and 2 μL of a DMSO solution of calcimycin (A23187) to give final concentrations of 5 and 7.6 μM, respectively. Five minutes later, the incubation was terminated by the addition of a 0.27-mL volume of CH_3CN/AcOH (100:3). The mixture was then clarified by precipitated protein by centrifugation. Analysis of 5-LO/CO pathway products was performed by HPLC. A 100-μL volume of clarified sample was injected onto a Radial Pak RP-18, OD 032 column (2.6 mm, i.d., Brownlee), and developed with a mobile phase of CH_3CN/H_2O/AcOH over a linear gradient from 35 to 65% acetonitrile for 1 min at a flow rate of 2 mL/min. The developing solvent was continued at 65% acetonitrile for another 4 min before being recycled to original conditions. Detection of product radioactivity was performed with the aid of a Berthold 504 radioactivity monitor equipped with an 800-μL flow cell mixing 2.4 mL/min OMNIFLUOR (New England Nuclear) with column effluent. Integration of peak areas was performed by a SP-4200 computing integrator (Spectra Physics). The radiolabeled product profile contained four major peaks. In order of elution, they were prostaglandin D_2, dihydroxy fatty acids, 5-HETE, and arachidonic acid. The area under the curve (AUC) as measured in integration units for each product was compared to the average AUC value for non-drug-treated samples. The results were expressed as "percent of control" and were plotted versus the log of drug concentration. The results (IC_{50}) in Tables III–VI are presented as the means of three determinations. In no case did the standard deviation exceed ±25%.

Carrageenan-Induced Rat Foot Edema. The standard procedure of Winter et al.[32,33] was employed to measure the inhibition of carrageenan-induced foot edema in rats. A 5-mL volume of drug solubilized in 0.1 M meglumine was administered orally to fasted male Sprague–Dawley rats (~200 g) in groups of five or six. One hour later, 0.05 mL of a carrageenan suspension (1% in water) was administered by subplantar injection into the hind paw. Paw volume was measured by mercury displacement immediately after injection and again at 3 h. The average foot swelling in a group of drug-treated animals ($n = 5$) was compared to that of a group of vehicle-treated animals ($n = 10$) and expressed as percent inhibition. When appropriate this was then plotted versus the log of drug concentration and the half-maximal (ED_{50}) value was estimated by linear-regression analysis.

Acknowledgment. We acknowledge Patricia Joseph for assistance in the CO/LO assay and Balys Kondratas for the RFE testing. We especially thank Deborah Sanford of our Document Preparation Center for her patience in processing this document.

Supplementary Material Available: Tables S1–S5 listing atomic coordinates, isotropic and anisotropic thermal parameters, bond lengths and angles, and H atom coordinates (5 pages). Ordering information is given on any current masthead page.

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Synthesis of 3-Arylecgonine Analogues as Inhibitors of Cocaine Binding and Dopamine Uptake

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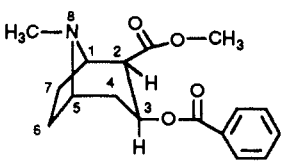
3-Arylecgonine analogues were synthesized and characterized by ^1H and ^{13}C NMR, IR, and MS. The compounds were synthesized as racemates from cycloheptatriene-7-carboxylic acid or enantiomerically from (−)-cocaine. These analogues were tested for their ability to inhibit [^3H]cocaine binding to bovine striatal tissue and to inhibit [^3H]dopamine uptake into striatal synaptosomes. Methyl (1*RS*-2-*exo*-3-*exo*)-8-methyl-3-phenyl-8-azabicyclo[3.2.1]octane-2-carboxylate was the most potent analogue. IC_{50} values for inhibition of cocaine binding and dopamine uptake were 20 and 100 nM, respectively. The racemates and the 1*R* isomers were equally potent inhibitors of binding and uptake. Methyl (1*RS*-2-*endo*-3-*exo*)-3-(2,4-dinitrophenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate was the least potent. IC_{50} for inhibition of both binding and uptake was 40 μM.

Introduction

Cocaine abuse has become a serious social problem in the U.S.[1] Initially believed to be nonaddictive, it is now accepted that cocaine is an additive drug.[2] Addiction to cocaine is driven by the powerful positive reinforcing properties of the drug.[3] There is strong evidence that the mesocorticolimbic dopamine (DA) system of the brain is the target site for cocaine and possibly other addictive drugs.[4-7] Cocaine (1) inhibits uptake of DA into the dopaminergic nerve terminals and has a DA-like effect on the firing rate of the mesocorticolimbic dopaminergic neurones.[8,9] Accordingly, one would expect to find cocaine receptors, which would bind [^3H]cocaine with high affinity, associated with that system. This has been verified by several groups.[10-12] One would also expect that cocaine analogues should show a behavioral order of potency which should closely approximate the binding order of potency. Actually, both the binding and uptake assays were used to evaluate the high-affinity binding site of brain striatum as the cocaine receptor responsible for the addictive properties of the drug.[13] For further pharmacological



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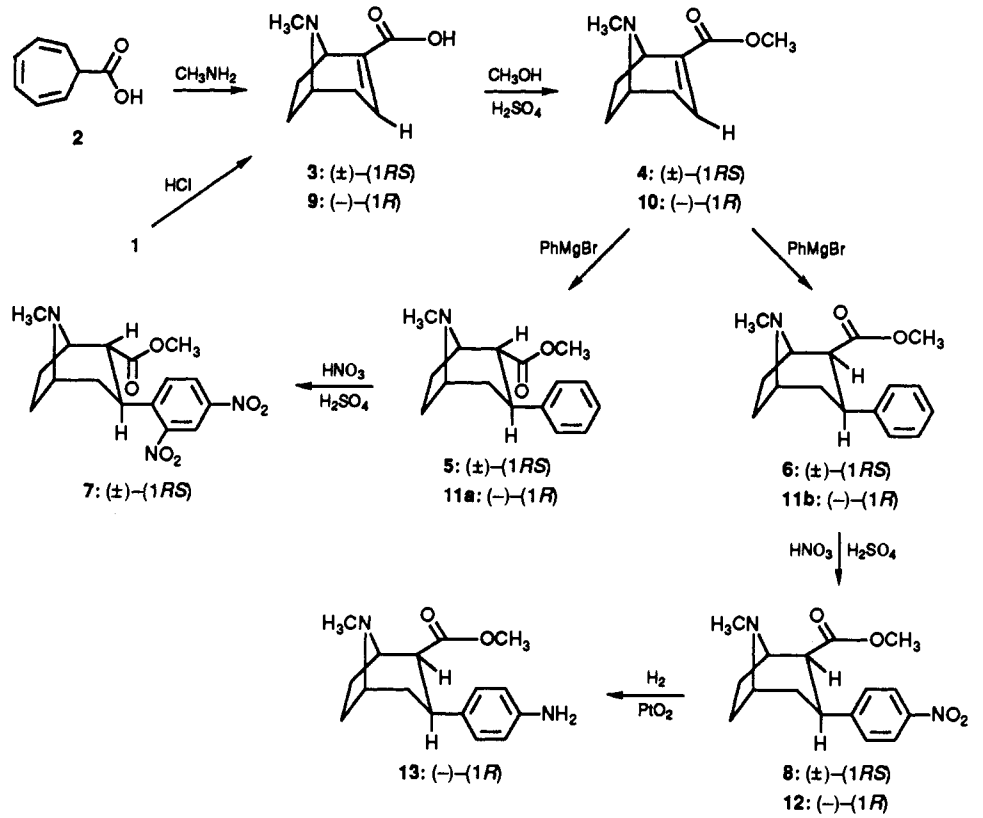
Scheme I



characterization of the cocaine receptor protein it became obvious that there is a need for cocaine analogues which may be used as photoaffinity labels or affinity ligands for receptor purification.

Cocaine chemistry was conceived in the late 1800's. The first synthetic scheme in which cocaine was synthesized from 2-carbomethoxytropinone was presented by Willstatter in 1923.[14] Although much was learned in those early years, interest in the pharmacological effects of the drug remained low until the middle part of this century. Much of the analogue design and synthesis has involved isomeric cocaine studies,[15,16] modification of the tropane moiety at the bridge nitrogen,[17] or modification of the C-2 position.[18] While some C-3 tropane derivatives have been synthesized,[19,20] very few 3-aryl analogues have been made and tested. Virtually none are available commercially or from NIDA. 3-Aryl analogues may be useful precursors to photoaffinity and affinity probes which are needed for isolation and characterization of the cocaine receptor. In this study we report the synthesis of several 3-arylecgonine analogues and their potencies for inhibiting cocaine binding to bovine striatal tissue and inhibiting dopamine uptake into striatal synaptosomes.

Results and Discussion

Chemistry. Racemic anhydroecgonine methyl ester **4** (Scheme I) was obtained by following the procedure of Doering et al.[21] and others.[22,23] Treatment of **4** with phenylmagnesium bromide and subsequent workup gave a mixture of α and β phenyl analogues **5** and **6** as reported by Clarke et al.[20] These were separated by column chromatography, and the products were nitrated by standard methods to give nitro compounds **7** and **8**, which crystallized readily.

The fact that under the same conditions nitration of **5** gave mainly a dinitro compound, and **6** gave mainly a mononitro was surprising. Stereochemically one would expect the α isomer **5** to be more hindered than **6** and thus less likely to be dinitrated. Possibly, the oxygen of the side chain carbonyl in position 2 assists in the approach of the electrophilic nitrating species in **5** and thus facilitates the substitution of a second nitro group.

(-)-Anhydroecgonine methyl ester **10** (Scheme I) was obtained from (-)-cocaine by hydrolysis and dehydration with concentrated hydrochloric acid,[19] followed by esterification.[19] Compounds **11a** and **11b** were obtained by the method of Clarke et al.[20] as described above. Instead of separating the mixture at this step, it was found to be more convenient to nitrate the mixture and to isolate the desired mononitro product **12** from the nitration mixture. This process gave a clean separation of **12** free of the unwanted dinitro contaminant in a much higher overall yield than was obtained from the separation of **11a** and **11b** followed by nitration of the pure materials.

Since the (±)-dinitro compound **7** had very low potency compared to that of mononitro (±)-**8**, it was decided that

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Table I. IC_{50} Data for Competitive Inhibition of [^{3}H]Cocaine Binding and [^{3}H]Dopamine Uptake in Bovine Striatal Tissue

corresponding analogue	IC_{50}[a] cocaine binding	IC_{50}[a] dopamine uptake
1	139 ± 38 nM	843 ± 116 nM
5	1.2 ± 0.7 μM	2.1 ± 0.1 μM
6	22 ± 4.7 nM	133 ± 15 nM
7	11 ± 0.8 μM	30 ± 1.1 μM
8	213 ± 68 nM	903 ± 59 nM
12	137 ± 37 nM	616 ± 84 nM
13	192 ± 42 nM	557 ± 79 nM

[a] Each value represents the mean ± SEM (n = 9).

the (−)-dinitro compound was not required for biological testing. This compound was therefore not isolated.

Catalytic reduction (PtO_2/H_2) of **12** gave the desired amino compound **13**. (Scheme I).

Pharmacology. It is generally accepted that the cocaine receptor in the brain, which mediates the reinforcement properties of the drug, is closely associated with the dopamine transporter. Evidence supporting this point of view include the following: (1) Cocaine is a potent inhibitor of dopamine uptake.[24] (2) Self-administration of cocaine is affected by lesions of dopaminergic terminal fields.[25,26] (3) Selective dopamine receptor blockers attenuate the rewarding impact of intravenous cocaine.[27] (4) The relative potency of several drugs in altering animal behavior and their ability to block dopamine uptake and inhibit binding of [^{3}H]mazindol, a selective dopamine transporter blocker, correlate very well.[28] (5) Several correlations between their affinity for the cocaine receptor (i.e. high-affinity [^{3}H]cocaine binding) and inhibition of [^{3}H]dopamine uptake into rat striatal synaptosomes have been demonstrated.[13]

Therefore, the newly synthesized cocaine analogues were assayed for their ability to inhibit [^{3}H]cocaine binding and [^{3}H]dopamine uptake. The racemate of β-phenylecgonine **6** was the most potent inhibitor of [^{3}H]cocaine binding (Table I). This supported previous conclusions that the β-phenyl analogue of cocaine was more potent in altering animal behavior.[20] In this study, the compound was found to be 4 times more potent in inhibiting [^{3}H]cocaine binding than (−)-cocaine itself. The α-phenyl racemate of ecgonine (**5**) was 50-fold less potent than the β-phenyl racemate. α-(Dinitrophenyl)ecgonine analogue **7** was much less potent than β-mononitro derivative **8** (Table I). The different compounds had similar rank order of potency in inhibiting [^{3}H]dopamine uptake (Table I). The correlation coefficient of inhibiting binding and uptake by the six compounds was very high (0.99).

(−)-β-(*p*-Aminophenyl)ecgonine **13** was the target compound sought after to use as an affinity ligand for purification of the cocaine receptor. This compound can be linked to activated affinity chromatography gels via the phenyl amino moiety. Its high affinity for the cocaine receptor (IC_{50} for inhibiting [^{3}H]cocaine binding = 110 nM) is an excellent feature. This compound is also a good inhibitor of dopamine uptake (Table I).

Experimental Section

Chemistry. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were obtained with a General Electric QE-300 spectrometer. Spectra were obtained with $CDCl_3$ or DMSO-d_6, with TMS as an internal standard, or D_2O with the solvent signal as the internal standard. Mass spectra were obtained with a Hewlett-Packard MSD spectrometer. Compounds were introduced into the mass spectrometer through a 15-m methyl silica gum GC capillary column (HP-1). Infrared spectra were recorded on an Analect FX-6160 FT-IR spectrophotometer. Crystalline compounds were analyzed with KBr disks while spectra of liquids were recorded as neat films. Chromatographic separations were performed on silica gel columns (70–230 mesh). Thin-layer chromatography was run with silica gel plates. The eluting solvents for both LC and TLC analyses were either (a) pentane/diethyl ether/isopropylamine (67:30:3) or (b) hexane/diethyl ether/isopropylamine (67:30:3). Elemental analyses were performed, on samples dried to constant weight, by Micro Analysis Inc. and elemental compositions agreed within 0.4% of the calculated values.

Methyl (1*RS*)-8-Methyl-8-azabicyclo[3.2.1]oct-2-ene-2-carboxylate (4). This compound was synthesized according to the procedure of Grundmann and Ottmann[23] with the following modifications. To a mixture of 3.6 g (26 mmol) of 2,4,6-cycloheptatriene-7-carboxylic acid (**2**)[23] and 12 g (0.3 mol) of NaOH in 50 mL of H_2O was added 18.26 g (0.27 mmol) of methylamine hydrochloride in 100 mL of H_2O. The solution was stirred with a magnetic stirrer and heated in a pressure bomb (2000 psi) in an oil bath at 150 °C for approximately 6 h. After cooling overnight, the solution was filtered, and the excess methylamine and H_2O were removed under vacuum. The dry residue was dissolved in 50 mL of 2 N H_2SO_4 and extracted with diethyl ether (3 × 50 mL) to remove any unreacted starting material. The aqueous phase was neutralized with 2 N NaOH and the H_2O was evaporated under vacuum, leaving inorganic salts and product. This residue was suspended in 50 mL of absolute MeOH and 10 g of concentrated H_2SO_4 was added. The mixture was heated under reflux for approximately 24 h. After this time the alcohol was removed under vacuum and the residue was dissolved in a minimum amount of H_2O. The aqueous solution was saturated with potassium carbonate, filtered, and extracted with ether (4 × 50 mL). The ether fractions were combined, dried over $MgSO_4$, filtered, and evaporated under vacuum, resulting in 2.9 g (60%) of a pale yellow, liquid product: IR (neat) 2950, 1713, 1640, 1436, 1364, 1283, 1254, 1140, 1085, 1049 cm^{-1}; ^{1}H NMR (300 MHz) (DMSO-d_6) δ 1.40–1.45 (m, 1 H, H_6), 1.60–1.70 (m, 1 H, H_6), 1.76–1.84 (dd, J = 20, 4 Hz, 1 H, H_{4eq}), 1.90–2.10 (m, 2 H, H_7), 2.20 (s, 3 H, NCH_3), 2.49–2.56 (d, J = 20 Hz, 1 H, H_{4ax}), 3.12–3.15 (m, 1 H, H_1), 3.57–3.59 (d, J = 4 Hz, 1 H, H_5), 3.65 (s, 3 H, OCH_3), 6.74–6.75 (m, 1 H, H_3); ^{13}C NMR (DMSO-d_6) δ 30.039 (C_6), 31.177 (C_7), 34.390 (C_4), 35.978 (NCH_3), 51.579 (OCH_3), 56.359 (C_5), 58.137 (C_1), 133.549 (C_2), 136.299 (C_3), 166.107 (C=O).

Methyl (1*RS*-2-*endo*-3-*exo*)-8-Methyl-3-phenyl-8-azabicyclo[3.2.1]octane-2-carboxylate (5) and Methyl (1*RS*-2-*exo*-3-*exo*)-8-Methyl-3-phenyl-8-azabicyclo[3.2.1]octane-2-carboxylate (6). These compounds were prepared according to the procedure of Clarke et al.[20] with the following modifications. Instead of making the aqueous solution (obtained during Grignard reaction workup) highly basic and extracting directly with diethyl ether, the aqueous mixture was first adjusted to pH = 8 with NH_4OH, extracted with ether, and then adjusted to pH = 11–12 with NH_4OH and extracted again with ether. The first extraction removed any residual starting material, a large portion of the unwanted α-isomer **5**, and a small amount of the desired β-isomer **6**. TLC using elution solvent a indicated that the second extraction yielded a much larger proportion of β-isomer **6**.

Compounds **5** and **6** were separated by the procedure of Clarke et al.[20] The mixture was chromatographed on silica gel (50 g/g of mixture) using elution solvent a. The β-isomer [mp = 81–83 °C (lit.[20] mp = 62–64.5 °C)] eluted first (R_f = 0.38), followed by the α-isomer [mp = 71–72 °C (lit.[20] mp = 70–72 °C)] (R_f = 0.27), and lastly the unreacted anhydroecgonine starting material (R_f = 0.16). The yields were similar to those reported by Clarke et al.[20]

Methyl (1*RS*-2-*endo*-3-*exo*)-3-(2,4-Dinitrophenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate (7). Compound **5** (0.1 g, 4 × 10^{-4} mol) was dissolved in 2 mL of concentrated H_2SO_4 and cooled to 0–4 °C in an ice/salt bath. Ice-cold 70%

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HNO_3 (1 mL) was added to the solution dropwise. After the addition was complete, the mixture was stirred and the temperature was kept between 0 and 4 °C, for an additional 1 h. The reaction mixture was then poured over ice (10 g) and made basic with concentrated NH_4OH. A yellow precipitate formed and was filtered under vacuum. This precipitate was recrystallized from acetone: mp = 127–130 °C; IR (KBr pellet) 3405, 3235, 2953, 1719, 1608, 1530, 1350 cm^{-1}; ^1H NMR (300 MHz) ($CDCl_3$) δ 1.58–2.20 (m, 6 H, H_1, H_5, H_6, H_7), 2.40 (s, 3 H, NCH_3), 3.10–3.15 (dd, J = 3 (vic), 12 Hz (gem), 1 H, H_{4ax}), 3.30–3.32 (m, 1 H, H_2), 3.51 (s, 3 H, OCH_3), 3.55–3.58 (m, 1 H, H_3), 3.73–3.83 (dt, J = 6 (vic), 12 Hz (gem), 1 H, H_{4eq}), 7.67–7.70 (d, J = 9 Hz, 1 H, $H_{6'}$), 8.33–8.37 (dd, J = 9, 2 Hz, 1 H, $H_{5'}$), 8.58–8.59 (d, J = 2 Hz, 1 H, $H_{3'}$); ^{13}C NMR ($CDCl_3$) δ 23.02 (C_6), 25.43 (C_7), 31.29 (C_5), 37.98 (C_4), 39.4, 51.14, 51.64, 60.85, 63.45 (OCH_3), 119.15, 123.00, 126.29, 129.76, 145.45, 150.22 (aromatics), 171.59 (C=O); MS (70 eV) m/z (relative intensity) 349 (M^+, 15), 290 (59), 126 (28), 97 (61), 96 (55), 83 (35), 82 (100). Anal. ($C_{16}H_{19}N_3O_6 \cdot 0.25H_2O$) C, H, N.

Methyl (1*RS*-2-*exo*-3-*exo*)-8-Methyl-3-(4-nitrophenyl)-8-azabicyclo[3.2.1]octane-2-carboxylate (8). Compound **6** (100 mg, 4×10^{-4} mol) was dissolved in 2.0 mL of concentrated H_2SO_4 and cooled to 0–4 °C in an ice/salt bath. To the stirring mixture was slowly added an ice-cold nitrating solution consisting of 1.0 mL of HNO_3 (70%) and 1.0 mL of concentrated H_2SO_4. The resulting yellow solution was stirred in the ice bath for an additional 30 min. At the end of this time the contents of the reaction vessel were poured over ice (25 g) and the vessel was rinsed three times with H_2O. The aqueous solution was then neutralized with $NaHCO_3$ (the temperature was kept between 0 and 4 °C) and extracted with diethyl ether (3 × 50 mL). The ether fractions were combined, washed twice with small portions of H_2O, dried over anhydrous $MgSO_4$, and filtered, and the ether was evaporated under vacuum. Recrystallization from pentane resulted in pale yellow crystals: 88 mg: 75%; mp = 95–97 °C; IR (KBr pellet) 3078, 2947, 1744, 1605, 1512, 1345 cm^{-1}; ^1H NMR (300 MHz) ($CDCl_3$) δ 1.58–1.78 (m, 3 H, H_6, H_5), 2.10–2.30 (m, 2 H, H_7), 2.24 (s, 3 H, NCH_3), 2.55–2.64 (dt, J = 3 (vic), 12 Hz (gem), 1 H, H_{4ax}), 2.97–2.99 (m, 1 H, H_1), 3.03–3.11 (m, 1 H, H_{4eq}), 3.37–3.41 (m, 1 H, H_2), 3.51 (s, 3 H, OCH_3), 3.61–3.37 (m, 1 H, H_3), 7.39–7.42 (m, 2 H, $H_{2'}$, $H_{6'}$), 8.11–8.15 (m, 2 H, $H_{3'}$, $H_{5'}$); ^{13}C NMR ($CDCl_3$) δ 24.74, 25.36 (C_6, C_7), 33.39 (C_4), 33.56, 41.48, 50.91, 52.20, 61.56, 64.78, 122.68, 122.73, 127.58, 127.66, 145.65, 151.01 (aromatics), 171.23 (C=O); MS (70 eV) m/z (relative intensity) 304 (M^+, 8), 273 (11), 245 (24), 126 (16), 97 (62), 96 (60), 84 (20), 83 (31), 82 (100). Anal. ($C_{16}H_{20}H_{20}N_2O_4 \cdot 0.25H_2O$) C, H, N.

Methyl (1*R*)-8-Methyl-8-azabicyclo[3.2.1]oct-2-ene-2-carboxylate Hydrochloride (9). Compound **9** was synthesized via the method of Zirkle et al.:[19] mp = 239–243 °C (lit.[19] mp = 240–244 °C); $[\alpha]^{21}_D$ = −50.76° (*c* 2.03, H_2O).

Methyl (1*R*)-8-Methyl-8-azabicyclo[3.2.1]oct-2-ene-2-carboxylate (10). Compound **9** (15 g, 74 mmol) was dissolved in 100 mL of absolute MeOH and 22.0 g of concentrated H_2SO_4 was added. The mixture was heated under reflux for approximately 24 h. At the end of this time the alcohol was removed by evaporation under vacuum, the residue then dissolved in a minimum amount of H_2O and carefully saturated with solid potassium carbonate. The aqueous solution was extracted with diethyl ether (3 × 50 mL). The ether fractions were combined, washed twice with small portions of H_2O, dried over anhydrous $MgSO_4$, filtered, and evaporated under vacuum, yielding **10** (7.92 g, 59%) as a pale yellow oil.

Methyl (1*R*-2-*endo*-3-*exo*)-8-Methyl-3-phenyl-8-azabicyclo[3.2.1]octane-2-carboxylate (11a) and Methyl (1*R*-2-*exo*-3-*exo*)-8-Methyl-3-phenyl-8-azabicyclo[3.2.1]octane-2-carboxylate (11b). Synthesis of these compounds was identical with that described for compounds **5** and **6**. Separation of the isomeric mixture formed during this reaction was not performed at this step. It was found that a more convenient isolation of **12** could be accomplished by chromatography of the nitration mixture resulting from the next step in the reaction sequence, rather than separating the starting materials.

Methyl (1*R*-2-*exo*-3-*exo*)-8-Methyl-3-(4-nitrophenyl)-8-azabicyclo[3.2.1]octane-2-carboxylate (12). A mixture of compounds **11a** and **11b** (100 mg, 0.40 mmol) was dissolved in 2.0 mL of concentrated H_2SO_4 and cooled to 0–4 °C in an ice/salt bath. To the stirring mixture was slowly added an ice-cold nitrating solution consisting of 1.0 mL of HNO_3 and 1.0 mL of concentrated H_2SO_4. The resulting yellow solution was stirred in the ice bath for an additional 30 min. At the end of this time the contents of the reaction vessel were poured over ice (25 g) and the flask was rinsed thoroughly with H_2O. The aqueous solution was neutralized with $NaHCO_3$, the temperature was kept between 0 and 4 °C, and then the mixture was extracted with diethyl ether (3 × 50 mL). The ether fractions were combined, washed twice with small portions of H_2O, dried over anhydrous $MgSO_4$, filtered, and evaporated under vacuum, leaving 100 mg of a dark yellow oil.

The resulting mixture was chromatographed on silica gel (50 g/g of mixture) using electron solvent b. β-Nitro isomer **12** eluted first (R_f = 0.33). Evaporation of the solvent mixture and recrystallization from pentane resulted in 80 mg of product **12**. Physical constants were the same as for compound **8**. The α-dinitro isomer was not isolated for reasons which are mentioned in the Discussion.

Methyl (1*R*-2-*exo*-3-*exo*)-3-(4-Aminophenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate Dibromide (13). Compound **12** (85 mg, 0.28 mmol) was dissolved in 50 mL of absolute MeOH and reduced over PtO_2 (20 mg) at 50 psi for approximately 3 h. After removal of the catalyst, the alcohol was evaporated under vacuum leaving 70 mg of a clear oil. The oil was dissolved in a minimum amount of anhydrous ether and 1 drop of 48% HBr was added. The resulting white precipitate was crystallized from methanol/ether: mp = 220–223 °C; IR (KBr pellet) 3421, 3091, 2901, 2592, 1721, 1708, 1518, 1436, 1235, 1142 cm^{-1}; MS (70 eV, TFA derivatized) m/z (relative intensity) 370 (M^+, 54), 339 (10), 311 (20), 270 (5), 155 (9), 97 (41), 96 (53), 83 (95), 82 (100). Anal. ($C_{16}H_{24}N_2O_2Br2 \cdot 1.5H_2O$) C, H, N.

Biology. Tissue Preparation. All tissue samples were prepared with striatum dissected from bovine brain. The dissected tissue included caudate, putamen, and globus pallidus, as well as nucleus accumbens. For [^{3}H]cocaine binding the striatal tissue was homogenized in ice-cold 10 mM Na_2HPO_4 buffer containing 0.32 M sucrose, pH = 7.4, using a P-10 Kinematica Polytron homogenizer (setting 5–6) for 15 s. The homogenate was then centrifuged for 20 min at 17000*g* in a Sorvall centrifuge. The supernatant was decanted and discarded. The resulting pellet was rehomogenized and centrifuged as described above. The final pellet was then resuspended in 20 volumes of the same phosphate–sucrose buffer to yield a protein concentration of approximately 3.0 mg/mL. The Lowry et al.[29] method was utilized to determine all final protein concentrations.

Tissue samples used for assaying [^{3}H]dopamine uptake was prepared as described by Richelson and Pfenning[24] with slight modifications. Striatal tissue was homogenized in ice-cold 0.3 M sucrose, 11 μM glucose (pH = 7.4) buffer using a glass Potter–Elvehjem homogenizer with Teflon pestle. The homogenate was centrifuged for 10 min at 3000*g* in a Sorvall centrifuge, and then the supernatant fraction was centrifuged for 20 min at 17000*g*. The resultant P_2 pellet was resuspended in 10 volumes of the same sucrose–glucose buffer.

[^{3}H]Cocaine Binding. Binding of [^{3}H]cocaine (*l*-[benzoyl-3,4-^{3}H(N)], 29.7 Ci/mmol; New England Nuclear, Boston, MA) to bovine striatal membranes was measured by filtration assay by using a previously reported method.[13]

[^{3}H]Dopamine Uptake. Uptake of [^{3}H]dopamine [(dihydroxyphenyl)ethylamine-3,4-d_2 30 Ci/mmol; New England Nuclear, Boston, MA] into bovine striatal synaptosomes was measured at 37 °C by filtration assay. A previously described method was utilized[13] except that the nonspecific uptake was measured at 4 °C.

Data Analysis. All assays were performed in triplicate, and mean values of three separate experiments were used. IC_{50} values were calculated using log–logit transformations of the raw data.

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