5a) was omitted. After 3 h, the reaction was stopped by addition of 0.08 mL of $HClO_4$. Alumina (0.3 g) was added; the suspensions were sonicated for 5 min, filtered through 0.2-μm Arco disk filters, and injected into the HPL column in 10.0-μL aliquots as described for the standards.

Registry No. 5a, 58-00-4; 5c, 641-36-1; (*RS*)-6, 99755-62-1; (*RS*)-7·HCl, 131792-44-4; (*RS*)-8·HCl, 131792-45-5; 9, 78136-55-7; 10, 121086-26-8; 11, 131792-46-6; (*RS*)-12·2HCl, 131792-47-7; (*RS*)-13·HCl, 131792-48-8; 14, 1195-09-1; 15, 4790-01-6; 16, 2495-83-2; 17, 131792-49-9; (*RS*)-18·HCl, 131792-50-2; (*RS*)-19·HCl, 131792-51-3; COMT, 9012-25-3; 2-methoxy-4-methylphenol, 93-51-6; isoquinoline methiodide, 3947-77-1; 3-hydroxy-4-methoxybenzaldehyde, 621-59-0.

The Enantiomeric Specificity of the Antihypertensive Activity of 1-(Phenylthio)-2-aminopropane, a Synthetic Substrate Analogue for Dopamine β-Monooxygenase

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We have found that (*R,S*)-1-(phenylthio)-2-aminopropane (**4a**), a synthetic alternate substrate for the terminal enzyme of norepinephrine biosynthesis, dopamine β-monooxygenase (DBM), is both an indirect sympathomimetic and a potent antihypertensive agent in spontaneously hypertensive rats. We demonstrate herein that there is a distinct enantiospecific difference in the activities of (*R*)-1-(phenylthio)-2-aminopropane (**4b**) and (*S*)-1-(phenylthio)-2-aminopropane (**4c**). We find that **4c**, the more potent DBM substrate analogue, exhibits both the indirect sympathomimetic activity and the antihypertensive activity previously observed for the racemate and inhibits the active transport of catecholamines at the nerve terminal. In contrast, **4b**, which is less potent as a DBM substrate or as an inhibitor of catecholamine uptake, does not exhibit an indirect sympathomimetic effect and is not an effective antihypertensive agent. These results suggest that the greater selectivity of the *S* enantiomer for both the catecholamine reuptake transporter and the target enzyme DBM accounts for its greater potency as an indirect-acting sympathomimetic agent as well as its activity as an antihypertensive agent. These results are also consistent with the hypothesized mechanism of action of this class of sulfur-containing DBM substrate analogues.

(*R,S*)-1-(Phenylthio)-2-aminopropane (**4a**, Scheme I) was synthesized and characterized in our laboratories as one derivative of a class of sulfur-containing substrate analogues for dopamine β-monooxygenase (EC 1.14.17.1, DBM), the terminal enzyme in the biosynthetic pathway to norepinephrine. As we have previously reported, **4a** is both an appreciable substrate for DBM and the most potent antihypertensive derivative of this class when administered in either acute or subchronic dosing protocols to spontaneously hypertensive rats (SHR).[1,2] In addition, **4a** is the most potent indirect sympathomimetic of all of the derivatives of this class, a characteristic which we have demonstrated is directly related to its ability to gain entrance to adrenergic neurons via the catecholamine reuptake mechanism.[2,3]

Although the exact in vivo mechanism of action is yet to be established, we have hypothesized that compounds of this class undergo adrenergic neuronal uptake, subsequent uptake into neurotransmitter vesicular stores, enzymatic oxygenation therein by DBM, and release of DBM-generated "false transmitter" sulfoxide products.[3] While **4a** was originally synthesized in order to assess the effects of monoamine oxidase catabolism on the biological half-life of compounds of this class, its potent antihypertensive activity suggested that the racemate be resolved and the enantiospecificity of the biological effects of **4b** and **4c** be examined. This was particularly important since it is known that α-methylation of monoamine analogues of catecholamines imparts adrenergic receptor specificity.[4]

In the present study, we have separately synthesized and characterized the *R* and *S* enantiomers of 1-(phenylthio)-2-aminopropane (**4b,c**) in order to determine which aspects of the enzymological and cardiovascular activities of these compounds are enantiospecific. We demonstrate herein that **4c** is both an indirect sympathomimetic and a potent antihypertensive agent in SHR. In contrast, **4b** is virtually inert in the cardiovascular assays employed. We also report herein that **4c** is ca. twice as potent as the *R* enantiomer, **4b**, in inhibiting dopamine uptake into rat brain striatal synaptosomes. Finally, we have found that

Scheme I. Synthetic Route to (*R*)- and (*S*)-1-(Phenylthio)-2-aminopropane (**4b,c**)

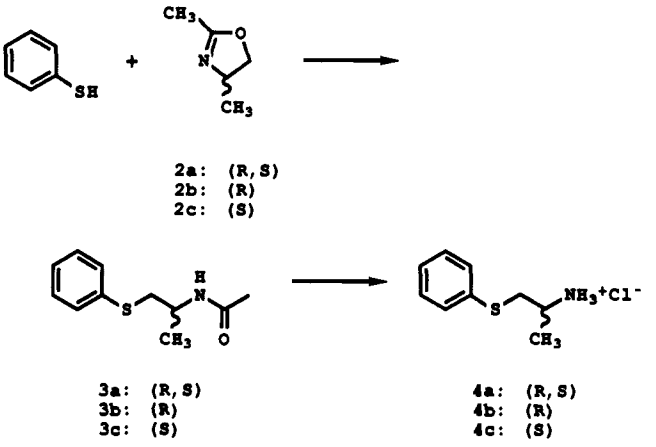

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Table I. DBM Kinetic Parameters[a]

compound	K_m, mM	k_{cat}, s^{-1}	k_{cat}/K_m, M^{-1} s^{-1}
(S)-1-(phenylthio)-2-aminopropane (4c)	7.7	37	4.8×10^3
(R)-1-(phenylthio)-2-aminopropane (4b)	19.0	13	0.7×10^3
tyramine[b,c]	2.0	121	6.0×10^4
phenylpropylamine[b]	20.4	17	0.8×10^3

[a] Kinetic assay conditions as detailed in the Experimental Section. [b] Kinetic values previously reported.[1] [c] The oxygenation by DBM of dopamine (the physiological substrate) has been reported to proceed at 93% of the activity of tyramine.[17]

4c is a more facile DBM substrate analogue in vitro than its enantiomer, 4b. These results suggest that the ability of the S enantiomer to be preferentially internalized by the nerve terminal, combined with its greater suitability as a DBM substrate, contributes to both the indirect-acting sympathomimetic activity as well as the antihypertensive properties of this compound.

Chemistry

The synthetic route to the N-acetyl derivatives (Scheme I) employed the Wehrmeister reaction[5,6] of thiophenol with the indicated 2,4-dimethyl-2-oxazoline. The chiral oxazolines were prepared by the method of Meyers et al.[7] beginning with either (R)- or (S)-2-amino-1-propanol. Hydrolysis of 3 in refluxing HCl gave the corresponding 1-(phenylthio)-2-aminopropane enantiomers, 4b and 4c. Derivatization of the isolated product with N-succinimidyl 2(S)-methoxy-2-phenylacetic acid ester (5) to form the diastereomeric adducts, followed by normal-phase HPLC separation,[8] allowed the determination that there was only a ca. 90% enantiomeric excess in either case, presumably as a result of enantiomeric contamination of the commercially obtained 2-amino-1-propanols. Since it would be important that the enzymological characterizations not be affected by enantiomeric impurities, semipreparative derivatization with compound 5, HPLC separation of the diastereomers, and inverse derivatization was employed in order to increase the enantiomeric purity. The resultant final preparations of both 4b and 4c were reanalyzed and each was found to have an enantiomeric excess of 99.8%.

Enzymatic Studies

The substrate kinetic parameters of 4b and 4c with DBM are shown in Table I. It is apparent that 4c is a more facile substrate than is 4b for DBM oxygenation, with their k_{cat}/K_m values differing by a factor of 7. By comparison, while these enantiomers are less effective DBM substrates than tyramine, a standard DBM assay substrate, 4c is a much better substrate for DBM than the corresponding carbon-containing analogue, 1-phenyl-3-propylamine. Nevertheless, 4b is still an appreciable substrate for DBM.

Bioassay Studies

A. Indirect Sympathomimetic Activity. As we have previously noted, all tested derivatives of this sulfur-containing class of DBM substrate analogues, when given systemically as a bolus in saline, cause the appearance of a transient indirect sympathomimetic effect.[1,2] 4a is the most potent derivative of this class in this regard.[2] When the individual R and S enantiomers, 4b and 4c, are compared for the dose–response nature of this transient initial hypertensive effect in normotensive rats (Figure 1), it is evident that 4c possesses marked activity at all dose levels which is indistinguishable from that of the racemate.[2] In contrast, the transient initial sympathomimetic activity of 4b is markedly lower than that of the S enantiomer, 4c, at all dose levels. A sympathomimetic effect such as that observed for 4c would be expected for either direct infusion of norepinephrine or for the infusion of a compound which is acting to cause the release of intraneuronal stores of norepinephrine (classical indirect sympathomimesis). We have previously demonstrated that the transient sympathomimesis exhibited by racemate 4a is classical indirect sympathomimesis and is blockable by cocaine,[2] suggesting that these compounds utilize the catecholamine transport system to gain access into the nerve terminal.

B. Antihypertensive Activity. The time course of the acute effects of either 4b or 4c on mean systemic blood

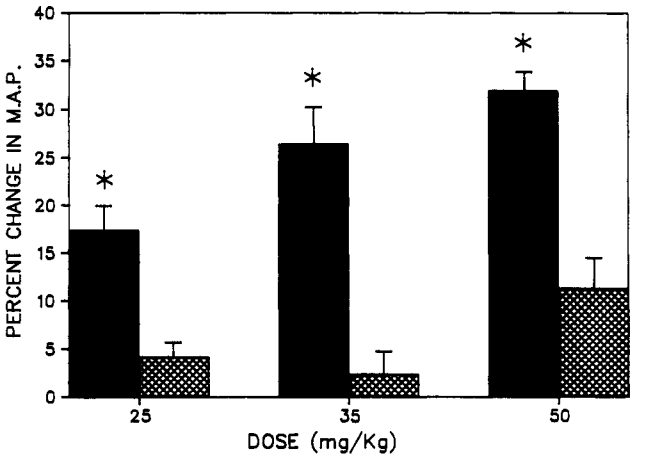

Figure 1. Transient initial sympathomimetic effect of (R)- and (S)-1-(phenylthio)-2-aminopropane (4b,c) on mean arterial pressure. Normotensive male WKY rats (avg wt 245 g) were administered a single ip dose of either 4c (dark bars) or 4b (hatched bars) while pulsatile blood pressure was monitored as noted in the Experimental Section. The indicated doses were administered as boli in 0.9% saline. Data are presented as mean values ($N = 5$); *, statistically significant difference ($p < 0.05$; two-tailed paired t test).

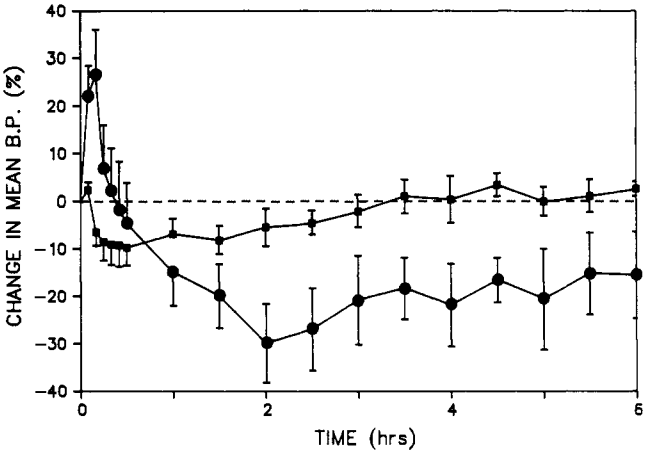

Figure 2. Effect of (R)- and (S)-1-(phenylthio)-2-aminopropane (4b,c) on mean arterial pressure in SHR. Male SHR rats (avg wt 255 g) were administered a 35 mg/kg dose, ip, of either 4c (filled circles) or 4b (filled squares) while pulsatile blood pressure was monitored as noted in the Experimental Section. The indicated doses were administered as boli in 0.9% saline. Data are presented as mean responses ± SEM ($N = 5$). Saline controls ($N = 27$) gave essentially no response (dotted line).

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Table II. Inhibition of Dopamine Uptake in Rat Brain Striatal Synaptosomes[a]

compound	IC_{50}, μM
(*S*)-1-(phenylthio)-2-aminopropane (**4c**)	6.5 ± 0.4[b]
(*R*)-1-(phenylthio)-2-aminopropane (**4b**)	9.8 ± 1.8[b]

[a] Assay conditions as detailed in the Experimental Section. [b] Average (N = 3) ± SEM. Values are statistically significantly different ($p < 0.02$; two-tailed paired t test).

pressure (bp) in spontaneously hypertensive rats (SHR) is presented in Figure 2. A 35 mg/kg dose of **4b** (ip bolus in saline) causes only a minor change in mean systemic bp with an maximum decrease in mean pressure of 10% and a duration of action of less than 3 h. In contrast, an identical dose of **4c** in SHR causes the development of a marked hypotension which is apparent within 1 h postinjection and which persists beyond the length of the measuring period. An average decrease in mean pressure of 25% was maintained for the terminal 5 h of the experiment. Note that the marked difference in the transient initial indirect sympathomimetic activity of each enantiomer previously noted in normotensive rats (Figure 1) also is apparent in SHR, with **4c** causing an initial increase in pressure which persists for the initial 20–30 min postinjection. The antihypertensive activity determined in these experiments for **4c** was virtually identical with that obtained in earlier work for a 50 mg/kg dose of racemate **4a**.[2] Control bioassay experiments in which normal saline is given in place of test compound gave essentially no effect on systemic pressure over the same time course (dotted line). A comparison of the time course of the antihypertensive activity of **4c** over a range of 25–50 mg/kg resulted in a shallow dose–response curve (data not shown), suggesting that maximal activity had been approached.

C. Effects on Dopamine Uptake. In view of the striking difference in the indirect sympathomimetic activities of the enantiomers, experiments were performed to determine whether or not these observed effects could be related to an enantiospecific difference in their ability to gain entrance to neuronal cells. In experiments in which the sodium-dependent uptake of [^{3}H]dopamine into striatal synaptosomes was quantitated,[9,10] both **4b** and **4c** were shown to be capable of inhibiting dopamine uptake. Analysis of inhibition curves (percent inhibition of [^{3}H]-dopamine uptake vs **4b** or **4c** concentration) allowed the determination of IC_{50} values for each enantiomer (Table II). It is clear that *S* enantiomer **4c** is ca. twice as potent as *R* enantiomer **4b** in inhibiting [^{3}H]dopamine uptake.

The results of these studies suggest that the previously noted antihypertensive activity of racemate[3] **4a** resides primarily with *S* enantiomer **4c**. The disparity in the activity of the two enantiomers of 1-(phenylthio)-2-aminopropane is most likely due to the fact that, in addition to being more readily transported into the sympathetic nerve terminals which modulate peripheral cardiovascular tone, *S* enantiomer **4c** is also converted more rapidly by DBM into the sulfoxide. The striking difference in the transient initial indirect sympathomimetic activity of the enantiomers (Figures 1 and 2) suggests that **4c** is capable of much more facile uptake into adrenergic neurons. This supposition is supported by the finding of a lower IC_{50} for the inhibition of [^{3}H]dopamine uptake into striatal synaptosomes by **4c** (Table II). Transport of [^{3}H]dopamine into striatal synaptosomes was studied because it provided a means to separate the effects of the enantiomers on catecholamine transport from their effects on DBM. Although their relative potencies may vary, most enantiomeric compounds which exhibit stereoselectivity at the norepinephrine transport complex demonstrate analogous stereoselectivity at striatal dopaminergic nerve terminals which contain no DBM.[11,12]

When considered alone, the observed differences in DBM substrate activity between enantiomers **4b** and **4c** (Table I) may appear insufficient to account for the observed differences in antihypertensive activity. It must be borne in mind, however, that the higher intraneuronal substrate concentration resulting from the more efficient transport of **4c** may well lead to an amplification in the amount of DBM-oxygenated product formed. We have previously shown that phenyl aminoethyl sulfide, the non-α-methylated parent compound of this class, is capable of adrenergic vesicular uptake and DBM oxygenation.[13] Thus, the differential responsiveness of these two critical processes to the stereoisomeric characteristics of the substrate may lead to marked differences in their antihypertensive efficacy. Finally, the kinetic disparity in the DBM-catalyzed turnover of enantiomers **4b** and **4c** must be highlighted. To our knowledge, this is the first example of an enantiomeric difference in DBM turnover as a result of substitution at a chiral center two atoms removed from the benzylic center of catalysis.

Experimental Section

Equipment. NMR spectra were obtained on a Varian T-60 NMR spectrometer. Mass spectra were obtained on a Finnigan MAT 112S instrument with a SS200 data system. A Laboratory Data Control (Riviera Beach, FL) high-performance liquid chromatograph was used for separations of enantiomers, with a Constametric III pump and a Spectro Monitor III (Model 1204D) variable-wavelength detector. Separations were achieved with either a 25 cm (21.4 mm i.d.) Dynamax Macro semipreparative silica column or a 25-cm silica column (5 μm, analytical) from Rainin (Woburn, MA). Radiolabeled samples were counted on a Beckman LS 8000 scintillation counter. Microanalyses were performed by Atlantic Microlabs, Atlanta, GA, and analytical results obtained were within ±0.4% of the theoretical values unless otherwise indicated.

Chemicals. (*R,S*)-, (*R*)-, and (*S*)-2-amino-1-propanol, thiophenol, phenylethylamine, and ethyl acetimidate were purchased from Aldrich Chemicals. Tyramine hydrochloride was from Sigma. All solvents were from Fisher.

(*R,S*)-2,4-Dimethyl-2-oxazoline (2a). This compound was synthesized according to the general method of Meyers et al.[6] (*R,S*)-2-Amino-1-propanol (70 mmol) was added to a stirred mixture of ethyl acetimidate hydrochloride (87 mmol) and dry methylene chloride (60 mL) at 0 °C under argon. The resulting solution was stirred at 0 °C for 6 h and was then poured into ice water (100 mL). The aqueous phase was extracted (3×) with 20 mL of CH_2Cl_2, and the combined extracts were dried over Na_2SO_4. The solution was distilled through a 6-in. column to yield the product (55%): bp 109–112 °C; ^{1}H NMR ($CDCl_3$) δ 4.39–3.48 (m, 3 H), 1.89 (s, 3 H), 1.17 (d, 3 H).

(*R*)- and (*S*)-2,4-Dimethyl-2-oxazoline (2b,c). These compounds were synthesized exactly as outlined above except that either (*R*)- or (*S*)-2-amino-1-propanol was employed. ^{1}H NMR data were identical with that above.

(*R,S*)-1-(Phenylthio)-2-aminopropane Hydrochloride (4a). This compound was prepared by a modification of the method of Wehrmeister.[4,5,7] (*R,S*)-2,4-Dimethyl-2-oxazoline (36 mmol) was added to thiophenol (40 mmol) under argon. After an initial

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exothermic reaction, the solution was warmed to 80–100 °C for 6 h to ensure complete formation of the acetamide of (*R,S*)-1-(phenylthio)-2-propylamine (**3a**). To effect hydrolysis of **3a**, 6 N HCl (300 mL) was added to the cooled reaction mixture and set to reflux for 6 h. The resulting solution was washed with $CHCl_3$ (3 × 50 mL) and the aqueous layer evaporated to dryness to give a white solid. The crude product was purified by recrystallization from ethanol/ether to yield the product (85%): mp 154–155.5 °C; ^1H NMR (D_2O) δ 7.45 (m, 5 H), 3.72–3.12 (m, 3 H), 1.38 (d, 3 H); mass spectrum (EI) *m/e* 167 (M^+); (*CI*) *m/e* 168 (M + 1). Anal. ($C_9H_{13}NS$·HCl) C, H, N.

(*R*)-1-(Phenylthio)-2-aminopropane (4b) was synthesized by using the synthetic scheme outlined above for **4a** with the appropriate chiral oxazoline and thiophenol. The HPLC purification to increase the enantiomeric purity was performed by derivatizing ca. 1 g of the free base of the synthesized enantiomer with 1.5 g of *N*-succinimidyl 2(*S*)-methoxy-2-phenylacetate (**5**) in refluxing THF for 1 h.[8] A semipreparative silica gel HPLC column equilibrated with 4:1 (v/v) hexane/ethyl acetate was used to separate the resultant diastereomers, with a repetitive series of injections and chromatographic separations (ca. 200 mg/injection). The pooled, separated diastereomers were collected, and the eluent was removed by evaporation. The desired diastereomer was hydrolyzed by refluxing overnight in 6 N HCl, the amine hydrochloride was recrystallized from Et_2O/EtOH, and analytical samples were reanalyzed for enantiomeric purity after derivatization with compound **5**: $[\alpha]^{25}_D$ −16.4° (*c* 1.0, H_2O); mp 111–112 °C; ^1H NMR data were identical with that of **4a**. Anal. ($C_9H_{13}NS$·HCl) C, H, N.

(*S*)-1-(Phenylthio)-2-aminopropane (4c) was synthesized by using the synthetic scheme outlined above for **4a** with the appropriate chiral oxazoline and thiophenol, followed by the enantiomeric purification procedure outlined for **4b**: $[\alpha]^{25}_D$ 18.8° (*c* 1.1, H_2O); mp 110–111 °C; ^1H NMR data were identical with that of **4a**. Anal. ($C_9H_{13}NS$·HCl) C, H, N.

DBM Assays and Determination of Kinetic Parameters. DBM activity was measured as described previously.[1] Kinetic constants were obtained by computer fit to the hyperbolic form of the Michaelis–Menten equation to obtain V_{max} and K_m,[14] and for the determination of k_{cat}, the molecular weight of DBM was taken to be 290 000.[15] Determination of the specific activity of the enzyme during each kinetic experiment, using 10 mM tyramine as substrate under standard assay conditions, allowed normalization to an arbitrary specific activity of 14.7 units/mg for DBM. Normalized k_{cat} values were thus obtained by multiplying the calculated k_{cat} values by a scaling factor.

Cardiovascular Assays. Spontaneously hypertensive rats (SHR) and normotensive WKY controls were obtained from Charles River Breeding Laboratories (Wilmington, MA). All tested animals were males (11–15 weeks of age, 250–300 g weight). Animals were housed in an appropriate caging facility, were maintained on a 12-h light cycle beginning at 6 a.m., were allowed food and water ad libitum, and were conscious and unrestrained except during surgery. Pulsatile arterial blood pressure measurement was effected by surgical implantation of carotid catheters as previously described.[16]

Dopamine Uptake Experiments. Striatal tissue from male Sprague–Dawley rats (150–250 g, Harlan Sprague-Dawley, Indianapolis, IN) was the source of synaptosomes for these experiments. Following sacrifice by rapid decapitation, the brain tissue was quickly removed, placed in 0.32 M sucrose, and striatum dissected from whole brain. [^3H]dopamine uptake into striatal synaptosomes was determined by a modification of the method of Harris and Baldessarini,[9] as previously described.[10] Briefly, 250 μL of an S_1 fraction (prepared via homogenization and centrifugation of dissected striatal tissue) diluted 4-fold with modified Krebs–phosphate solution (120 mM NaCl, 4.9 mM KCl, 1.2 mM $MgSO_4$, 11 mM glucose, 160 μM Na_2EDTA, 1.1 mM ascorbic acid, 10 μM parglyine, and 15.5 mM Na_2PO_4, equilibrated with 95% O_2–5% CO_2 and adjusted to pH 7.4 with HCl) was preincubated with 1100 μL of the Krebs–phosphate solution and 100 μL of vehicle or test compound for 10 min at 37 °C prior to the addition of 50 μL of [^3H]dopamine [(3,4-dihydroxy[2,5,6-^3H]phenyl)-ethylamine hydrochloride, specific activity 52.6 Ci/mmol, Du Pont/NEN]. The [^3H]dopamine was diluted with unlabeled dopamine to 10% of its original specific activity; the final concentration of dopamine in the assays was ca. 30 nM. Uptake of dopamine into synaptosomes was terminated by filtration through Whatman GF/C filters under vacuum exactly 2 min after addition of radiolabel, and the filters were washed (2×) with 5 mL of chilled Krebs–phosphate solution, shaken with 8 mL of Beckman Ready-Protein liquid scintillant, and counted in a Beckman liquid scintillation counter. Non-energy-dependent accumulation of [^3H]dopamine was determined by incubating samples at 0 °C rather than at 37 °C.

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Registry No. **4a**, 91281-31-1; **4b**, 122673-69-2; **4c**, 122673-70-5; DBM, 9013-38-1.

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