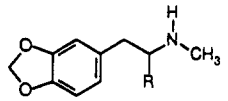
Nonneurotoxic Tetralin and Indan Analogues of 3,4-(Methylenedioxy)amphetamine (MDA)

David E. Nichols,* William K. Brewster, Michael P. Johnson, Robert Oberlender, and Robert M. Riggs

Departments of Medicinal Chemistry and Pharmacognosy and Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907. *Received June 19, 1989*

Four cyclic analogues of the psychoactive phenethylamine derivative 3,4-(methylenedioxy)amphetamine were studied. These congeners, 5,6- and 4,5-(methylenedioxy)-2-aminoindan (**3a** and **4a**, respectively), and 6,7- and 5,6-(methylenedioxy)-2-aminotetralin (**3b** and **4b**, respectively) were tested for stimulus generalization in the two-lever drug-discrimination paradigm. Two groups of rats were trained to discriminate either LSD tartrate (0.08 mg/kg) from saline, or (±)-MDMA·HCl (1.75 mg/kg) from saline. In addition, a 2-aminoindan (**5a**) and 2-aminotetralin (**5b**) congener of the hallucinogenic amphetamine 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) were also evaluated. None of the methylenedioxy compounds substituted in LSD-trained rats, while both **3a** and **3b** fully substituted in MDMA-trained rats. Compounds **4a** and **4b** did not substitute in MDMA-trained rats. Compounds **5a** and **5b** did not substitute in MDMA-trained rats, although **5a** substituted in LSD-trained rats, but with relatively low potency compared to its open-chain counterpart. In view of the now well-established serotonin neurotoxicity of 3,4-(methylenedioxy)amphetamine and its *N*-methyl homologue **1**, **3a** and **3b** were evaluated and compared to **1** for similar toxic effects following a single acute dose of 40 mg/kg sc. Sacrifice at 1 week showed that neither **3a** nor **3b** depressed rat cortical or hippocampal 5-HT or 5-HIAA levels nor were the number of binding sites (B_{max}) depressed for [^{3}H]paroxetine. By contrast, and in agreement with other reports, **1** significantly depressed all three indices of neurotoxicity. These results indicate that **3a** and **3b** have acute behavioral pharmacology similar to **1** but that they lack similar serotonin neurotoxicity.

We have recently described the effects of 1-(1,3-benzodioxol-5-yl)-2-(methylamino)butanamine (**2**; MBDB) and compared them with those of the hallucinogens LSD and MDA and with those of the novel psychoactive agent 3,4-(methylenedioxy)methamphetamine (**1**; MDMA).[1,2]



1 R = CH_3

2 R = CH_2CH_3

The latter has become popular as a recreational substance and is available on the illicit market as "ecstasy." However, anecdotal reports,[3] and our own studies of MDMA and MBDB[1,2,4,5] led us to propose that these are representatives of a novel psychoactive drug class to be known as entactogens. The obvious need for new *types* of therapeutic substances for use in psychiatry demands that close scrutiny be given to any potential new class of psychoactive agent.[6]

Critical to any definition of a novel pharmacological class is the demonstration that its members cannot be fit within the structure–activity relationships of currently existing drug categories. In particular, because of the genesis of **1** from the class of hallucinogenic amphetamines, it must be shown that it or a pharmacologically similar substance is not a hallucinogen. Furthermore, because of the phenylisopropylamine skeleton of **1**, characteristic of many sympathomimetic agents, it must be shown that these substances are not simply additional members of the broad class generically known as central nervous system (CNS) stimulants. Thus, to be considered a new class, MDMA-like substances must also be shown to differ in some significant way from central stimulants such as amphetamine and cocaine. These would seem to be the very minimal essential findings to establish a new pharmacological category.

Although MDMA was advocated as a useful adjunct to psychotherapy,[3] its nonpatentability, recreational popularity, and production of serotonin neutrotoxicity in rodents[7,8] and primates[9] make it unlikely that MDMA will be employed clinically. Therefore, we have begun to search for substances that may have similar therapeutic potential, but which will lack neurotoxicity in animal models.

We were encouraged in this endeavor by the findings that both **1** and a related serotonin neurotoxin *p*-chloroamphetamine (PCA), caused brain 5-HT levels to decrease in two temporal phases; an acute reversible phase and a later irreversible phase.[10-13] In fact, the neurotoxicity of **1** has been compared to that of PCA.[14] While pretreatment with a serotonin uptake inhibitor such as fluoxetine (5 or 10 mg/kg 90 min prior to testing) does not block the discriminative stimulus properties of **1** or **2** (Oberlender and Nichols, unpublished), it is effective in preventing the long-term serotonin neurotoxicity caused by **1**[10] or PCA,[12] even when administered as long as 6 h after treatment with the neurotoxin. Furthermore, the acute animal behavioral effects of **1** are manifested quite rapidly, while Battaglia et al.[15] have shown that repeated injections are required to produce a significant reduction in the number of [^{3}H]paroxetine-labeled 5-HT uptake sites, a putative measure of serotonin neuron terminal loss.

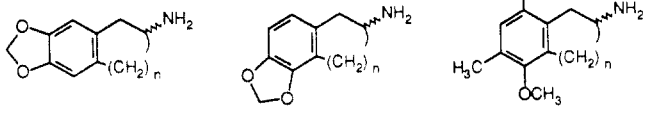
(1) Nichols, D. E.; Hoffman, A. J.; Oberlender, R. A.; Jacob, P., III.; Shulgin, A. T. *J. Med. Chem.* **1986**, *29*, 2009.
(2) Oberlender, R.; Nichols, D. E. *Psychopharmacology* **1988**, *95*, 71.
(3) Greer, G.; Tolbert, R. *J. Psychoact. Drugs* **1986**, *18*, 319.
(4) Johnson, M. P.; Hoffman, A. J.; Nichols, D. E. *Eur. J. Pharmacol.* **1987**, *132*, 269.
(5) Steele, T. D.; Nichols, D. E.; Yim, G. K. W. *Biochem. Pharmacol.* **1987**, *36*, 2297.
(6) Nichols, D. E. *J. Psychoact. Drugs* **1987**, *19*, 33.
(7) Schmidt, C. J.; Wu, L.; Lovenberg, W. *Eur. J. Pharmacol.* **1986**, *124*, 175.
(8) Stone, D. M.; Stahl, D. L.; Hanson, G. R.; Gibb, J. W. *Eur. J. Pharmacol.* **1986**, *128*, 41.
(9) Ricaurte, G. A.; DeLanney, L. E.; Irwin, I.; Langston, J. W. *Brain Res.* **1988**, *446*, 165.
(10) Schmidt, C. J. *J. Pharmacol. Exp. Ther.* **1987**, *240*, 1.
(11) Schmidt, C. J.; Taylor, V. L. *Pharmacologist* **1987**, *29*, Abstract 224.
(12) Fuller, R. W.; Perry, K. W.; Molloy, B. B. *Eur. J. Pharmacol.* **1975**, *33*, 119.
(13) Fuller, R. W.; Wong, D. T.; Snoddy, H. D.; Bymaster, F. P. *Biochem. Pharmacol.* **1977**, *26*, 1333.
(14) Stone, D. M.; Stahl, D. C.; Hanson, G. R.; Gibb, J. W. *Eur. J. Pharmacol.* **1986**, *128*, 41.
(15) Battaglia, G.; Yeh, S. Y.; DeSouza, E. G. *Pharmacol. Biochem. Behav.* **1988**, *29*, 269.

0022-2623/90/1833-0703$02.50/0

One approach seemed to be to search for nonneurotoxic analogues of structurally related known neurotoxins. Perhaps one of the most striking of such examples to be found is that of PCA.[13] A nonneurotoxic congener of PCA, 6-chloro-2-aminotetralin, has been reported to produce an acute reduction in brain serotonin and 5-HIAA levels, but does not lead to the long-lasting neurotoxicity characteristic of PCA.[16,17]

On the basis of this reasoning, it seemed likely that derivatives of (methylenedioxy)-2-aminotetralins might possess an MDMA-like action but, analogous to the chloro analogue, might not produce long-term serotonin neurotoxicity. Furthermore, such cyclic analogues might be expected to lack hallucinogenic effects.[18]

This communication reports the results of studies directed toward a test of this hypothesis. Compounds **3a,b** and **4a,b** were prepared and tested for stimulus generalization in rats trained to discriminate LSD (0.08 mg/kg) from saline and in rats trained to discriminate (±)-MDMA (1.75 mg/kg) from saline. Compounds **5a,b** are the corresponding rigid analogues of the hallucinogenic phenylisopropylamine 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM), which have previously been found to lack DOM-like activity in rats in a conditioned-avoidance paradigm[18] but have not been evaluated in the drug-discrimination paradigm. These analogues were reexamined to determine whether they would possess LSD-like activity in this assay.



3a n = 1
3b n = 2

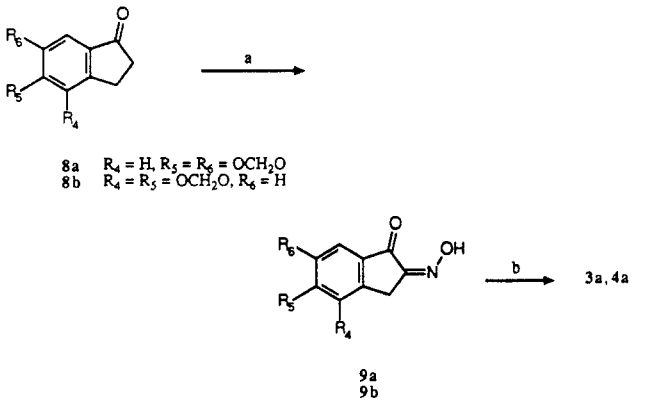
4a n = 1
4b n = 2

5a n = 1
5b n = 2

Although **1** is a secondary amine, it has been shown fairly clearly in animal studies[1,19] that the effect of the *N*-methyl is to attenuate the hallucinogenic activity of its *R*(−)-enantiomer so that racemic **1** lacks the hallucinogenic properties that are prominent in the action of the primary amine 3,4-(methylenedioxy)amphetamine (MDA). However, as we had shown with **5a** and **5b**, incorporation of the side chain into a carbocyclic ring also seemed to abolish hallucinogenlike action.[18] Thus, results obtained with primary amines **3a,b** and **4a,b** might be expected to be similar to those of secondary amines more structurally analogous to **1** and **2**.

When tests of the discriminative stimulus properties of **3a,b** and **4a,b** were completed, compounds which fully substituted for the training drug **1** were evaluated for serotonin neurotoxicity. This was assessed by analysis of brain levels of serotonin (5-HT) and its major metabolie 5-HIAA and radioligand binding studies utilizing [^{3}H]paroxetine to label brain 5-HT uptake sites[20] in rats that had been treated acutely with a high dose of drug and sacrificed one week later.

Scheme I[a]

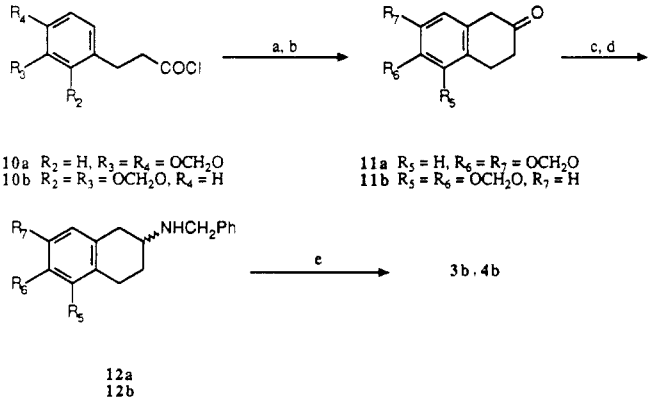


8a $R_4 = H, R_5 = R_6 = OCH_2O$
8b $R_4 = R_5 = OCH_2O, R_6 = H$

9a
9b

[a] (a) $(CH_3)_2CHCH_2CH_2ONO$, HCl, CH_3OH; (b) H_2, 10% Pd-C, CH_3COOH, H_2SO_4.

Scheme II[a]



10a $R_2 = H, R_3 = R_4 = OCH_2O$
10b $R_2 = R_3 = OCH_2O, R_4 = H$

11a $R_5 = H, R_6 = R_7 = OCH_2O$
11b $R_5 = R_6 = OCH_2O, R_7 = H$

12a
12b

[a] (a) CH_2N_2, $(CH_3CH_2)_2O$; (b) $[Rh(OCOCH_3)_2]_2$, CH_2Cl_2, reflux; (c) $PhCH_2NH_2$, toluene, reflux; (d) H_2, 5% Pt-C, absolute CH_3CH_2OH; (e) H_2, 10% Pd-C, 95% CH_3CH_2OH.

Chemistry

Indan derivative **3a** was prepared from indanone **8a**. The latter was prepared in 85% yield by an intramolecular cyclization of 3-[3,4-(methylenedioxy)phenyl]propionyl chloride. Treatment of the indanone with amyl nitrite in methanol with HCl afforded the hydroxyimino ketone (Scheme I).[21] This was reduced to the 2-aminoindan following a modification of our earlier method.[18]

Compound **4a** was prepared by a similar sequence. Methylenation of 2,3-dihydroxybenzaldehyde was accomplished by treatment with methylene bromide in DMF with potassium carbonate and a catalytic amount of cupric oxide.[22] This aldehyde was readily converted into cinnamic acid **6** by Knoevenagel condensation with malonic acid, which was reduced to dihydrocinnamic acid **7** by catalytic hydrogenation. However, the intramolecular cyclization of the corresponding acid chloride to indanone **8b** gave very poor yields, under a variety of conditions. This was attributed to the formation of polymers, due to the high reactivity of the positions para to the dioxole oxygens. However, cyclization of the carboxylic acid using P_2O_5 in benzene did afford the desired indanone, albeit in poor yield. This was converted to the hydroxyimino ketone, as above, which on catalytic hydrogenation gave the desired amino compound **4a**.

Compound **3b** was prepared from the 2-tetralone, as shown in Scheme II. The synthesis was uneventful. The

(16) Fuller, R. W.; Perry, K. W.; Baker, J. C.; Molloy, B. B. *Arch. Int. Pharmacodyn. Ther.* **1974**, *212*, 141.
(17) Fuller, R. W.; Wong, D. T.; Snoddy, H. D.; Bymaster, F. P. *Biochem. Pharmacol.* **1977**, *26*, 1333.
(18) Nichols, D. E.; Barfknecht, C. F.; Long, J. P.; Standridge, R. T.; Howell, H. G.; Partyka, R. A.; Dyer, D. C. *J. Med. Chem.* **1974**, *17*, 161.
(19) Glennon, R. A.; Young, R.; Rosecrans, J. A.; Anderson, G. M. *Biol. Psychiatry* **1982**, *17*, 807.
(20) Battaglia, G.; Yeh, S. Y.; O'Hearn, E.; Molliver, M. E.; Kuhar, M. J.; DeSouza, E. B. *J. Pharmacol. Exp. Ther.* **1987**, *242*, 911.
(21) Perkin, W. H., Jr.; Robinson, R. *J. Chem. Soc.* **1907**, *91*, 1073.
(22) Smidrkal, J.; Trojanek, J. *Z. Chem.* **1973**, *13*, 214.

Table I. Drug-Discrimination Data in LSD-Trained Rats

compd	dose μmol/kg	dose mg/kg	n^a	%D[b]	%SDL[c]	ED_{50} (95% CI)
saline			8	0	0	
LSD	0.012	0.005	8	0	13	0.048 μmol/kg
	0.023	0.01	8	0	0	(0.031–0.076)
	0.046	0.02	9	0	44	
	0.093	0.04	8	0	88	0.021 mg/kg
	0.186	0.08	8	0	100	(0.013–0.033)
3a	0.59	0.125	11	27	13	
	1.17	0.25	16	50	50	
	2.34	0.50	10	20	38	
	4.68	1.0	15	47	25	
	9.36	2.0	6	91	D[d]	
3b	2.20	0.50	15	47	25	
	4.39	1.0	11	27	38	
	8.78	2.0	16	50	63	
	13.18	3.0	15	67	D	
	17.57	4.0	21	64	D	
4a	1.17	0.25	10	20	38	
	2.34	0.50	13	44	25	
	4.68	1.0	10	20	13	
	9.36	2.0	10	20	25	
	18.72	4.0	5	100	D	
4b	0.55	0.125	9	11	0	
	1.10	0.25	9	11	13	
	2.20	0.50	8	0	25	
	2.74	0.625	8	0	25	
	4.39	1.0	12	33	25	
	6.59	1.5	15	47	38	
	8.78	2.0	11	55	D	
5a	4.10	1.0	10	0	20	8.94 μmol/kg
	6.15	1.5	11	27	38	(5.98–13.35)
	8.21	2.0	8	0	38	
	16.41	4.0	11	27	63	2.18 mg/kg
	18.46	4.5	9	0	89	(1.46–3.25)
5b	3.88	1.0	10	20	25	
	5.82	1.50	11	27	38	
	7.77	2.0	15	47	13	
	9.70	2.5	13	69	0	
	10.67	2.75	5	100	D	

[a] Number of animals tested. [b] Percentage of animals disrupted. [c] Percentage of animals selecting drug lever. [d] Disruption; >50% of the animals did not finish 50 presses on one lever within the 5-min test period.

rearrangement of the diazo ketone, under the influence of rhodium acetate,[23] afforded β-ketone **11a** in modest yield, which was isolated as its bisulfite addition product. Following liberation of the free ketone, formation of the enamine with benzylamine, followed by reduction and catalytic N-debenzylation, afforded **3b**.

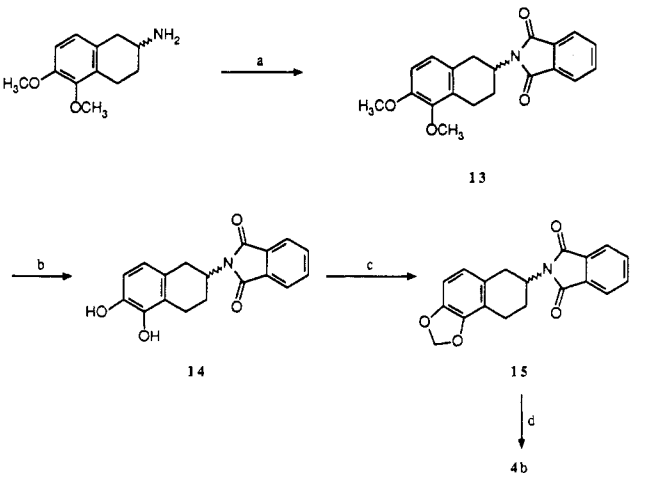
Compound **4b** was prepared by a similar sequence, although the yields were not as high for the formation of ketone **11b**. An alternate route was therefore employed in an attempt to improve the overall yield, as outlined in Scheme III. However, the yield was not improved, and in retrospect Scheme II must be preferred for its simplicity. Compounds **5a** and **5b** were resynthesized as described in an earlier report.[18]

Pharmacology

All compounds were evaluated in the two-lever drug-discrimination assay, in groups of rats trained to discriminate saline from injections of LSD tartrate (0.08 mg/kg ip), or saline from (±)-MDMA hydrochloride (**1**·HCl; 1.75 mg/kg ip) by using methods previously described.[1,2] This was done to determine which, if any, of the compounds possessed discriminative stimulus properties similar to LSD ("hallucinogenic") or MDMA ("entactogenic").

On the basis of complete substitution in the drug discrimination assay, compounds **3a** and **3b** were tested for

Scheme III[a]



[a] (a) phthalic anhydride, melt; (b) BBr_3, CH_2Cl_2; (c) CH_2Br_2, K_2CO_3, CuO, DMF, reflux; (d) $NH_2NH_2 \cdot H_2O$, 95% CH_3CH_2OH, reflux.

the production of serotonin neurotoxicity. Acute administration of the drugs to rats was followed at 1 week by sacrifice. One hemisphere of the brains was analyzed for catecholamines, serotonin, and their metabolites, and the other hemisphere was analyzed by radioligand-binding techniques using the serotonin uptake inhibitor [^{3}H]paroxetine to determine the affinity (K_D) and number of

(23) McKervey, M. A.; Tuladhar, S. M.; Twohig, M. F. *J. Chem. Soc., Chem. Commun.* **1984**, 129.

Table II. Drug-Discrimination Data in MDMA-Trained Rats

compd	dose μmol/kg	dose mg/kg	n^a	%D[b]	%SDL[c]	ED_{50} (95%CI)
saline			8	0	0	
1	0.95	0.22	8	0	0	2.65 μmol/kg
	1.90	0.44	8	0	25	(1.96–3.57)
	3.81	0.88	8	0	75	0.64 mg/kg
	7.62	1.75	8	0	100	(0.046–0.82)
3a	1.17	0.25	8	0	13	2.66 μmol/kg
	2.34	0.50	10	20	50	(1.59–4.44)
	4.68	1.0	8	0	63	0.58 mg/kg
	9.36	2.0	10	10	100	(0.34–1.00)
3b	2.20	0.50	9	11	13	5.78 μmol/kg
	4.39	1.0	9	11	50	(3.24–10.32)
	8.78	2.0	8	0	63	1.32 mg/kg
	17.57	4.0	11	27	88	(0.74–2.35)
4a	1.17	0.25	6	0	0	
	2.34	0.50	8	13	29	
	4.68	1.0	10	20	13	
	9.36	2.0	9	11	25	
	18.72	4.0	5	0	0	
	28.08	6.0	4	50	D[d]	
4b	1.10	0.25	8	38	0	
	2.20	0.50	11	27	63	
	2.74	0.625	12	33	63	
	3.29	0.75	10	20	63	
	4.39	1.0	9	11	50	
	6.59	1.50	9	11	75	
	7.69	1.75	12	33	75	
	8.78	2.0	12	42	71	
	13.18	3.0	5	100	D	
5a	4.10	1.0	8	0	13	
	8.21	2.0	9	11	38	
	12.31	3.0	8	0	25	
	16.41	4.0	11	27	75	
	17.44	4.25	11	27	50	
	20.51	5.0	7	71	D	
5b	1.94	0.50	10	20	38	
	3.88	1.0	11	27	50	
	7.76	2.0	11	27	38	
	11.64	3.0	6	83	D	

[a] Number of animals, tested. [b] Percentage of animals disrupted. [c] Percentage of animals selecting drug lever. [d] Disruption; >50% of the animals did not finish 50 presses on one lever within the 5-min test period.

binding sites (B_{max}) for serotonin. This latter measurement has been shown to correlate with loss of serotonin neuron terminals.[20]

Results and Discussion

The results of the drug discrimination studies in rats are presented in Tables I and II. In the LSD-trained rats, stimulus generalization did not occur with any of the compounds **3a,b**, **4a,b** or **5b**. These results are consistent with our previous findings,[18] that tetralin congeners of hallucinogenic amphetamine derivatives lack hallucinogenlike activity. It is also doubtful that these analogues would be hallucinogenic in man. Although this cannot be determined with absolute certainty in the absence of clinical studies, the drug discrimination paradigm has so far yielded only false positives in studies with hallucinogens. That is, drug-discrimination testing in rats has not given results suggesting a compound to be inactive, when in fact it was known that the compound was active in man.

However, indan **5a** gave full substitution, with an ED_{50} = 2.18 mg/kg, approximately $^1/_{15}$ the potency of the hallucinogen DOM in this assay.[24] Earlier studies of this compound, using disruption of a conditioned-avoidance response, did not produce results suggestive of hallucinogenlike activity.[18] The fact that neither of the methylenedioxy-substituted indans substituted for LSD indicates that they would probably lack hallucinogenic activity.

However, compounds **3a** and **3b** do produce an MDMA-like discriminative stimulus (Table II), with **3a** having potency comparable to that of the training drug **1**. However, it should be noted that **3a** has a plane of symmetry, thus has no enantiomers, and pharmacologically behaves as a pure enantiomer, while the training drug is racemic. Therefore, in terms of receptor kinetics, one could estimate that the potency of **3a** is about $^1/_2$ that of the biologically more active *S* enantiomer of **1**.

We have previously shown that extension of the α-methyl of **1** to an α-ethyl (**2**) gives a compound which also retains MDMA-like activity.[1] Yet, a similar transformation in the hallucinogenic phenethylamines leads to complete loss of hallucinogenic activity.[25]

Thus, in considering the structure–activity relationships of the hallucinogenic phenethylamines, it is quite clear that very limited side-chain modification is tolerated. α-Ethyl or cyclic tetralin congeners of hallucinogenic phenethylamines appear to lack LSD-like discriminative stimulus properties. In addition, although indan **5a** has an LSD-like discriminative cue, its potency is greatly reduced compared to its open-chain parent, while **3a** retains significant **1**-like activity. On the other hand, the MDMA cue is elicited by a variety of structures, including the ones in this study, which clearly lie outside of the range of allowable structural

(24) Oberlender, R. A.; Kothari, P. J.; Nichols, D. E.; Zabik, J. E. *J. Med. Chem.* **1984**, *27*, 788.

(25) Standridge, R. T.; Howell, H. G.; Gylys, J. A.; Partyka, R. A.; Shulgin, A. T. *J. Med. Chem.* **1976**, *19*, 1400.

Table III. The Effects of 1, 3a, and 3b on Levels of Monoamines and Metabolites in Rat Brain[a]

treatment	monoamine levels, pg/mg wet wt (% remaining)					
	NE	5-HT	5-HIAA	DA	DOPAC	HVA
			Cortex			
saline	383 ± 20	379 ± 26	326 ± 14	127 ± 25	168 ± 12	264 ± 85
1	375 ± 16	161 ± 19[b] (43)	230 ± 10[b] (71)	151 ± 22	212 ± 24	318 ± 81
3a	395 ± 15	330 ± 12	329 ± 18	130 ± 38	187 ± 16	193 ± 33
3b	367 ± 16	356 ± 13	370 ± 25	151 ± 43	213 ± 20	262 ± 33
			Hippocampus			
saline	513 ± 28	296 ± 33	479 ● 36	ND	ND	ND
1	429 ± 20	105 ± 11[b] (53)	256 ± 18[b] (53)	ND	ND	ND
3a	459 ± 21	273 ± 17	473 ± 33	ND	ND	ND
3b	484 ± 22	256 ± 27	549 ± 33	ND	ND	ND

[a] One injection of saline or 40 mg/kg of 1, 3a, or 3b was administered sc. Animals were sacrificed one week later. Frontal cortex and hippocampus brain regions were removed and stored at −70 °C until assay. Monoamines and their metabolite levels were determined by HPLC-EC. The number of uptake sites was estimated by [^{3}H]paroxetine binding (see the Experimental Section). Values are presented as the mean ± SE for $n = 8$. [b] Significantly decreased from control ($p < 0.001$, Student's t test). ND indicates not determined.

Table IV. Effect of 1, 3a, or 3b on Binding of [^{3}H]Paroxetine to Rat Brain Cortical or Hippocampal Homogenates[a]

treatment	cortex		hippocampus	
	K_d, nM	B_{max}, fmol/g wet wt	K_d, nM	B_{max}, fmol/g wet wt
saline	0.103 ± 0.011	957 ± 47	0.118 ± 0.013	660 ± 25
1	0.123 ± 0.015	643 ± 53[b]	0.195 ± 0.036	348 ± 21[b]
4a	0.104 ± 0.008	918 ± 43	0.127 ± 0.013	675 ± 53
4b	0.102 ± 0.015	964 ± 40	0.118 ± 0.016	778 ± 58

[a] Values are mean ± SEM for $n = 8$. [b] $p < 0.0001$, Student's t test versus saline treatment.

variation for hallucinogenic amphetamines.

Thus, these studies further reinforce the view that 1, 2, and chemically related congeners cannot be pharmacologically classified as "hallucinogenic agents", unless there is very compelling justification for redefining the structure–activity relationships of that class.

Based on other studies, there is some evidence to suggest that 1 has amphetamine-like pharmacological properties.[26,27] However, we have shown previously in biochemical pharmacology assays using inhibition of monoamine uptake into rat brain synaptosomes and drug-stimulated release from prelabeled slices of rat brain caudate that compound 2 appears to have no significant impact on dopaminergic systems.[4,5] More recently, we have shown that 1 and 2 do not produce symmetrical substitution in drug discrimination studies in (+)-amphetamine-trained rats.[28] Furthermore, in a separate study we have tested 3a at four doses in rats trained to discriminate 1.0 mg/kg of (+)-amphetamine sulfate from saline. Doses of 0.25, 0.50, and 1.0 mg/kg resulted in saline-lever selection in all rats responding at each dose level, while five of five rats treated with 2 mg/kg of 3a were disrupted (Oberlender and Nichols, unpublished study). Thus, additional evidence is accumulating that compounds such as 1 and 2 (and 3a) also do not really fit within the class of CNS stimulants. These findings strengthen support for our hypothesis that 1 and 2 belong to an entirely new pharmacological class.

The results of the studies of serotonin neurotoxicity are most interesting (Tables III and IV). Table III reports levels of 5-HT, 5-HIAA, and catecholamines and their metabolites, measured at 1 week following acute drug administration. Neither 3a nor 3b depressed levels of any monoamine or its metabolites, nor were the K_D or B_{max} altered in the radioligand-binding studies with [^{3}H]paroxetine (Table IV). The latter indicates that no loss of serotonin uptake sites has occurred, presumably reflecting the absence of serotonin terminal degeneration following treatment with 3a or 3b. By contrast, 1 significantly depressed 5-HT and 5-HIAA levels and decreased the B_{max} for [^{3}H]paroxetine binding. Recent studies have shown that 2 also produces serotonin neurotoxicity similar to 1.[29]

Thus, we have demonstrated, for the first time, that compounds may possess acute behavioral pharmacology similar to 1 (i.e. substitution in MDMA-trained rats) while lacking long-term serotonin neurotoxicity. Previously, the *N*-ethyl homologue of 1,[30] as well as 2,[2] and fenfluramine[31] have been shown to possess behavioral properties similar to 1. However, all of these selectively destroy serotonin neurons in rat brain.

Experimental Section

Melting points were taken on a Mel-Temp apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian FT-80, or Chemagnetics A-200 spectrometer. Chemical shifts are reported in δ values (parts per million) relative to an internal reference of Me_4Si in $CDCl_3$. Microanalysis was performed at the Purdue Microanalysis Laboratory, and all values were within 0.4% of the calculated composition.

2,3-(Methylenedioxy)cinnamic Acid (6). A mixture of 20 g (0.133 mol) of 2,3-(methylenedioxy)benzaldehyde,[22] malonic acid (25.9 g, 0.25 mol), and 1.1 mL of piperidine in 60 mL of pyridine was stirred on the steam bath for 3 h. The mixture was poured into a mixture of 93 mL of concentrated HCl and 350 mL of ice/water. The flocculent precipitate was collected by suction filtration and washed by resuspension and stirring for 10 min in 250 mL of water. The precipitate was collected by filtration, dried, and recrystallized from MeOH/H_2O. A second recrystallization from MeOH and decolorization with charcoal afforded 24.7 g

(26) Evans, S. M.; Johanson, C. E. *Drug Alcohol Depend.* **1986**, *18*, 159.
(27) Kamien, J. B.; Johanson, C. E.; Schuster, C. R.; Woolverton, W. L. *Drug Alcohol Depend.* **1986**, *18*, 139.
(28) Nichols, D. E.; Oberlender, R. A. *NIDA Res. Monog.* **1989**, in press.
(29) Johnson, M. P.; Nichols, D. E. *Pharmacol. Biochem. Behav.* **1989**, *33*, 105.
(30) Boja, J. W.; Schechter, M. D. *Pharmacol. Biochem. Behav.* **1987**, *28*, 153.
(31) Schechter, M. D. *Pharmacol. Biochem. Behav.* **1986**, *24*, 1533.

(96%) of a white crystals: mp 194–195 °C (lit.[32] mp 194 °C); ^{1}H NMR ($CDCl_3$) δ 7.71 (d, 1, =CH, J = 16.1 Hz), 6.87 (m, 3, ArH), 6.66 (d, 1, =CHCO, J = 16.1 Hz), 6.08 (s, 2, OCH_2O).

3-[2,3-(Methylenedioxy)phenyl]propanoic Acid (7). Acid **6** (6.3 g, 32.8 mmol) was taken up into 100 mL of 95% ethanol and shaken in a Parr apparatus at 60 psig of H_2 over 0.3 g of 10% Pd–C. After the theoretical amount of H_2 was absorbed (5 h), the mixture was filtered and concentrated under vacuum. An analytical sample was crystallized from benzene to provide a white crystalline solid: mp 79–80 °C (lit.[33] mp 78–79 °C); ^{1}H NMR ($CDCl_3$) δ 11.64 (s, 1, COOH), 6.72 (m, 3, ArH), 5.90 (s, 2, OCH_2O), 2.91 (t, 2, $ArCH_2$, J = 8 Hz), 2.70 (t, 2, CH_2, J = 8 Hz).

5,6-(Methylenedioxy)-1-indanone (8a). A mixture of 4.7 g (24.2 mmol) of 3-[3,4-(methylenedioxy)phenyl]propanoic acid[34] and 6 mL (83 mmol) of thionyl chloride in 50 mL of dry benzene was stirred at reflux for 3 h. The benzene was removed by rotary evaporation and the crude acid chloride was redissolved in 50 mL of dichloromethane and cooled with an ice bath to 0 °C. A solution of $SnCl_4$ (3.6 mL, 30.8 mmol) in 20 mL of dichloromethane was added over 10 min. Stirring was continued for 10 min, the ice bath was removed, and the reaction was stirred for an additional 0.5 h. The mixture was poured over 15 g of crushed ice, and the phases were separated. The organic layer was washed with 3 N HCl (2 × 60 mL) and water (100 mL). The solution was dried ($MgSO_4$), filtered, and then passed through a short pad of silica gel to remove residual tin salts. After solvent removal, the residue was recrystallized from benzene/hexanes to yield 3.58 g (84%): mp 163–164 °C (lit.[35] mp 163–164 °C); ^{1}H NMR ($CDCl_3$) δ 7.10 (s, 1, ArH), 6.83 (s, 1, ArH), 6.06 (s, 2, OCH_2O), 3.03 (m, 2, CH_2), 2.65 (m, 2, CH_2).

4,5-(Methylenedioxy)-1-indanone (8b). To a vigorously stirred mixture of 15 g of P_2O_5 in 100 mL of benzene, at reflux, was added dropwise, over 10 min, a solution of 5.0 g (26 mmol) of **7** in 50 mL of benzene. An additional 10 g of P_2O_5 was added after 45 min. One hour later, the reaction was poured into 200 mL of cold water. The two-phase mixture was transferred to a separatory funnel. Residual P_2O_5 in the reaction flask was treated with water and benzene, and the solvents were transferred to the separatory funnel. The aqueous phase was discarded, and the organic layer was washed with 1 N NaOH and brine and then dried ($MgSO_4$). Filtration, concentration, and recrystallization from benzene afforded 1.42 g (31%) of the desired indanone: mp 163–166 °C (lit.[33] mp 164–167 °C); ^{1}H NMR ($CDCl_3$) δ 7.39, 6.88 (2 d, 2, ArH, J = 8 Hz), 6.10 (s, 2, OCH_2O), 3.06 (t, 2, $ArCH_2$, J = 5.6 Hz), 2.70 (m, 2, CH_2CO).

2-(Hydroxyimino)-5,6-(methylenedioxy)-1-indanone (9a). A suspension of 0.5 g (2.8 mmol) of **8a** in 45 mL of MeOH was heated to 45 °C to effect solution. To this was added 0.42 mL (3.11 mmol) of isoamyl nitrite. Then 0.27 mL of concentrated HCl was added over 1 min, at which point a pale yellow precipitate began to form. After 45 min of stirring, an additional 0.2 mL each of isoamyl nitrite and concentrated HCl was added. After a total of 75 min of stirring, the yellow precipitate was collected by suction filtration, washed on the filter with cold methanol (2 × 5 mL) and ether (2 × 5 mL), and air-dried to afford 0.44 g (75.5%) of product, mp 216–217 °C (lit.[21] mp 230 °C dec). An additional 67 mg of product was recovered from the filtrate following concentration and crystallization from MeOH (total yield 86.9%): ^{1}H NMR (DMSO-d_6) δ 8.83 (br s, 1, NOH), 7.15, 7.12 (2 s, 2, ArH), 6.18 (s, 2, CH_2), 3.64 (s, 2, CH_2).

2-(Hydroxyimimo)-4,5-(methylenedioxy)-1-indanone (9b). A procedure identical with that for the preparation of **9a**, starting with **8b**, yielded 0.49 g (85%) of product, mp 240–241 °C (lit.[33] mp 245–250 °C). An additional 34 mg of product was recovered from the filtrate following concentration and crystallization from MeOH (total yield 90.5%): ^{1}H NMR (DMSO-d_6) δ 12.60 (s, 1, OH), 7.41 (d, 1, ArH, J = 8.1 Hz), 7.07 (d, 1, ArH, J = 8.1 Hz), 6.22 (s, 2, OCH_2O), 3.68 (s, 2, CH_2).

5,6-(Methylenedioxy)-2-aminoindan Hydrochloride (3a). With a modification of an earler procedure,[18] 1.1 g (5.4 mmol) of hydroxyimino ketone **9a** was dissolved by warming in 50 mL of acetic acid. To the solution was added 0.6 mL (10.8 mmol) of concentrated H_2SO_4 and 250 mg of 10% Pd–C. The mixture was shaken at 50 psig of H_2 for 18 h. After removal of the catalyst by filtration, the solvent was removed by rotary evaporation. The residue was dissolved in 50 mL of water and this solution was washed with 3 × 100 mL of ethyl acetate and 100 mL of ether. The aqueous layer was basified with 20% NaOH and the liberated amine was extracted into 3 × 50 mL of dichloromethane. The organic solution was dried ($MgSO_4$), filtered, and concentrated. The residual oil was dissolved in 2 mL of EtOH, acidified with concentrated HCl, and ether was added. The resulting crystalline product was collected by filtration to yield 641 mg (55%): mp 276 °C; ^{1}H NMR ($CDCl_3$; free base) δ 6.67 (s, 2, ArH), 5.88 (s, 2, CH_2), 3.80 (m, 1, CH), 3.10 (dd, 2, 2 × CH, J = 15.4 Hz, 6.6 Hz), 2.54 (dd, 2, 2 × CH, J = 15.4 Hz, 5.1 Hz), 1.48 (s, 2, NH_2). Anal. ($C_{10}H_{12}ClNO_2$) C, H, N.

4,5-(Methylenedioxy)-2-aminoindan Hydrochloride (4a). Similarly to the preparation of **3a**, 0.25 g of **9b** was reduced catalytically over 0.11 g of 10% Pd–C in 10 mL of AcOH containing 0.3 mL of concentrated H_2SO_4. Following solvent removal and neutralization, the free base was purified by radial chromatography on a silica rotor and elution with 2.5% MeOH–CH_2Cl_2 under an NH_3 atmosphere to afford 0.167 g (77.3%) of amine: ^{1}H NMR ($CDCl_3$) δ 6.68; 6.64 (2 d, 2, ArH, J = 7.8 Hz), 5.92 (d, 2, OCH_2O), 3.87 (m, 1, CH), 3.16 (dd, 1, ArCH, J = 6.5 Hz, 4.5 Hz), 3.08 (dd, 1, ArCH, J = 6.4 Hz, 4.0 Hz), 2.65 (d, 1, ArCH, J = 5.0 Hz, 2.3 Hz), 2.57 (dd, 1, ArCH, J = 4.8 Hz, 2.0 Hz), 1.47 (s, 2, NH_2). The base was converted to the hydrochloride salt and recrystallized from ethanol/ethyl acetate to provide colorless crystals, mp 235–236 °C. Anal. ($C_{10}H_{12}ClNO_2$) C, H, N.

6,7-(Methylenedioxy)-2(1*H*)-naphthalenone (11a). Fifteen grams (77 mmol) of 3-[3,4-(methylenedioxy)phenyl]propanoic acid[34] was converted to its acid chloride with thionyl chloride, which was then vacuum distilled. A solution of 11.04 g (52 mmol) of this acyl chloride (**10a**) in 40 mL of dry ether was prepared and cooled in an ice bath. Into this solution was directly distilled approximately 6.9 g (164 mmol) of diazomethane,[36] as an ether azeotrope. This solution was stirred overnight and was then concentrated under vacuum. The resulting diazo ketone was dissolved in 100 mL of CH_2Cl_2 and this solution was added slowly to a solution of 110 mg of $[Rh(OCOCH_3)_2]_2$ in 100 mL of CH_2Cl_2[23] heated at reflux. After complete addition, heating was continued for 10 min, and one drop of CF_3COOH was added, followed by 10 min of additional reflux. The solution was cooled, washed with aqueous $NaHCO_3$ and brine, dried, and concentrated. The crude residue was shaken with saturated $NaHSO_3$ to afford the bisulfite adduct, which was washed with ethanol and ether and dried. Liberation of the ketone by treatment of the bisulfite adduct with Na_2CO_3 solution afforded 3.51 g (35.5%) of the pure tetralone: mp 97–98 °C (lit.[37] mp 99 °C); ^{1}H NMR ($CDCl_3$) δ 6.69, 6.58 (2 s, 2, ArH), 5.92 (s, 2, OCH_2O), 3.46 (s, 2, $ArCH_2$), 2.96 (m, 2, $ArCH_2$), 2.50 (m, 2, $COCH_2$).

5,6-(Methylenedioxy)-2(1*H*)-naphthalenone (11b). Acid **7** was converted to the acid chloride with $SOCl_2$ and 1.61 g (7.5 mmol) of the resultant acid chloride (**10b**) was dissolved in 25 mL of ether. Into this solution was distilled approximately 1.0 g of ethereal diazomethane. After stirring overnight, the ether was removed by rotary evaporation. The residual diazo ketone was taken up into 15 mL of CH_2Cl_2 and this solution was added, dropwise, over 10 min, to a rapidly stirred solution of 15 mg of $[Rh(OCOCH_3)_2]_2$ in 15 mL of CH_2Cl_2.[23] After 10 min of reflux, 1 drop of CF_3COOH was added and heating was continued for 10 min. The reaction was cooled, washed with aqueous $NaHCO_3$ and brine, and dried ($MgSO_4$). After filtration and evaporation, the residue was shaken with saturated $NaHSO_3$. The bisulfite adduct was washed with ethanol and ether, and was then dried. Solution of the adduct in water, decomposition with Na_2CO_3, extraction with ether, workup, and Kugelrohr distillation afforded 0.30 g (21%) of the desired ketone: mp 88–91 °C (lit.[38] no melting

(32) Perkin, W. H., Jr.; Trikojus, V. M. *J. Chem. Soc.* **1926**, 2925.
(33) Irie, H.; Kishimoto, T.; Uyeo, S. *J. Chem. Soc. C* **1968**, 3051.
(34) Haworth, R. D.; Perkin, W. H., Jr.; Rankin, J. *J. Chem. Soc.* **1924**, *125*, 1686.
(35) Reeve, W.; Myers, H. *J. Am. Chem. Soc.* **1951**, *73*, 1371.
(36) DeBoer, T. J.; Backer, H. J. *Organic Syntheses*; Wiley: New York, 1963; Collect. Vol. 4, 250.
(37) Dran, Mme. R.; Prange, T. *Bull. Soc. Chim. Fr.* **1967**, 4469.
(38) Clark, R. D.; Caroon, J. M.; Repke, D. B.; Strosberg, A. M.; Whiting, R. L.; Brown, C. M. *J. Pharm. Sci.* **1986**, *75*, 80.

point reported); IR (KBr) 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.70, 6.59 (2 d, 2, ArH, J = 7.9 Hz), 5.97 (s, 2, OCH_2O), 3.53 (s, 2, $ArCH_2$), 3.02 (t, 2, $ArCH_2$, J = 6.7 Hz), 2.54 (t, 2, $COCH_2$, J = 6.7 Hz). Anal. ($C_{11}H_{10}O_3$) C, H.

2-(*N*-Benzylamino)-6,7-(methylenedioxy)-1,2,3,4-tetrahydronaphthalene Hydrochloride (12a). Tetralone **11a** (3.0 g, 15.8 mmol) and benzylamine (1.78 g, 16.6 mmol) were dissoved in 150 mL of toluene and heated at reflux with continuous water removal. After 1.5 h, the toluene was removed by rotary evaporation. The residual brown oil was dissolved in 350 mL of ethanol and the mixture was reduced over 1 g of 5% Pt–C in a Parr shaker at 50 psig. After 24 h, the mixture was filtered through Celite, acidified with concentrated HCl, and cooled overnight. The precipitated salt was collected by suction filtration to afford 2.80 g (52.5%) of product, mp 270 °C. An additional 280 mg of salt was obtained from the mother liquor (total yield 58%): ^1H NMR (DMSO-d_6) δ 9.53 (s, 2, NH_2^+), 7.63 (m, 2, ArH), 7.42 (m, 3, ArH), 6.64, 6.62 (2 s, 2, ArH), 5.93 (s, 2, OCH_2O), 4.25 (s, 2, $ArCH_2$), 3.40 (m, 1, CHN^+), 3.19 (m, 1, ArCH), 2.95 (m, 1, ArCH), 2.77 (m, 2, ArCH), 2.32 (m, 1, CH), 1.87 (M, 1, CH). Anal. ($C_{18}H_{20}ClNO_2$) C, H, N.

2-(*N*-Benzylamino)-5,6-(methylenedioxy)-1,2,3,4-tetrahydronaphthalene Hydrochloride (12b). In a procedure similar to that for **12a**, 0.375 g (1.97 mmol) of tetralone **11b** and 0.222 g (2.07 mmol) of benzylamine were converted to the enamine. Concentration and catalytic reduction at low pressure over 110 mg of 5% Pt–C in ethanol, with workup as above, gave 0.57 g (90.4%) of the salt: mp 268–269 °C; ^1H NMR (DMSO-d_6) δ 9.54 (b s, 2, NH_2^+), 7.63 (d, 2, ArH), 7.44 (m, 3, ArH), 6.75, 6.63 (2 d, 2, ArH, J = 8 Hz), 5.99, 5.97 (2 s, 2, OCH_2O), 4.25 (s, 2, $ArCH_2$), 3.41 (m, 1, CHN), 3.21 (m, 1, ArCH), 2.85 (m, 2, ArCH), 2.60 (m, 1, ArCH), 2.37 (m, 1, CH_2), 1.85 (m, 1, CH_2). Anal. ($C_{18}H_{20}ClNO_2$) C, H, N.

6,7-(Methylenedioxy)-2-aminotetralin (3b). A solution of 1.05 g (3.3 mmol) of the *N*-benzyl compound **12a** in 50 mL of 95% ethanol containing 150 mg of 10% Pd–C was shaken in a Parr apparatus at 50 psig of H_2. After shaking overnight, the reaction was filtered through Celite and concentrated under vacuum to give a quantitative yield of the primary amine salt. A sample was recrystallized from ethanol: mp 282–283 °C, ^1H NMR ($CDCl_3$, free base) δ 6.51, 6.50 (2 s, 2, ArH), 5.83 (s, 2, OCH_2O), 3.09 (m, 1, CHN), 2.84 (dd, 1, ArCH, J = 15.8 Hz, 4.6 Hz), 2.74 (m, 2, $ArCH_2$), 2.43 (dd, 1, ArCH, J = 15.8 Hz, 9.3 Hz), 1.90 (m, 1, CHCN), 1.53 (m, 3, CHCN, NH_2). Anal. ($C_{11}H_{14}ClNO_2$) C, H, N.

5,6-(Methylenedioxy)-2-aminotetralin (4b). Method A. In a procedure similar to that for **3b**, 0.30 g (0.94 mmol) of **12b** in 50 mL of ethanol containing 50 mg of 10% Pd–C was shaken at 50 psig of H_2 for 8 h to afford a quantitative yield of the primary amine salt, after workup. Recrystallization was done with ethanol/ethyl acetate: mp 280–282 °C; ^1H NMR (500 MHz, $CDCl_3$, free base) δ 6.64, 6.56 (2 d, 2, ArH, J = 8 Hz), 5.93, 5.91 (2 d, 2, OCH_2O, J = 1.5 Hz), 3.14 (m, 1, CHN), 2.94 (dd, 1, ArCHCN, J = 15.8 Hz, 4.7 Hz), 2.86 (qd, 1, ArCH), 2.66 (qd, 1, ArCH), 2.49 (dd, 1, ArCHCN, J = 15.8 Hz, 9.4 Hz), 2.10 (m, 1, CHCN), 1.56 (m, 1, CHCN). Anal. ($C_{11}H_{14}ClNO_2$) C, H, N.

5,6-Dimethoxy-2-phthalimido-1,2,3,4-tetrahydronaphthalene (13). The hydrochloride salt of 5,6-dimethoxy-2-amino-1,2,3,4-tetrahydronaphthalene[39] (2.89 g, 11.9 mmol) was converted to the free base and combined with 1.81 g (12.21 mmol) of phthalic anhydride. This mixture was brought to a melt, under N_2, in an oil bath at 190 °C. After heating for 2 h, the mixture was cooled, 120 mL of 2-propanol was added, and the solution was heated to reflux. The phthalimido product crystallized on cooling to afford material with mp 168–169 °C, in a total yield of 2.87 g (71.8%): IR (KBr) (C=O) 1750, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.83 (m, 2, ArH), 7.71 (m, 2, ArH), 6.77, 6.76 (2 d, 2, ArH, J = 9.1 Hz), 4.52 (m, 1, CHN), 3.85, 3.82 (2 s, 6, OCH_3), 3.58 (m, 1, ArCH), 3.18 (m, 1, ArCH), 2.79 (m, 2, ArCH), 2.64 (m, 1, CHCN), 2.05 (m, 1, CHCN). Anal. ($C_{20}H_{19}NO_4$) C, H, N.

5,6-Dihydroxy-2-phthalimido-1,2,3,4-tetrahydronaphthalene (14). To a solution of 2.74 g (8.11 mmol) of **13** in 100 mL of dry CH_2Cl_2 under N_2 and cooled in a solid CO_2/acetone bath was added 40.6 mL of a 1.0 M solution of BBr_3 in CH_2Cl_2. The reaction was stirred overnight, while being warmed to room temperature, and then poured into 50 mL of ice/water. The resulting precipitate was collected by suction filtration, washed with water, and dried at high vacuum to afford 2.43 g (97%) of a white solid: mp 302–306 °C; ^1H NMR (DMSO-d_6) δ 8.95, 8.15 (2 s, 2, OH), 7.85 (s, 4, ArCH), 6.58, 6.36 (2 d, 2, ArCH, J = 8.2 Hz), 4.28 (m, 1, CHN), 3.31 (m, 1, ArCH) 2.93 (m, 1, ArCH), 2.71 (m, 1, ArCH), 2.42 (m, 2, ArCH, CHCN), 2.02 (m, 1, CHCN). Anal. ($C_{18}H_{15}NO_4$) C, H, N.

5,6-(Methylenedioxy)-2-phthalimido-1,2,3,4-tetrahydronaphthalene (15). Dihydroxy compound **14** (0.249 g, 0.8 mmol) was dissolved in 2 mL of dry DMF. After addition of 0.42 g (2.41 mmol) of CH_2Br_2, 0.334 g (2.41 mmol) of powdered K_2CO_3, and 4 mg of CuO,[22] the mixture was stirred and heated at reflux under N_2. An additional 0.42 g of CH_2Br_2 was added after 4 h, and heating was continued for 20 h. The reaction was cooled, and the volume was reduced by rotary evaporation. To the residue was added 25 mL of water, and the product was extracted into CH_2Cl_2 (5 × 10 mL). The organic extract was washed with 5% KOH and brine and dried ($MgSO_4$). The solution was filtered and concentrated under vacuum, and the residue was purified by radial chromatography on a silica gel rotor, and elution with dichloromethane. The product was recrystallized from 2-propanol to provide 0.101 g (39%) of material: mp 207–208 °C; ^1H NMR ($CDCl_3$) δ 7.85 (m, 2, ArH), 7.73 (m, 2, ArH) 6.65, 6.55 (2 d, 2, ArH, J = 8 Hz), 5.97, 5.96 (2 d, 2, OCH_2O, J = 1.4 Hz), 4.54 (m, 1, CHN), 3.57 (m, 1, ArCH), 3.03–2.56 (m, 4, ArCH, CHN), 2.05 (m, 1, CHCN). Anal. ($C_{19}H_{15}NO_4$) C, H, N.

5,6-(Methylenedioxy)-2-amino-1,2,3,4-tetrahydronaphthalene Hydrochloride (4b). Method B. Phthalimido derivative **15** (90 mg, 0.28 mmol) was suspended in 8 mL of 95% EtOH and hydrazine hydrate (60 mg, 1.2 mmol) was added. The mixture was stirred and heated at reflux overnight under N_2. The cooled reaction mixture was poured into water, basified with NaOH, and extracted with CH_2Cl_2. The organic extracts were washed with brine and dried ($MgSO_4$). After filtration and concentration under vacuum, the free base was acidified with HCl; this salt was dried several times by azeotropic distillation of ethanol and was crystallized from 2-propanol/ether to afford a yield of 41 mg (64%), with properties indentical with the material prepared by method A.

Pharmacology Methods. Drug-Discrimination Studies. Male, Sprague–Dawley rats, weighing approximately 200 g at the beginning of the study, were obtained from Murphy Breeding Labs, Inc., Plainfield, IN. Except for the first week, all rats were housed individually in a temperature-controlled room (25 °C) with an 0600–2000 lights on, 2000–0600 lights off schedule. Twenty-four drug naive rats were used for the LSD studies, and 15 rats were used that had been trained with MDMA in a previous study.[2]

Immediately following scheduled discrimination sessions the animals were returned to their home cages. Food was provided to maintain each rat at about 80% of the free-feeding weight. On Sundays, no sessions were run and animals were allowed to feed at their regularly scheduled time. Water was available ad lib, except during the training and testing periods.

Apparatus. Six identical standard operant chambers (Coulbourn Instruments) equipped with two response levers separated by a food pellet delivery system were employed. Food pellets (Bioserve, 45 mg, dustless) were used as reinforcement. Chambers contained a white house light and masking white noise and were enclosed in ventilated, sound-attenuated cubicles. The operant chambers were controlled by solid-state logic interfaced through a Coulbourn Instruments Dynaport to an IBM-PC located in an ajdacent control room. Data acquisition and control were handled by the IBM-PC using locally developed software.

Drug Administration. The training dose of *d*-LSD tartrate (NIDA) (185.5 nM/kg, 0.08 mg/kg), (±)-MDMA·HCl (1.75 mg/kg), or appropriate test drug doses were administered in saline in a volume of 1.0 mL/kg of body weight. Dosages refer to the salt forms, and all test compounds except LSD were administered as hydrochloride salts. All injections were administered intraperitoneally 30 min prior to the start of discrimination sessions.

Discrimination Training. To avoid positional preference, half of the animals were trained to press TRAINING DRUG-L

(39) McDermed, J. D.; McKenzie, G. M.; Phillips, A. P. *J. Med. Chem.* **1975**, *18*, 363.

and SAL-R, while the other half were trained vice versa. Rats were trained on an FR50 schedule with 15-min maintenance sessions. No significant difference in responding rate was seen between the training dose of LSD or MDMA and saline ($p > 0.05$, Student's t test). The complete training procedure has been published in a previous article.[2]

Stimulus Generalization. Testing sessions were run on Wednesdays and Saturdays only. Training sessions were held the rest of the week with Sundays off. On test days, the animal was placed into the operant chamber 30 min after injection. Test sessions lasted until the rat emitted 50 responses on either lever or until 5 min had passed, whichever came first. If the rat did not emit 50 responses on either lever within 5 min, he was scored as disrupted and was not included in the calculations. In either case, no reinforcement was given. In order to receive a test drug, the animals were required to satisfy the 85% correct lever response criterion on each of the two preceding training sessions. Also, following the procedure of Colpaert et al.,[40] test data were discarded and the test condition later retested if the test session was followed by failure to meet the 85% criterion in either of the two subsequent training sessions. This procedure was employed to increase the reliability of the individual test data. It has been reported[40] that incorrect lever selections in trained rats typically occur in bursts of one to three sessions. This correction procedure assists in avoiding the contamination of test data that may occur during such bursts. If the animal was not included in the testing procedure on a given day, the session was used for training.

Several preliminary experiments to determine appropriate dosages for new compounds were carried out; these data were discarded. Drug dosages were based on these initial experiments. The drug treatments in this study were randomized within each group of rats tested (LSD-trained or MDMA-trained). At least eight animals were tested at each dose, except in cases where very high doses produced an excessive number of disruptions.

Data Analysis. Animals were scored as drug positive if they selected the LSD or MDMA-appropriate lever, for LSD or MDMA-trained rats, respectively (i.e. if they emitted 50 responses on the drug lever). If generalization occurred (greater than 80% of the rats selected the drug-appropriate lever at some dose), these quantal data were analyzed by the method of Litchfield and Wilcoxon[41] to determine an ED_{50}.

Pharmacological Methods. Neurotoxicity Studies. Male Sprague–Dawley rats (Harlan Industries, Indianapolis, IN), weighing 175–200 g, were individually housed and given free access to food and water. Eight animals were used for each treatment. Rats were injected once, subcutaneously, with 40 mg/kg of the hydrochloride salts of racemic **1**, **3a**, or **3b** and were sacrificed at 1 week post drug administration. All drugs were dissolved in saline vehicle. The frontal cortex and hippocampus brain regions[42] were dissected over ice and immediately frozen in liquid nitrogen for storage at −70 °C until assay.

HPLC-EC Determination of Monoamines. The brain regions were weighed and homogenized with a motor-driven Teflon mortar and pestle in 1.0 mL of 0.4 N $HClO_4$ containing 0.05% Na_2EDTA, 0.1% $Na_2S_2O_5$, and 50 ng/mL of dihydroxybenzylamine (DHBA) as an internal standard. The samples were then centrifuged at 11000g for 20 min, and the supernatant was assayed for catecholamines, 5-HT, and their metabolites by HPLC-EC. A reverse-phase C_{18} cartridge column (Brownlee Laboratories, Ann Arbor, MI) and a mobile phase containing 0.05 M NaH_2PO_4, 0.03 M citric acid, 0.1 mM Na_2EDTA, 0.25% sodium octyl sulfate, and 25% methanol (pH = 2.85) were used to separate the monoamines and metabolites. A Hitachi D-2000 integrator was used for quantitation. A flow rate of 0.7 mL/min gave the following retention times: NE, 5.10 min; DOPAC, 5.95 min; DHBA, 6.50 min; 5-HIAA, 6.95 min; DA, 7.85 min; HVA, 10.86 min; and 5-HT, 13.20 min.

[^{3}H]Paroxetine Binding. The procedure of Habert et al.[43] was employed, with minor modification. The brain regions were thawed and weighed and homogenized in 15 mL of 50 mM Tris, containing 120 mM NaCl and 5 mM KCl (pH = 7.4), with a Brinkman polytron (setting 6, 2 × 20 s). The homogenates were centrifuged twice at 30000g for 10 min, with an intermittent wash in the same buffer.

Increasing concentrations of [^{3}H]paroxetine (0.1–2.5 mM) in the presence or absence of 10 μM fluoxetine were incubated in a total volume of 2 mL, containing 200–400 μg of protein. The tubes were allowed to equilibrate for 1 h at 24 °C before being filtered through a Brandel cell harvester using GF/C filters presoaked with 0.05% polyethyleneimine. The tubes were then washed twice with 5 mL of cold buffer and the filters were air-dried. Filters were placed into scintillation vials, aqueous counting scintillant (ACS; Amersham) was then added, and the samples were allowed to sit overnight at room temperature before counting at 46% efficiency in a Packard 4000 scintillation counter. The concentration of radioligand was determined by counting the dpm's added. Data analysis utilized the least squares curve fitting routines embodied in the programs EBDA and LIGAND as adopted for the IBM PC by McPherson.[44] The concentration of protein per tube was determined by using the method of Bradford.[45]

Acknowledgment. Support for this work was provided by USPHS Grant DA-04758 from the National Institute on Drug Abuse.

(40) Colpaert, F. C.; Niemegeers, C. J. E.; Janssen, P. A. J. *J. Pharmacol. Exp. Ther.* **1982**, *221*, 206.
(41) Litchfield, J. T., Jr.; Wilcoxon, F. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99.
(42) Glowinski, J.; Iverson, L. L. *J. Neurochem.* **1966**, *13*, 665.
(43) Habert, E.; Graham, D.; Tahraoui, L.; Claustre, Y.; Langer, S. Z. *Eur. J. Pharmacol.* **1985**, *118*, 107.
(44) McPherson, G. A. *J. Pharmacol. Methods* **1985**, *14*, 213.
(45) Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.