the reaction was allowed to warm to 25 °C, resulting in a yellow solution. After 30 min, a solution of ethyl 2,5-dimethoxy-4-methylcinnamate (0.836 g, 3.34 mmol) in 2.0 mL of DMF was added. The mixture was stirred at 25 °C for 5 days. The reaction was cooled in an ice bath, and excess H_2O was added. The solution was extracted several times with ether. The ether extract was washed (brine), dried ($MgSO_4$), and filtered, and the filtrate was concentrated to yield 0.88 g of liquid residue. This material was taken up into a minimum amount of ether and crystallized to remove solid byproducts. Concentration of the mother liquor yielded 0.54 g of a yellow liquid. NMR and TLC analysis of this mixture showed its components to be identical with the cyclopropyl esters obtained by the diazoacetate procedure.

Conversion of Ethyl 2-(2,5-Dimethoxy-4-methylphenyl)-3-methylcyclopropanecarboxylates to the Hydrazide 7a or 7b. The mixture of ethyl 2-(2,5-dimethoxy-4-methylphenyl)-3-methylcyclopropanecarboxylates (obtained from the above reaction) was treated with 99% hydrazine hydrate as described above. Purification of the crude hydrazide over a silica gel column by eluting with 2% MeOH in $CHCl_3$ gave two major components, which had NMR and IR spectra and TLC properties identical with the hydrazides (**7a** and **7b**) obtained from esters formed by the diazoacetate method.

Rat Fundus Preparation. Responses to drugs were studied in the isolated rat fundus, prepared from 250- to 350-g male Sprague-Dawley rats which had been fasted overnight but allowed free access to water. Following the method of Vane,[12] strips of fundus, approximately 3 × 10 mm, were suspended in 25-mL organ baths containing Tyrode's solution, maintained at 37 °C, and oxygenated with 95% O_2–5% CO_2. The bathing solution contained 10^{-7} M iproniazid and scopolamine. Strips were placed under a 1-g initial tension, and contractions were measured using a Grass FT03 force-displacement transducer and recorded with a Gould preamplifier and recorder.

A complete dose–response curve was obtained to serotonin for each tissue. Dose–response curves were obtained in a noncumulative manner, with each drug concentration washed out before proceeding to a higher concentration. This gave more reliable responses and kept spontaneous activity of the tissue to a minimum. Only one test drug was used in each preparation. Responses are reported as the percent of the maximum contraction obtainable with serotonin. Points on the dose–response curves in Figure 1 are the means, with vertical bars indicating the standard error.

Mouse Ear Scratch. This was a modification of our previously published procedure.[11] Male, albino, Swiss-Webster derived mice (Laboratory Supply Co., Indianapolis, IN), 25–40 g, were group housed at 22–24 °C under a 12–12 h light–dark cycle with free access to food and water. Mice were given ip drug injections and were observed in groups of three. Drugs were injected in a volume of saline equal to 0.1 mL/10 g of body weight. Mice were observed continuously for 30 min, and the total number of scratches were recorded. A scratching episode, as described by Kulkarni,[14] consisted of "hindleg scratching of the back of the ear and subsequent return of the hindleg to the floor". Significant differences between treatment responses were identified using Student's *t* test.

Acknowledgment. Support for this work was provided by USPHS Grant DA02189 from the National Institute on Drug Abuse. We also acknowledge the excellent technical assistance provided by Diane Tonner and Geraldine Lumsden.

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Effects of Certain Hallucinogenic Amphetamine Analogues on the Release of [^{3}H]Serotonin from Rat Brain Synaptosomes

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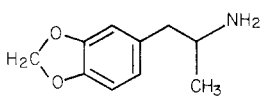
The enantiomers of 3,4-(methylenedioxy)amphetamine (MDA), *p*-methoxyamphetamine (PMA), and *N*-Me-MDA (MDMA), along with their α,α-dimethylated derivatives, were evaluated for an effect on the release of [^{3}H]serotonin from rat whole brain synaptosomes. The amphetamine isomers were all potent in inducing the release of [^{3}H]serotonin at bath concentrations of 1 and 10 μM but were inactive at 0.1 μM. No significant difference in isomer potency was observed at the 10-μM concentration. However, at 1 μM the (+) isomer of MDMA was more effective in inducing release than was the (−) isomer. Since it is the (+) isomer which is clinically active, this result suggests that transmitter release may play a role in the biological activity of MDMA. By contrast, the α,α-dimethyl compounds were not effective in releasing serotonin, even at the highest bath concentration.

Hallucinogenic phenethylamine derivatives produce their central effects through a stereoselective process. In those cases which have been examined, the pharmacological effects are selectively elicited by the isomer possessing the *R* absolute configuration.[1–6] N-Methylation considerably attenuates or abolishes activity in this series, with the notable exception of 3,4-(methylenedioxy)amphetamine (MDA, **1**).[7] In the case of MDA a curious reversal



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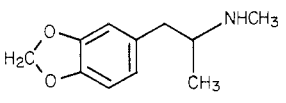

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of stereoselectivity is observed; (*S*)-(+)-*N*-Me-MDA [(*S*)-**2**, (*S*)-(+)-MDMA] is more potent than its optical isomer. Further, cross tolerance does not develop between **1** and **2**, possibly indicating different mechanisms of action.[7] It was previously argued that while (*R*)-**1** might possess a direct receptor action, the effects of (*S*)-**2** might be mediated by release of endogenous neurotransmitter.[7] This was based partly on the proposal by Cheng et al.[8] that

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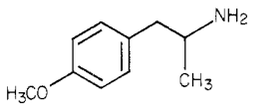
hallucinogenic phenethylamines could be divided into two classes, those with a direct receptor effect and those which stimulated transmitter release. Several other investigators have offered similar suggestions.[9-12] Within this framework, hallucinogenic phenethylamines could elicit actions ranging from LSD-like to amphetamine-like, depending on the aromatic substituents and how they affect the various components of the mechanism of action. Particular compounds which have prompted this assessment are **1** and *p*-methoxyamphetamine (PMA, **3**). Both **1** and **3**



3

have appreciable cardiovascular effects[13-16] and their toxicity in mice has been compared.[14,16] These actions appear largely due to a release of endogenous catecholamines. Both compounds have been described as having amphetamine-like stimulant effects.[11,14,17-19] In addition to actions on adrenergic systems, Tseng et al.[9,20,21] have provided impressive evidence which shows that **3** is a potent releaser of serotonin (5-HT) both in vivo and in vitro. However, neither has been shown to possess significant direct in vitro activity at serotonin receptors.[2,10,22]

In view of the widely accepted role of 5-HT in the action of hallucinogens,[23-29] it was of interest to compare isomers and selected congeners of **1–3** for an ability to release 5-HT from nerve terminals. In particular, it was decided to study the *R* and *S* isomers of **1–3**, as well as their α,α-dimethyl homologues **4–6**. Selection of these latter compounds for study was based on the known stimulant and anorexigenic effect of various substituted phenetermine derivatives, which is presumably due to a transmitter-releasing effect.[30] However, α,α-dimethylation seems to abolish any direct effect of such compounds at 5-HT receptors.[12,31] Compounds **1–6** and their isomers, where applicable, were therefore studied for an ability to release [^{3}H]5-HT from rat brain synaptosomes.

Scheme I

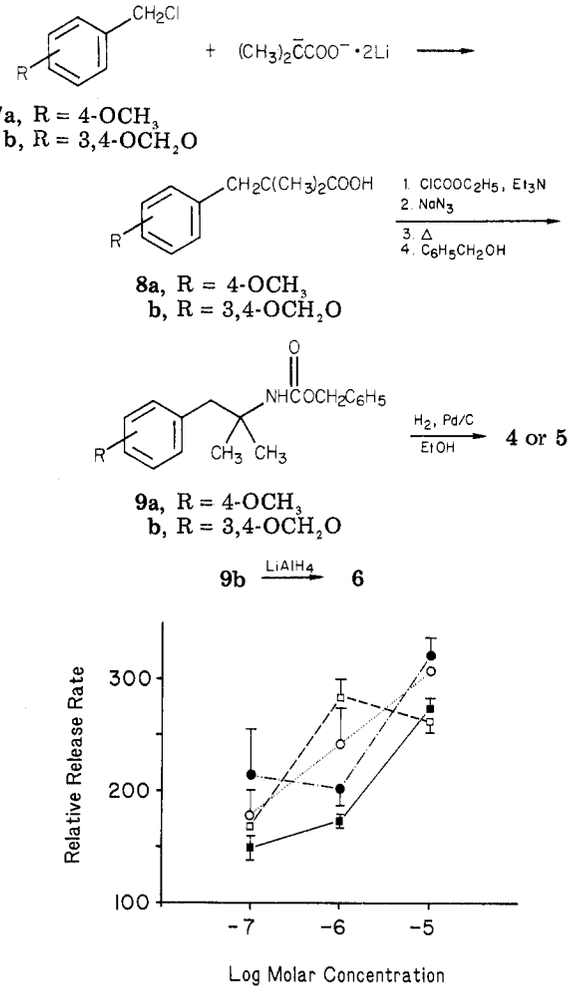
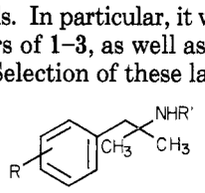


Figure 1. Effect of concentration on release of [^{3}H]serotonin (5-HT) from whole brain rat synaptosomes for (+)-**1** (○), (−)-**1** (●), (+)-**2** (□), and (−)-**2** (■). Although the relative potencies do not vary appreciably for the isomers of **1**, the (+) isomer of **2** is clearly more potent at 10^{-6} M than is the (−) isomer.



4, R = 4-OCH_3; R′ = H
5, R = 3,4-OCH_2O; R′ = H
6, R = 3,4-OCH_2O; R′ = CH_3

Chemistry. The *R* and *S* isomers of **1** and **3** were obtained from the National Institute on Drug Abuse. The

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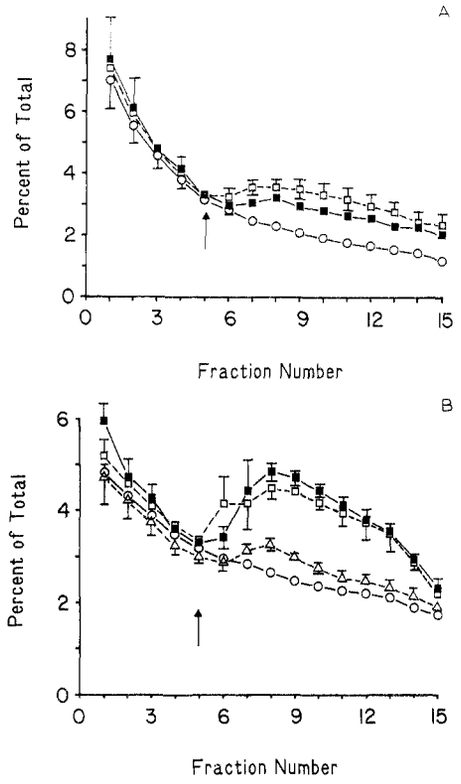

Figure 2. (A) Effects of (*R*)-(−)-1 (■) and (*S*)-(+)-1 (□) at 10^{-6} M bath concentration on release of [^{3}H]5-HT from rat whole brain synaptosomes, as compared to nondrug control efflux (○). The arrow marks the point of drug addition. Points are the mean of three experiments with standard error bars. (B) Effects of (*R*)-(−)-1 (■), (*S*)-(+)-1 (□), and **5** (△) at 10^{-5} M bath concentration on release of [^{3}H]5-HT from rat whole brain synaptosomes, as compared to nondrug control efflux (○). The arrow marks the point of drug addition. Points are the mean of four experiments with standard error bars.

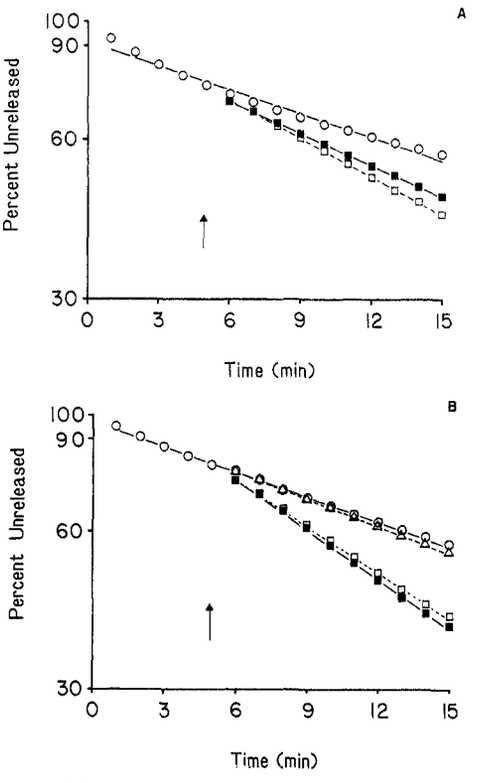

Figure 3. (A) Semilog plot of the efflux data shown in Figure 2A. The rates of release for the two isomers are similar, with the rate for (*R*)-(−)-1 being 202 min^{-1} and for (*S*)-(+)-1 being 242 min^{-1}. (B) Semilog plot of the efflux data shown in Figure 2B. The rates of release for the two isomers are similar, with the rate for (*R*)-(−)-1 being 321 min^{-1}; for (*S*)-(+)-1 being 308 min^{-1}, and for **5** being 174 min^{-1}.

R and *S* isomers of **2** were prepared by a previously described route.[7] The α,α-dimethyl compounds **4–6** were prepared as shown in Scheme I. The carboxylic acids **8a** and **8b** were prepared by the general method of Pfeffer et al.[32] These were subjected to conditions of the Weinstock modification of the Curtius rearrangement,[33] followed by subsequent treatment of the resulting isocyanates with benzyl alcohol to give the *N*-carbobenzoxy derivatives **9a** and **9b**. Hydrogenolysis of these gave **4** and **5**, respectively. Reduction of **9b** with $LiAlH_4$ afforded the *N*-methyl derivative **6**.

Pharmacology. All compounds were examined for their ability to induce [^{3}H]5-HT release from prelabeled rat whole brain synaptosome preparations. The experimental apparatus used was that described by Raiteri et al.[34] Synaptosomes were prepared by the method of Whittaker and Barker.[35]

Results and Discussion.

Figure 1 illustrates the dose–response curves for the isomers of 3,4-(methylenedioxy)amphetamine (MDA) and it's N-methylated derivative MDMA. The isomers of both MDA and MDMA appear equally efficacious at bath concentrations of either 10^{-5} or 10^{-7} M. However at 10^{-6} M the ability of the isomers of MDMA, but not MDA, to release [^{3}H]5-HT is significantly different. A 2 pt × 2 pt parallel line bioassay, using the release rates at the two lowest bath concentrations, gave a potency difference between the MDMA isomers of about 8.5. However, the dose–response curves deviate significantly from parallelism ($p < 0.005$). Similar comparison between the isomers of MDA, between (−)-MDA and (−)-MDMA and between (+)-MDA and (+)-MDMA using all three dose–response points, revealed that the only significant effect of N-methylation was to reduce the potency of the (−) isomer ($p < 0.025$). That is, (+)-MDA and (+)-MDMA have similar potency, while (−)-MDA and (−)-MDMA differ in potency by a factor of 6.5.

Figure 2A,B presents the raw release data from [^{3}H]5-HT-labeled rat whole brain synaptosomes, following treatment with 10^{-6} and 10^{-5} M bath concentrations, respectively, of the isomers of MDA (**1**). Figure 2B also illustrates the low potency of the α,α-dimethylated derivative **5**, even at 10^{-5} M. This latter observation was typical of the low activity noted for both **4** and **6**, also α,α-dimethylated compounds.

Since it is difficult to make quantitative comparisons using raw release data, Figure 3A, B represents analysis of the data using semilog plots of the data in Figure 2A,B, respectively. Regression analysis gives "best fit" lines with slopes which correspond to rates of release. These rates were used in potency comparisons.

Figure 4A,B contains raw release data for the isomers of MDMA (**2**) at bath concentrations of 10^{-6} and 10^{-5} M, respectively. Figure 5A,B contains the semilog plots of the data shown in Figure 4A, B. Similar release curves were obtained for the isomers of **3**. The release data for the

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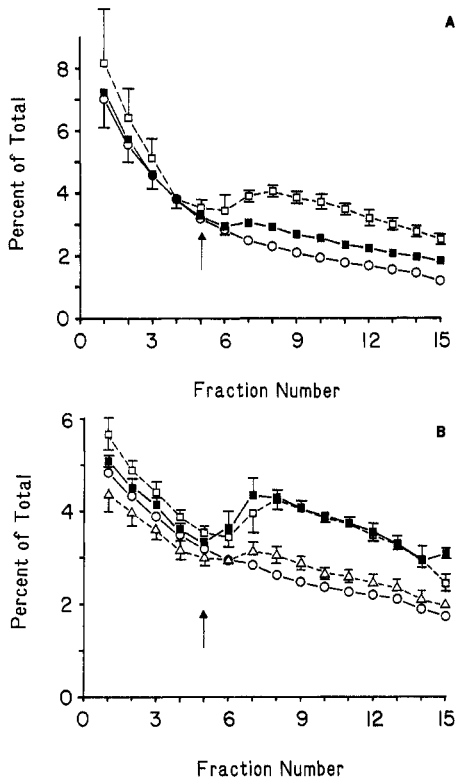

Figure 4. (A) Effects of (*R*)-(−)-**2** (■) and (*S*)-(+)-**2** (□) at 10^{-6} M bath concentration on release of [^{3}H]5-HT from rat whole brain synaptosomes, as compared to nondrug control efflux (○). The arrow marks the point of drug addition. Points are the mean of three experiments with standard error bars. (B) Effects of (*R*)-(−)-**2** (■), (*S*)-(+)-**2** (□), and **6** (△) at 10^{-5} M bath concentration on release of [^{3}H]5-HT from rat whole brain synaptosomes, as compared to nondrug control efflux (○). The arrow marks the point of drug addition. Points are the mean of four experiments, with standard error bars.

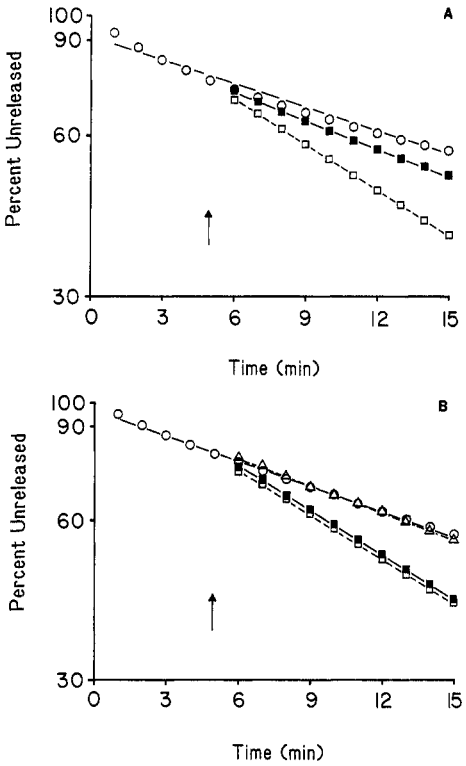

Figure 5. (A) Semilog plot of the efflux data shown in Figure 4A. The rates obtained for these plots are: (*R*)-(−)-**2**, 173 min^{-1} (■); (*S*)-(+)-**2**, 284 min^{-1} (□); control rate determined as 125 min^{-1}. (B) Semilog plot of the efflux data shown in Figure 4B. The rates of release for the MDMA isomers at these higher concentrations are nearly identical. The observed release rate for (*R*)-(−)-**2** was 274 min^{-1} and for (*S*)-(+)-**2** was 262 min^{-1}. The release rate for **6** is not different from control.

Table I. Summary of [^{3}H]Serotonin Release from Rat Brain Synaptosomes by Amphetamine Isomers

treatment	concn, M	*N*	*K* (±SE), $min^{-1} \times 10^4$
control		5	125 (8)
(*R*)-(−)-**1**	10^{-7}	3	214 (41)
	10^{-6}	3	202 (15)
	10^{-5}	3	321 (16)
(*S*)-(+)-**1**	10^{-7}	3	178 (23)
	10^{-6}	3	242 (32)
	10^{-5}	3	307 (26)
(*R*)-(−)-**2**	10^{-7}	3	149 (11)
	10^{-6}	3	173 (6)
	10^{-5}	3	274 (9)
(*S*)-(+)-**2**	10^{-7}	3	168 (22)
	10^{-6}	3	284 (16)
	10^{-5}	3	262 (10)
(*R*)-(−)-**3**	10^{-7}	3	139 (11)
	10^{-6}	3	228 (17)
	10^{-5}	3	307 (6)
(*S*)-(+)-**3**	10^{-7}	3	134 (2)
	10^{-6}	3	255 (7)
	10^{-5}	3	230 (6)

isomers of **1**–**3** are summarized in Table I.

None of the α,α-dimethyl compounds showed appreciable potency at low concentrations and were compared only at the 10^{-5} M bath strength. Thus, data for compounds **4**–**6** are presented in Table II, along with rates for control and 10^{-5} M PMA (**3**), for comparison. These data were obtained from a separate experiment.

The data clearly show that the isomers of both **1** and **2** are potent in inducing the release of [^{3}H]5-HT from synaptosomes. To our knowledge this is the first time that this type of action has been demonstrated for **1**, although its effects on catecholamines have been previously reported.[16,19,36,38] The relative rates also indicate that the isomers of **1** are slightly more potent in inducing release of 5-HT than are the isomers of **3**.

Table II. Summary of [^{3}H]Serotonin Release Data from Rat Whole Brain Synaptosomes for **4**-**6** (Phentermines) at 10^{-5} M Bath Concentration of Drug

treatment	*N*	% of total released ± SEM	*K*, $min^{-1} \times 10^4$	rel rate
control	13	43.41 (1.31)	159	1.00
4	4	45.05 (2.61)	168	1.06
5	4	45.25 (1.48)	175	1.09
6	4	44.30 (2.58)	169	1.06
(*S*)-(+)-**3**	4	57.78 (1.15)	280	1.76

While these studies do not prove the hypothesis that the (+) isomer of MDMA (**2**) owes it's in vivo biological activity to transmitter release, the data are consistent with this hypothesis. Based on the higher potency of (+)-MDA when compared with (−)-MDA in inducing the release of norepinephrine from synaptosomes,[38] it seems likely that other transmitter systems may also be involved. It should be noted that amphetamine itself, as well as all examples of ring-substituted derivatives of amphetamine so far reported, all possess the *S*-(+) configuration. Since (+)-

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amphetamine is well known to be a more potent releasing agent of catecholamines from nerve terminals than (−)-amphetamine, postulation of an indirect mechanism for (+) isomers of substituted amphetamines seems logical.

Experimental Section

Chemistry. Melting points were determined in open glass capillaries using a Mel-Temp apparatus and are uncorrected. IR spectra were recorded on a Beckman IR-33 instrument and are reported in reciprocal centimeters. NMR spectra were recorded on a Varian EM-360 or FT-80 spectrometer. Chemical shifts are reported in δ units (parts per million) with Me_4Si as the internal reference. The multiplicities are expressed as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Elemental analyses were performed by the Purdue Microanalytical Laboratory and were within ±0.4% of the calculated values.

2,2-Dimethyl-3-(4-methoxyphenyl)propionic Acid (8a) and 2,2-Dimethyl-3-[3,4-(methylenedioxy)phenyl]propionic Acid (8b). A solution of 11.2 g (110 mmol) of diisopropylamine in 150 mL of dry THF was stirred at −78 °C under N_2 in a dry ice/*i*-PrOH bath. After the dropwise addition of 48.0 mL of a 2.3 M hexane solution of *n*-BuLi (110 mmol), the reaction flask was removed from the bath and allowed to stir for 30 min. The flask was reimmersed in the cooling bath for 15 min, following which 4.4 g (50 mmol) of isobutyric acid was added, dropwise, followed by 10.5 mL (60 mmol) of hexamethylphosphoramide. The cooling bath was removed and the mixture was stirred for 30 min. *p*-Methoxybenzyl chloride (7.9 g, 50 mmol) or 3,4-(methylenedioxy)benzyl chloride (8.5 g, 50 mmol) was then added dropwise, and the reaction was stirred overnight at room temperature.

The mixture was poured into 100 mL of 10% HCl, and the THF was removed under vacuum. The resulting solution was extracted twice with 150 mL of Et_2O. The combined extracts were washed twice with 10% HCl. The product was then extracted from the Et_2O with 3 × 75 mL portions of 4 N Na_2CO_3. The combined carbonate solutions were acidified, and the product was extracted into Et_2O and dried ($MgSO_4$). The ethereal solution was filtered and concentrated under reduced pressure to afford a light yellow oil. Both **8a** and **8b** were recrystallized from hexane to yield 7.6 g (73%) and 6.5 g (58%), respectively. **8a**: mp 59–61 °C; IR (KBr) 3600–2400 (br, OH), 1685 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 10.40 (br s, 1 H, COOH), 7.15 (d, 2 H, Ar H), 6.85 (d, 2 H, Ar H), 3.84 (s, 3 H, OCH_3), 2.85 (s, 2 H, CH_2), 1.20 (s, 6 H, CH_3). Anal. ($C_{12}H_{16}O_3$) C, H. **8b**: mp 71–73 °C; IR (KBr) 3600–2200 (br, OH), 1690 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 10.20 (br s, 1 H, COOH), 6.70 (s, 3 H, Ar H), 5.95 (s, 2 H, OCH_2O), 2.80 (s, 2 H, CH_2), 1.18 (s, 6 H, CH_3). Anal. ($C_{12}H_{14}O_4$) C, H.

1-[*N*-(Benzyloxycarbonyl)amino]-1,1-dimethyl-2-(4-methoxyphenyl)ethane (9a) and 1-[*N*-(Benzyloxycarbonyl)amino]-1,1-dimethyl-2-[3,4-(methylenedioxy)phenyl]ethane (9b). Triethylamine (5.4 g, 53 mmol) and 10.4 g of **8a** or 11.1 g of **8b** (50 mmol) were dissolved in 10 mL of H_2O, and enough acetone was added to maintain solubility at 0 °C (ice bath). Ethyl chloroformate (6.4 g, 59 mmol) in 40 mL of Me_2CO was added over a period of 30 min, followed by the dropwise addition of 4.1 g (63 mmol) of NaN_3 in 30 mL of H_2O. The mixture was allowed to warm to room temperature and was stirred for an additional 45 min.

The intermediate acyl azide was extracted into 100 mL of toluene. This solution was washed with H_2O and dried ($MgSO_4$). The solution was filtered, and then the filtrate was transferred to a flask fitted with a drying tube and heated on a steam bath for 30 min, at which time nitrogen evolution had ceased. Infrared analysis showed the disappearance of the azide absorption at 2110 cm^{-1} and the presence of the isocyanate absorption at 2230 cm^{-1}.

The toluene was removed under vacuum and replaced with 30 mL of dry benzyl alcohol, and heating on the steam bath was continued overnight. Unreacted benzyl alcohol was removed by gentle high-vacuum distillation to leave the benzyl carbamates as amber oils, which were used without further purification. **9a**: yield 12.3 g (79%); mp 52–53 °C; IR (KBr) 3330 (NH), 1710 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 7.45 (s, 5 H, Ar H), 7.05 (d, 2 H, ArH), 6.80 (d, 2 H, Ar H), 5.10 (s, 2 H, OCH_2O), 4.80 (br s, 1 H, NH), 3.73 (s, 3 H, OCH_3), 2.87 (s, 2 H, CH_2), 1.20 (s, 6 H, CH_3). Anal. ($C_{19}H_{23}NO_3$) C, H, N. **9b**: yield 13.5 g (83%); IR (neat) 3340 (NH), 1710 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 7.45 (s, 5 H, Ar H), 6.70 (m, 3 H, Ar H), 5.95 (s, 2 H, OCH_2O), 5.15 (s, 2 H, CH_2), 4.65 (br s, 1 H, NH), 2.95 (s, 2 H, CH_2), 1.30 (s, 6 H, CH_3). Anal. ($C_{19}H_{21}NO_4$) C, H, N.

1,1-Dimethyl-2-(4-methoxyphenyl)ethylamine Hydrochloride (4) and 1,1-Dimethyl-2-[3,4-(methylenedioxy)phenyl]ethylamine Hydrochloride (5). The benzyl carbamates (10 mmol; 3.13 g of **9a** or 3.27 g of **9b** were dissolved in 250 mL of absolute EtOH to which 0.5 g of 10% Pd/C was added. The solution was shaken under 35 psig of H_2 for 24 h. The mixture was filtered through Celite, and the filtrate was acidified with concentrated HCl. Solvent removal and recrystallization from EtOH–EtOAc yielded 1.45 g (67%) of **4** and 1.63 g (71%) of **5**. **4**: mp 176–178 °C; NMR ($CDCl_3$) δ 8.65 (br s, 3 H, NH_3), 7.25 (d, 2 H, Ar H), 6.90 (d, 2 H, Ar H), 3.85 (s, 3 H, OCH_3), 3.10 (s, 2 H, CH_2), 1.45 (s, 6 H, CH_3). Anal. ($C_{11}H_{18}NOCl$) C, H, N. **5**: mp 180–181 °C; NMR ($CDCl_3$) δ 8.63 (br s, 3 H, NH_3), 6.80 (s, 3 H, Ar H), 6.00 (s, 2 H, OCH_2O), 3.05 (s, 2 H, CH_2), 1.45 (s, 6 H, CH_3). Anal. ($C_{11}H_{16}NO_2Cl$) C, H, N.

***N*-Methyl-1,1-dimethyl-2-[3,4-(methylenedioxy)phenyl]ethylamine Hydrochloride (6).** A solution of 1.64 g of **9b** (5.0 mmol) in 10 mL of dry THF was added slowly to a stirred suspension of 0.38 g of $LiAlH_4$ (10.0 mmol) in 25 mL of dry THF under N_2. After the solution was refluxed for 24 h, the excess hydride was destroyed by the dropwise addition of 1.5 mL of H_2O. The mixture was then basified with dilute NaOH solution and stirred until a flocculent precipitate was obtained. After the solution was filtered and the THF was removed under vacuum, the residue was dissolved in 100 mL of Et_2O and washed twice with 10% $NaHCO_3$ solution. The ethereal solution was dried ($MgSO_4$), filtered, and reduced under vacuum to yield a light yellow oil, which was then dissolved in 50 mL of absolute EtOH and acidified with concentrated HCl. Solvent removal and recrystallization from EtOH–EtOAc afforded 0.84 g (69%) of **6**: mp 206–207 °C; NMR ($CDCl_3$) δ 6.80 (s, 3 H, Ar H), 6.05 (s, 2 H, OCH_2O), 3.10 (s, 2 H, CH_2), 2.70 (s, 3 H, NCH_3), 1.38 (s, 6 H, CH_3). Anal. ($C_{12}H_{18}NO_2Cl$) C, H, N.

Synaptosome Preparation. Synaptosomes were prepared by the method of Whittaker and Barker.[35] Male Sprague–Dawley rats weighing 220–240 g, allowed access to food and water ad libitum, were decapitated. The whole brain was removed and placed on ice. Immediately after weighing, the brain was homogenized in 20 volumes of ice-cold 0.32 M sucrose solution.

The crude homogenate was centrifuged in a Sorvall RC2-B centrifuge (rotor #SM-24) at 3000 rpm ($1000g_{max}$) and 4 °C for 11 min. The pellet was discarded, and the supernatant was then centrifuged at 12 000 rpm ($17\,000g_{max}$) for 30 min. The pellet was gently resuspended in 3.0 mL of ice-cold 0.32 M sucrose solution and carefully layered onto a two-step discontinuous sucrose gradient. The gradient consisted of 1.2 and 0.8 M sucrose solutions. The sucrose gradient was placed in a Beckman L5-65 ultracentrifuge and centrifuged at 21 000 rpm ($55000g_{av}$) in a swinging bucket rotor (SW-41) for 2 h at 4 °C. The synaptosomal subfraction was then harvested at the interface of the 1.2 and 0.8 M sucrose solutions.

The resulting subfraction was resuspended in ice-cold 0.32 M sucrose solution and centrifuged at 20 000 rpm ($48200g_{max}$) and 4 °C for 30 min. The pellet was gently resuspended in ice-cold 0.32 M sucrose solution to bring the total volume of the synaptosome suspension to 4–5 mL. The solution was placed on ice for immediate use in the synaptosome superfusion procedure. Protein concentration was determined by the method of Lowry[37] and was in the range 8–16 mg of protein/mL.

Synaptosome Superfusion Procedure. A 0.4-mL aliquot of the above-prepared synaptosome suspension was added to 3.6 mL of Krebs–Henseleit buffer in a jacketed bath at 37 °C. The supension was agitated under an atmosphere of 95% O_2/5% CO_2 for 5 min, at which time [^{3}H]5-HT (14 Ci/mmol, Amersham) was added to make the solution 10^{-7} M in serotonin. Incubation was continued for an additional 5 min. To each of four superfusion chambers,[34] previously fitted with 0.65 μm Millipore filters, a 0.4-mL aliquot of the ^{3}H-labeled synaptosome suspension was added. The synaptosomes were pulled down on the filters with mild suction and then washed with approximately 50 mL of fresh oxygenated buffer to remove unbound [^{3}H]5-HT.

Fresh buffer (10 mL) was placed in each chamber, and oxygenation with 95% O_2/5% CO_2 was maintained. Using a per-

istaltic pump, we perfused the synaptosomes at a rate of 0.5 mL/min for 5 min without collecting the superfusate. Serial 1-min samples (0.5 mL) were then collected directly into liquid scintillation vials for the next 5 min. At this point, 25-μL aliquots of previously prepared solutions of **1–6** were added to produce the desired drug concentrations in the superfusion chambers. One-minute samples (0.5 mL) of superfusate were collected for the remaining 10 min. The filters were aspirated to dryness and collected for counting. Treatment order was randomized, and treatments were randomized across the baths. Four experiments were run for each drug.

To each of the scintillation vials was added 5 mL of 2-ethoxyethanol, followed by 10 mL of 0.5% PPO/toluene. The vials were counted for tritium. Activity for each sample was expressed as a percent of the total radioactivity (total eluted plus residual activity on the filter). These data are plotted in Figures 2A,B and 4A,B. A plot of the $\log_{10}$ (percent radioactivity unreleased) vs. time gave linear regressions which closely approximated first-order disappearance kinetics (Figures 3A,B and 5A,B). Squared correlation coefficients (r^2) for all regressions were in the range 0.9976–0.9997. Rates for release were taken as the negative slopes of these lines and are expressed in Tables I and II as $K \times 10^4$. These served as convenient measures of relative potency for release, whereas the raw release data were difficult to quantitate. Comparison of these gave relative release rates, with the control release rate defined as 1.00.

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A New, Potent, Conformationally Restricted Analogue of Amphetamine: 2-Amino-1,2-dihydronaphthalene[1,2]

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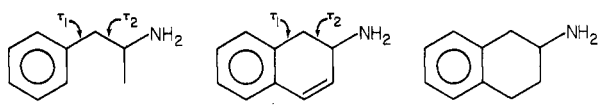
A new stimulant compound, 1,2-dihydro-2-naphthalenamine (2-amino-1,2-dihydronaphthalene, 2-ADN), was prepared as an analogue of amphetamine and of 2-aminotetralin. The optical isomers of 2-ADN were obtained by chemical resolution, and the absolute configuration was determined to be *R*-(+) and *S*-(−). Preliminary pharmacological evaluation revealed that racemic 2-ADN is approximately one-fourth as potent as (+)-amphetamine as a stimulant in mice. The *S*-(−) isomer of 2-ADN was found to be solely responsible for the stimulant effects of the racemate. Both reserpine and α-methyl-*p*-tyrosine antagonized the stimulation produced by 2-ADN.

In this paper, we report the synthesis and preliminary pharmacology of a new, potent, conformationally restricted analogue of amphetamine (**1**), 2-amino-1,2-dihydronaphthalene (2-ADN, **2**). This compound is conformationally restricted in the sense that there is no longer free rotation about the carbon–carbon bonds labeled τ_1 and τ_2 in amphetamine, since these are now part of a ring in **2**, as shown above. One can also view 2-ADN as an analogue of 2-aminotetralin (**3**). The introduction of a double bond into the 3,4 position of **3** should serve to "flatten" the reduced ring, and it was of interest to see how this change would affect biological activity. We previously reported that **3** was inactive as a stimulant in mice at doses up to 8 mg/kg (44 μmol/kg),[3,4] although **3** has been reported by other workers to be a stimulant with one-tenth the activity of amphetamine.[5]



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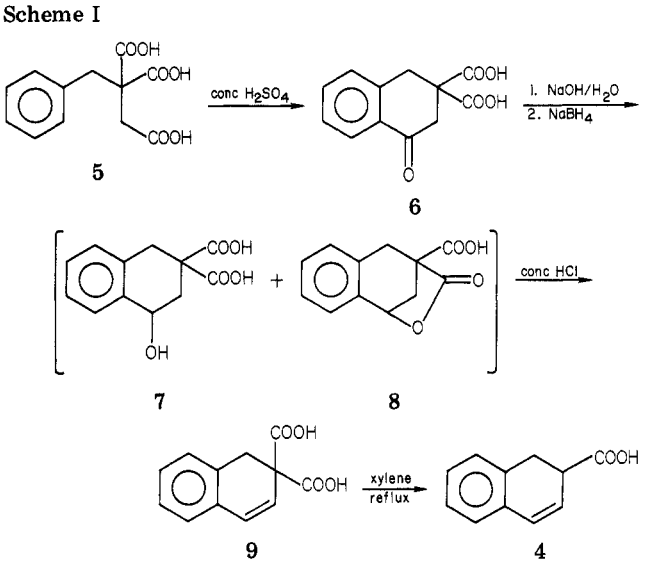
Chemistry. Although a few aryl-substituted derivatives of 2-ADN had been reported in the literature,[6] the unsubstituted 2-ADN apparently had not been synthesized. Our initial approach was directed toward the synthesis of **4** (Scheme I). Since a general synthesis for a series of aryl-substituted derivatives of 2-ADN was desired, the published route to **4**, utilizing sodium amalgam reduction of 2-naphthalenecarboxylic acid, appeared unsatisfactory.[7] Therefore, a new synthesis of **4** was developed. Triacid **5**[8] was cyclized to keto diacid **6**, which was reduced with $NaBH_4$ to a mixture of hydroxy diacid **7** and lactone acid **8**, as determined by NMR analysis of the crude reaction mixture. Treatment of the mixture with concentrated HCl gave diacid **9**. Thermal decarboxylation of **9** in refluxing

Scheme I



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(1) Taken, in part, from the Ph.D. Thesis of B.A.H., Purdue University, 1980.
(2) A preliminary report of this work was presented at the 10th Annual Meeting of the Society for Neuroscience, Cincinnati, OH, 1980, Abstr 245.15.
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