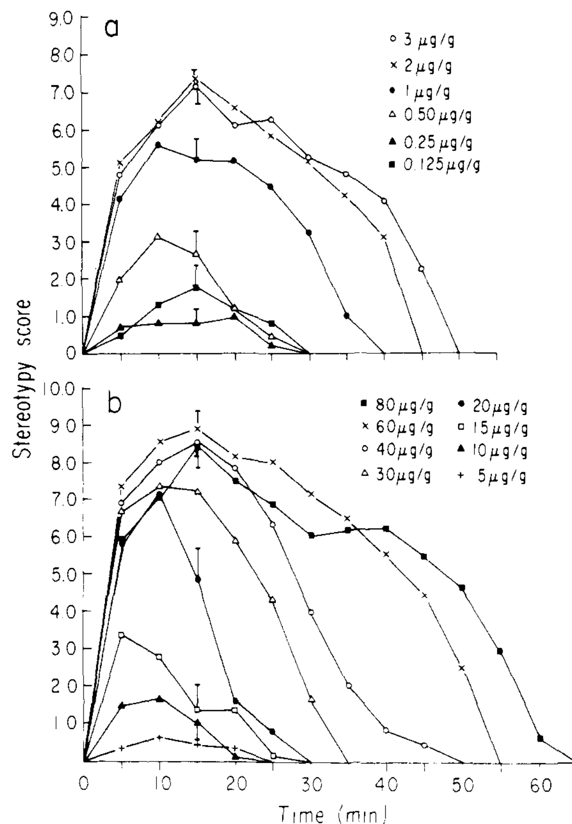


**Figure 3.** (a) Stereotypy scores on intact rats following ip injections of various amounts of **3** plotted against time. (b) Stereotypy scores on intact rats following ip injections of various amounts of **2** plotted against time. In both figures, each point represents the mean of scores on six animals. Scoring was done every 5 min after drug administration. Vertical bars indicating SEM are shown only for scores at 15 min.

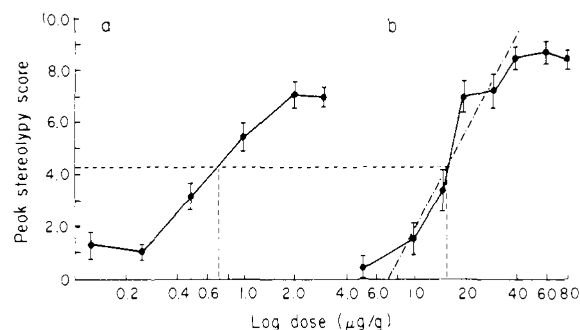


**Figure 4.** (a) Peak stereotypy scores on intact rats following ip injections of **3** plotted against the corresponding log dose. The regression line through the points in the figure corresponding to doses 0.25–1.0 μg/g determined by the method of least squares coincides with the line joining these points. (b) Peak stereotypy scores on intact rats following ip injections of **2** plotted against the corresponding log dose. Line ·–·– is the regression line ($y = 11.80x - 9.94$) determined by the method of least squares for the points in the figure corresponding to doses of 10.0–40.0 μg/g. The dose of **2** required to achieve half the maximum peak score of stereotypy (4.9) induced by it is 17.5 μg/g, about 21 times smaller than that of **3** (0.83 μg/g) required to achieve the same effect. In both figures, each point represents the mean score on six animals. Vertical bars represent the SEM.

vasculature[24a,b] and the rat vas deferens.[24c] This is also consistent with our previous findings[2b] which showed that when **3** and **2** were compared with DA for their ability to stimulate DA-sensitive adenylate cyclase of mouse homogenates of caudate nuclei at 10 μM, **3** was found to be only about 40% as active while **2** was shown to be equipotent

Table III. Effect of Pyrogallol on **2**-Induced Stereotyped Behavior in the Rat

| Treatment | No. of rats | Duration of stereotypy, mean ± SE, min |
|---|---|---|
| **2** | 6 | 32.2 ± 2.0[a] |
| **2** + pyrogallol | 6 | 58.7 ± 3.5 |
| Controls[b] | 12 | |

[a] **2** vs. **2** + pyrogallol; $p < 0.001$. [b] None of the rodents treated with the carrier or the inhibitor and carrier showed stereotypy.

with DA. However, additional supportive evidence is required to demonstrate conclusively that **3** acts as a partial agonist in the CNS. Such agonists can act as antagonists to drugs of greater intrinsic activity. It would be relevant to show whether **3** antagonizes **2** when given together in appropriate ratios. (6) The dose of **2** required to achieve half the maximum peak score of stereotypy (4.5) induced by it is 17.5 μg/g, about 21 times larger than that of **3** (0.83 μg/g) required to achieve the same effect.

**Inhibition of COMT and Stereotyped Behavior.** The suggestion that O-methylation of **2** may be an important metabolic pathway in vivo led us to study the effect of pyrogallol, a COMT inhibitor, on the duration of **2**-induced stereotypy in rats. Rats were pretreated with pyrogallol or with the diluent in the manner described by Missala et al.[12a] followed by the ip injection of **2** (25 μg/g). Table III shows that the mean duration of stereotypy of rats treated with **2** following pretreatment with the diluent was 32.2 min, while pretreatment with pyrogallol prolonged the stereotypy effects to 58.7 min ($p < 0.001$). Control animals injected with the diluent after pretreatment with the inhibitor showed sedation but no evidence of stereotyped behavior.

**Rotation in Nigra-Lesioned Rats.** Unilateral lesions with 6-OHDA in the substantia nigra of rats cause contralateral rotation when DA agonists are administered. Therefore, such nigra-lesioned rats are frequently used for assessing DA agonists.[2b,25] A comparison of the rotary behavior induced by **3** (1 μg/g, ip) and **2** (25 μg/g, ip) showed the following [Figure 2 (b)]. (1) Onset was almost immediate for both drugs. (2) The peak turning rate was reached within 4–5 min after the administration of either drug. In contrast, peak intensity of stereotypy in intact rats required 10 min to develop. (3) There were no significant differences between **2** and **3** with respect to either turning rate or duration.

Figure 2 shows that the peak blood concentration of **2** in the two intact rats and the peak turning rate in the lesioned rats were attained simultaneously, 4–5 min after injection. However, the intensity of stereotypy caused by **2** in intact rats required 10 min to peak. The decrease of rotation closely paralleled the fall in blood concentrations. The Spearman rank correlation test[26] yielded a correlation coefficient of 0.98 ($p < 0.0001$) between the mean number of rotations in nigra-lesioned rats induced by **2** (25 μg/g, ip) and its blood concentrations in the two rats. This high correlation coefficient indicates a direct relation between CNS effects and blood concentration of PBD. Moreover, the correspondence of the drug's peak concentration in blood with the peak rotational rate suggests that the equilibration of 2 between CNS and blood was rapid. This is consistent with the fact that the peak of radioactivity of [$^3$H]-**3** (1 μg/g, ip) injected in mice was attained in brains 5 min following its administration and coincided with the peak of its behavioral effects.[6]

In contrast to rotatory behavior, the intensity of stereotypy induced by **2** (25 μg/g, ip) in intact rats showed a low correlation coefficient of 0.59 ($p < 0.06$) with **2** concentration in rat blood. This is consistent with the hypothesis that rotatory and stereotyped behavior result from stimulation of two functionally different dopaminergic receptors.[27]

## Experimental Section

Uncorrected melting points were determined on a Thomas-Hoover apparatus. $^1$H NMR spectra were recorded on a Varian T-60 in $CDCl_3$ or $Me_2SO$-$d_6$ with $(Me)_4Si$ as the internal standard. Eastman chromagram sheets (6060 silica gel with fluorescent indicators) were used for TLC. The following TLC solvent systems were used: (A) cyclohexane–EtOAc, 4:1; (B) 1:1; (C) 1:4; (D) $C_6H_5$–MeOH, 4:1; (E) 3:2; (F) 1-butanol–$H_2O$–AcOH, 4:1:1; (G) $CHCl_3$–MeOH–AcOH, 17:2:1; (H) 1-propanol–$H_2O$, 89:28. Visualization was done with UV and/or $I_2$ vapors. Infrared spectra were obtained on a Perkin-Elmer 267 grating spectrometer. A gas chromatograph, Hewlett-Packard 7610, with a flame ionization detector and a 6 ft × 2.0 mm column (5% silicone OV-17 on 80–100 Chromosorb W-HP) was used for separating and identifying the *O*-methyl isomers. Elemental analyses were done by Galbraith Labs, Inc. Radioactivity was measured in Aquasol with [$^{14}$C]toluene as an internal standard in a Beckman LS-100C liquid scintillation counter. Where analyses are indicated by symbols of the elements, the analytical results were within ±0.4% of the theoretical values.

**Materials:** *S*-adenosyl-L-methionine-*methyl*-$^{14}C$ sulfate ($^{14}$C-AMe) (sp act. 56.7 mCi/mmol), Aquasol, [$^{14}$C]toluene (sp act. 9.9764 × $10^4$ dpm/g) (New England Nuclear); S-adenosyl-L-methionine sulfate (AMe) (Boehringer, Mannheim); NPA (Winthrop Sterling); 3-methoxy-4-hydroxyphenylacetic acid (Sigma Chemical Co.); 3-hydroxy-4-methoxybenzaldehyde (K & K Laboratories, Inc.); *N*-*n*-propyl-*N*-*n*-butylamine (ICN Pharmaceuticals); (–)-apomorphine hydrochloride (Merck & Co.); *N*-*n*-propyl-(–)-norapomorphine hydrochloride (Sterling Winthrop Research Institute); catechol, Eastman Chromagram sheets, alumina (No. 6063) and silica gel (No. 6060) (Eastman Organic Chemicals).

**Animals.** Intact (200–250 g) and nigra-lesioned rats (150 ± 5 g at the time of operation) were male, Sprague–Dawley. Nigra-lesioned rats were prepared by injecting stereotaxically 6-OHDA in the zona compacta of the right substantia nigra[25a] according to Ungerstedt et al.[28] Four weeks later, successfully lesioned rats rotated contralaterally to the lesion when tested with **3** (1 μg/g).

**Benzyl 3-Methoxy-4-benzyloxyphenylacetate (6).** The preparation of **6** was carried out under anhydrous conditions. To 25 mL of absolute methanol was added with stirring 1.3 g (0.055 mol) of freshly cut Na metal over a period of 10–15 min, followed by the addition of 5.0 g (0.027 mol) of 3-methoxy-4-hydroxyphenylacetic acid, most of which went into solution upon heating. To this was added dropwise 9.4 g (0.055 mol) of benzyl bromide over a period of 15–20 min while heating. After refluxing for 5 h, the reaction mixture was allowed to stand overnight and then filtered. The addition of 100 mL of ether caused additional precipitation of solid material which was removed by filtration. The ether layer was dried over anhydrous $MgSO_4$ after it was washed with a cold $Na_2CO_3$ solution followed by water. During the removal of the ether under reduced pressure, a white solid crystallized out, yielding a crude product of 6.3 g: mp 57–62 °C. A recrystallization of this from EtOH–$H_2O$ yielded a final product of 5.3 g: mp 68–69 °C; TLC (A, B, and C) showed only one spot; IR (KBr) 1726 cm$^{-1}$ (C=O stretch, ester), OH stretching band absent; the $^1$H NMR spectrum ($CDCl_3$) was consistent with its structure. Anal. ($C_{23}H_{22}O_4$) C, H.

**3-Methoxy-4-benzyloxyphenylacetic Acid (7).** A solution of 25 mL of MeOH, 12 mL of $H_2O$, 0.9 g of KOH, and 3.62 g (0.01 mol) of **6** was refluxed for 2 h. After the $CH_3OH$ was removed under reduced pressure, the oily residue was diluted with 40 mL of water, acidified with 6 N aqueous HCl, and extracted with ether (2 × 40 mL). The combined ether layers were washed with water and dried over anhydrous $MgSO_4$. The solvent was partially removed followed by the slow addition of ligroine (bp 30–40 °C fraction) until crystallization started. A yield of 2.3 g (94%) was obtained: mp 119–120 °C. Recrystallization from benzene-ligroine (bp 30–40 °C fraction) did not improve the melting point: TLC (D, E, and F) showed one spot; IR (KBr) 1700 cm$^{-1}$ (C=O stretch,

carboxyl); the $^1H$ NMR spectrum ($CDCl_3$) was consistent with its structure. Anal. ($C_{16}H_{16}O_4$) C, H.

**3-Methoxy-4-benzyloxyphenylacetyl chloride** was prepared in the same manner as 3,4-dimethoxyphenylacetyl chloride.[2b] The solvent and excess $SOCl_2$ were removed initially under reduced pressure and finally under high vacuum. The yield of a dark red oil was quantitative and the crude product was used in the next step without further purification: IR (film) 1790 $cm^{-1}$ (C=O stretch, acyl chloride), OH stretching band absent.

***N*-*n*-Propyl-*N*-*n*-butyl-$\beta$-(3-methoxy-4-benzyloxyphenyl)acetamide (8).** 8 was prepared in the same manner as *N*-methyl-*N*-*n*-propyl-$\beta$-(3,4-dimethoxyphenyl)acetamide[2b] using, however, freshly distilled dimethylformamide instead of chloroform as a solvent. The amide product, an oil, weighed 4.36 g (99%): TLC (A, B, and C) showed only one spot; IR (film) 1635 $cm^{-1}$ (C=O stretch, tertiary amide), OH, NH, and COCl stretching bands completely absent; the $^1H$ NMR spectrum ($CDCl_3$) was consistent with its structure.

***N*-*n*-Propyl-*N*-*n*-butyl-$\beta$-(3-methoxy-4-benzyloxyphenyl)ethylamine Hydrochloride (9).** 9 was prepared in the same manner as *N*-methyl-*N*-*n*-propyl-$\beta$-(3,4-dimethoxyphenyl)ethylamine hydrochloride.[2b] The crude product was recrystallized from $CH_2Cl_2$–EtOAc: wt 2.1 g (83%); mp 98–100 °C; TLC (G, H) showed one spot; IR (KBr) showed complete absence of the NC=O stretch band; the $^1H$ NMR spectrum ($CDCl_3$) was consistent with its structure. Anal. ($C_{23}H_{34}ClNO_2$) C, H, Cl, N.

***N*-*n*-Propyl-*N*-*n*-butyl-$\beta$-(3-methoxy-4-hydroxyphenyl)ethylamine Hydrochloride (4).** A solution of 2.09 g (0.005 mol) and 50 mL of MeOH was hydrogenated over 0.3 g of 10% Pd/C in a Parr shaker hydrogenator at 0 psig and room temperature. The reaction was complete in 2 h as shown by the amount of $H_2$ absorbed. The catalyst was filtered off with the aid of "filter aid", and the solvent was removed under reduced pressure. The solid residue was crystallized from absolute EtOH–ether, filtered, washed with ether, and dried to a constant weight: 1.48 g (93%); mp 116–118 °C; TLC (F, G, and H) showed one spot; the $^1H$ NMR spectrum ($CDCl_3$) was consistent with its structure. Anal. ($C_{16}H_{28}ClNO_2$) C, H, Cl, N.

***N*-*n*-Propyl-*N*-*n*-butyl-$\beta$-(3-hydroxy-4-methoxyphenyl)ethylamine Hydrochloride (5).** 5 was prepared in the same manner as 4 using, however, as a starting material 3-hydroxy-4-methoxyphenylacetic acid prepared from 3-hydroxy-4-methoxybenzaldehyde.[13] Upon refluxing the borane complex of *N*-*n*-propyl-*N*-*n*-butyl-$\beta$-(3-benzyloxy-4-methoxyphenyl)ethylamine with methanolic HCl (Scheme I, step 5), the product was found by TLC and $^1H$ NMR to be partially debenzylated. Because the resultant mixture could not be separated by crystallization, it was subjected to hydrogenolysis yielding 5. The solid residue was crystallized from EtOH–ether, filtered, washed, and dried to constant weight: 1.15 g (72%); mp 144.5–146 °C; TLC (F, G, and H); the $^1H$ NMR spectrum ($CDCl_3$) was consistent with its structure. Anal. ($C_{16}H_{28}ClNO_2$) C, H, Cl, N.

**Preparation of COMT.** Rat liver was used according to Nikodejevic et al.[29] but, in the final purification step, the enzyme was dialyzed against 0.001 N $Na_2HPO_4$ instead of being passed through a chromatography column.

**O-Methylation.** In the method used,[7a] mercaptoethanol was replaced with $Na_2S_2O_5$. The incubation medium consisted of 0.1 mL of COMT (approximately 1.5 mg of protein determined by the Lowry method),[30] 125 $\mu$mol/0.5 mL of potassium phosphate buffer (pH 7.9), 20 $\mu$mol/0.2 mL of $MgCl_2$, 0.0088 $\mu$mol/0.1 mL (1:4 dilution) of $^{14}C$-AMe (sp act. 56.7 mCi/mmol), 0.05 $\mu$mol/0.1 mL of AMe, 0.0002–0.004 $\mu$mol of 2 in 0.2 mL water, or 0.2 mL of heparinized whole blood with 2.5% $Na_2S_2O_5$ (w/v). Results were also compared and assays made with catechol, 1, 3, 4, and 5 (0.004 $\mu$mol/0.2 mL of water) (Table II). Blanks were incubated in which the substrate was replaced with 0.2 mL of its vehicle.

The incubation was initiated by the addition of the COMT and carried out in a shaker–incubator at 37 °C. The incubation time varied from 10 to 150 min and was stopped with 0.5 M borate buffer, pH 10.0. Each sample was extracted twice with 3 mL of heptane–isoamyl alcohol (3:1). Aliquots (1 mL) were assayed for radioactivity after evaporation.

**TLC and Autoradioassay.** Twenty micrograms of either 4 or 5 were required for visualization under UV on silica chromagrams. Accordingly, 0.211 $\mu$mol of $^{14}C$-AMe, 1.19 $\mu$mol of "cold" AMe, and 0.5 $\mu$mol of 2 were added to the incubation mixture and incubated for 2 h. The samples were thereafter extracted twice, and the extracts were combined and gently evaporated under vacuum at room temperature. The residues were dissolved in 0.1 mL of methanol and used to spot silica gel plates. 4 and 5 of approximately the same amount were spotted on the same plate. Solvent systems F and H were used to develop the plates followed by visualization with UV and $I_2$ vapors. TLC plates were exposed to photographic film for 3.5 h.

**Gas Chromatography.** The separation of 4 and 5 was achieved by GC with corresponding retention times of 8.66 and 7.68 min using a flow rate of helium of 30 mL/min, injection port temperature 270 °C, flame temperature 270 °C, and oven temperature 175 °C. The mean ratio of peak heights ($R_1$) of 5 to 4 determined on a total of four samples consisting of equal amounts of the two synthetic isomers was equal to 1.11. The mean ratio of peak heights ($R_2$) of the extracted products of four incubations was equal to 1.55. The actual ratio of the two isomers was calculated from $R_2/R_1$.

**Disappearance Rate of PBD in Rat Blood.** The mean percent recovery of 2 and its SE (82.7 ± 1.8%) was determined on seven rat blood samples to which 0.18 $\mu$g of 2 was added per milliliter of sample. Thereafter, two male intact rats weighing 400–440 g were injected ip with a normal saline solution of the HCl salt of 2 (25 $\mu$g/g) and six to nine blood samples (100-$\mu$L sample) were removed from the tail during the ensuing 1 h. The samples were transferred to the incubation mixtures. Blanks were blood samples removed prior to injections of 2. Results, corrected for recovery, were plotted as micrograms of 2 per milliliter of blood as a function of time [Figure 2 (a)].

**Stereotyped Behavior (Intact Animals).** Test animals were maintained on a 12-h light cycle in an air-conditioned room and testing was carried out in a diffusely illuminated room at 24 ± 1 °C, between 10.00 and 12.00 h. Each rat tested was housed individually in a transparent plastic box (45 × 23 × 20 cm) and allowed to adapt to its surroundings for 20 min prior to the drug injection. A total of 36 rats was tested. Following drug injection, each rat was scored at 5-min intervals for sniffing, licking, biting and/or gnawing, head tremor, ptosis, and rearing and/or locomotor activity of a repetitive nature. Each item was graded for intensity as 0 for none, 1 for moderate and/or intermittent, and 2 for intense and/or continuous. Scores were summed, and means and SE were calculated for each group and plotted as a function of time (Figure 3). Each rat was injected ip with a normal saline solution of the HCl salts of 3 (0.125–3.0 $\mu$g/g, ip), 2 (5–80 $\mu$g/g, ip), and with the vehicle on separate occasions with a 1-week interval between injections. Animals were selected and injected at random in groups of three, followed by blind scoring.

**Inhibition of COMT and Stereotyped Behavior.** Pyrogallol dissolved in physiological saline was injected ip in intact rats in doses of 250 $\mu$g/g at 60 min and 10 $\mu$g/g at 30 min before ip injection of 2 (25 $\mu$g/g). Control rats were treated identically but with either the omission of the drug or of the drug and the inhibitor. Animals were injected in groups of three and observed for stereotypy under conditions described in the previous section. The duration of stereotypy was recorded, and means and SE were computed (Table III).

**Rotatory Behavior of Nigra-Lesioned Rats.** The number of turns induced by 2 (25 $\mu$g/g, ip) and 3 (1.0 $\mu$g/g, ip) was measured in the same six rats with a 1-week interval between injections in automatic recording rotometers.[25a] The means and SE of their rotations were recorded and plotted as turns per minute vs. time [Figure 2 (b)]. In each test, a lesioned rat injected ip with normal saline solution served as a control; controls showed either occassional random or no turning.

**Testing of 4 and 5 in Nigra-Lesioned Rats.** Normal saline solutions of the HCl salts of either 4 or 5 (105 $\mu$g/g, ip) were injected in the same group of four lesioned rats with a 1-week interval between injections.

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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*-propylnoraporphine (**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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