Pharmacology Procedures. Procedures for the biological testing reported herein have been published.[5,6,11]

(Aminoalkyl)indole Binding Assay. Radioligand binding studies were performed as described[11] using male Sprague-Dawley rat cerebellar membranes from a 48000*g* pellet which was washed twice by suspension in 20 mM HEPES buffer, pH 7. The pellet was suspended (1:120 w/v) in buffer, kept on ice, and used within 1 h.

The assay was started with the addition of homogenate containing 100-120 μg of cerebellar membrane protein. The 1-mL final assay volume contained: 20 mM HEPES pH 7; 0.5 nM [^{3}H]-(*R*)-(+)-**21** (59-60 Ci/mmol, 99% purity, Du Pont/NEN); 1 mg/mL BSA (Sigma A-7030); 100-120 μg of cerebellar membrane protein; and varying concentrations of competing compounds. Nonspecific binding was determined in the presence of 1 μM unlabeled (*R*)-(+)-**21**.

Compounds were solubilized in (i) a mixture of methanesulfonic acid/ethanol, (ii) ethanol, or (iii) DMSO. The experiments were controlled for vehicle effects. Further dilutions of compounds or radioligand were in buffer containing 5 mg/mL BSA to prevent absorption to glass.

The incubation was carried out at 30 °C for 90 min and stopped by rapid filtration and rinsing with 20 mL of 20 mM HEPES, pH 7.0, containing 0.5 mg/mL BSA over Whatman GF/B filters (presoaked in 5 mg/mL BSA-Buffer) on a 48-channel cell harvester. Radioactivity on the filters was measured by liquid scintillation spectrometry. Specific binding was defined as the difference in binding in the presence and absence of 1 μM (*R*)-(+)-**21**. Assays were performed in triplicate, and each experiment was repeated at least three times.

Binding data was analyzed using the radioligand binding analysis program EBDA[47] and LIGAND.[48] Protein was determined by the method of Lowry et al.[49]

Acknowledgment. We are grateful to John Herrmann, Garry Pilling, and Dick Philion for the preparation of bulk intermediates, to Steve Clemans and his staff for spectroscopic measurements, and to Sol Daum, Lynn McNaughton, and Jeff Abt for technical assistance.

Supplementary Material Available: (1) NMR chemical shift data for **20**, **21**, **24**, **29**, and **40** and (2) tables listing atomic coordinates, bond distances and angles, and thermal parameters for the (-)-dibenzoyl-L-tartaric acid salt complex of (*R*)-(+)-**14** (1:2) (10 pages). Ordering information is given on any current masthead page.

(47) McPherson, G. A. Analysis of Radioligand Binding Experiments. A Collection of Computer Programs for the IBM PC. *J. Pharmacol. Methods* **1985**, *14*, 213-228.
(48) Munson, P. J.; Rodbard, D. LIGAND: A Versatile Computerized Approach for Characterization of Ligand-binding Systems. *Anal. Biochem.* **1980**, *107*, 220-239.
(49) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, 265-275.

2β-Substituted Analogues of Cocaine. Synthesis and Inhibition of Binding to the Cocaine Receptor

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The potencies of a series of 2β-substituted cocaine analogues to displace [^{3}H]-3β-(*p*-fluorophenyl)tropane-2β-carboxylic acid methyl ester binding in rat striatal membranes demonstrate the requirement for a 2β-substituent with two hydrogen-bond acceptors. The insensitivity of the ester moiety to steric and electronic factors suggests its modification to provide site-specific irreversible ligands.

The natural component of coca leaves (*Erythroxylum coca*), known as (-)-cocaine, is a psychostimulant and a powerful reinforcer[1,2] known to bind to specific sites in mammalian brain.[3-7] A correlation of the potencies of cocaine and cocaine analogues in drug self-administration with their potencies to inhibit dopamine uptake and with their binding affinities has supported the existence of a cocaine receptor at the dopamine transporter.[8] To elucidate the nature of this putative pharmacophore, we initiated a program to systematically examine the effects of structure variation on binding affinity. Recently, we reported on the stereoselectivity of the cocaine binding site,[9] the effect of substitution at C-3,[10-12] and the effects of the location of the nitrogen atom and of its substitution pattern.[13] As part of this continuing investigation, we now report on the effect of substitution at the 2-position.

Results

Synthesis. The cocaine analogues, **2-20**, which were synthesized and studied are listed in Table I. Scheme I summarizes the procedures utilized to prepare **2-15**. Hydrolysis of (-)-cocaine (**1**) gave benzoylecgonine (**10**);[14] reduction of **10** with diborane afforded the alcohol **12** which, when treated with acetic anhydride, gave **13**. Treatment of **10** with *N,N*-formyldiimidazole or thionyl chloride gave the imidazolide **22a** or acid chloride **22b**,

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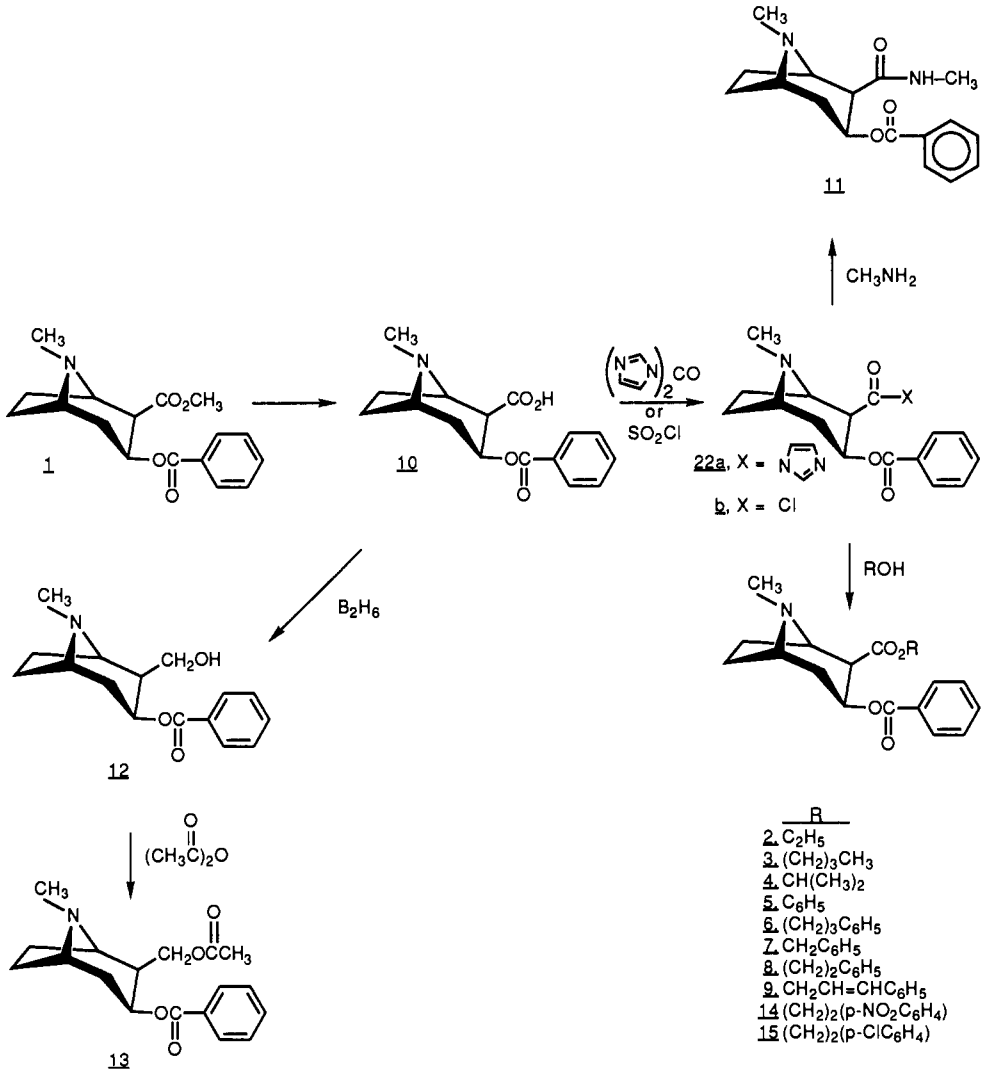
(1) Griffiths, R. R.; Bigelow, G. E.; Henningfield, J. E. Similarities in Animal and Human Drug-Taking Behavior. In *Advances in Substances Abuse*; Mello, N. K., Ed.; JAI Press Inc.: Vol. 1, pp 1-90.
(2) Johanson, C. E.; Fischman, M. W. The Pharmacology of Cocaine Related to Its Abuse. *Pharmacol. Rev.* **1989**, *41*(1), 3-52.
(3) Calligaro, D. O.; Eldefrawi, M. E. Central and Peripheral Cocaine Receptors. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 61-67.
(4) Calligaro, D. O.; Eldefrawi, M. E. High Affinity Stereospecific Binding of [^{3}H]Cocaine in Striatum and Its Relationship to the Dopamine Transporter. *Membr. Biochem.* **1988**, *7*, 87-106.
(5) Madras, B. K.; Bergman, J.; Fahey, M. A.; Canfield, D. R.; Spealman, R. D. Effects of Cocaine and Related Drugs in Nonhuman Primates. I. [^{3}H]Cocaine Binding Sites in Caudate-Putamen. *J. Pharmacol. Exp. Ther.* **1988**, *251*, 131-141.
(6) Reith, M. E. A.; Sershen, H.; Lajtha, A. Saturable [^{3}H]Cocaine Binding in Central Nervous System of Mouse. *Life Sci.* **1980**, *27*, 1055-1062.
(7) Schoemaker, H.; Pimoule, C.; Arbilla, S.; Scatton, B.; Javoy-Agid, F.; Langer, S. Z. Sodium Dependent [^{3}H]Cocaine Binding Associated with Dopamine Uptake Sites in the Rat Striatum and Human Putamen Decrease After Dopaminergic Denervation and in Parkinson's Disease. *Naunyn-Schmiedbergs Arch. Pharmacol.* **1985**, *329*, 227-235.
(8) Ritz, M. C.; Lamb, R. J.; Goldberg, S. R.; Kuhar, M. J. Cocaine Receptors on Dopamine Transporters are Related to Self Administration of Cocaine. *Science* **1987**, *237*, 1219-1223.

0022-2623/92/1835-0135$03.00/0

Scheme I



respectively. Addition of methylamine to a solution of **22a** gave the amide **11**; the esters **2–9**, **14**, and **15** were obtained by treating **22a** or **22b** with the appropriate alcohol.

Compound **14** was used to prepare **16–20** (Scheme II). Catalytic reduction of **14** using platinum oxide catalyst gave the *p*-amino analogue **16**. Diazotization of **16** followed by treatment with sodium azide yielded **18**. Reaction of **16** with thiophosgene, bromoacetyl bromide, or ethylsuccinoyl chloride afforded the *p*-isothiocyanate **17** and the amides **19** and **20**, respectively.

Receptor Binding Studies. The IC_{50} values[15] for inhibition of [^{3}H]-3β-(*p*-fluorophenyl)tropane-2β-carboxylic acid methyl ester (**23**) binding in rat striatal membranes are shown in Table I. The largest effects were reduction of affinity relative to cocaine (**1**) (IC_{50} 0.102 μM) by replacement of the 2-carbomethoxy group by a carboxyl substituent (**10**) (IC_{50} 195 μM), by hydrogen (**31**) (IC_{50} 5.18 μM), or by an *N*-methylcarbamoyl moiety (**11**) (IC_{50} 3.18 μM). Enhanced activities were observed for the phenethyl ester analogues substituted with *p*-amino (**16**) (IC_{50} 0.072 μM), *p*-α-bromoacetamido (**19**) (IC_{50} 0.061 μM), and *p*-(ethylsuccinoylamido) (**20**) (IC_{50} 0.086 μM).

Discussion

It has been shown by us[9] and others[16,17] that the stere-

(9) Carroll, F. I.; Lewin, A. H.; Abraham, P.; Parham, K.; Boja, J. W.; Kuhar, M. J. Synthesis and Ligand Binding of Cocaine Isomers at the Cocaine Receptor. *J. Med. Chem.* **1991**, *34*, 883–886.

(10) Carroll, F. I.; Gao, Y.; Rahman, M. A.; Abraham, P.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. Synthesis and Ligand Binding, QSAR and CoMFA Study of 3β-(*p*-Substituted phenyl)tropan-2β-carboxylic Acid Methyl Esters, *J. Med. Chem.* **1991**, *34*, 2719–2725.

(11) Boja, J. W.; Carroll, F. I.; Rahman, M. A.; Abraham, P.; Lewin, A. H.; Kuhar, M. J. New, Portent Cocaine Analogs: Ligand Binding and Transport Studies in Rat Striatum. *Eur. J. Pharmacol.* **1990**, *184*, 329–332.

(12) Boja, J. W.; Patel, A.; Carroll, F. I.; Rahman, M. A.; Abraham, P.; Lewin, A. H.; Kopajtic, T. A.; Kuhar, M. J. [^{125}I]RTI-55: A Potent Ligand for Dopamine Transporters. *Eur. J. Pharmacol.* **1991**, *194*, 133–134.

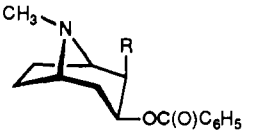
(13) Abraham, P.; Pitner, J. B.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J.; Carroll, F. I. *N*-Modified Analogues of Cocaine: Synthesis and Inhibition of Binding to the Cocaine Receptor. *J. Med. Chem.*, in press.

(14) Findlay, The Three Dimensional Structure of Cocaine. 1. Cocaine and Pseudococaine. *J. Am. Chem. Soc.* **1954**, *76*, 2855.

(15) Boja, J. W.; Rahman, M. A.; Philip, A.; Lewin, A. H.; Carroll, F. I.; Kuhar, M. J. Isothiocyanate Derivatives of Cocaine: Irreversible Inhibition of Ligand Binding at the Dopamine Transporter. *Mol. Pharmacol.* **1991a**, *39*, 339–345.

(16) Ritz, M. C.; Cone, E. J.; Cone, M. J. Cocaine Inhibition of Ligand Binding at Dopamine, Norepinephrine and Serotonin Transporters: A Structure-Activity Study. *Life Sci.* **1990**, *46*, 635–645.

Table I. Physicochemical and Pharmacological Data for Cocaine Analogues



compd	R	salt	mp, °C	recryst solvent	yield (%)	optical rotation $[\alpha]_D$ (c solvent)	method	IC_{50} (μM)
1	CO_2OH_3							0.102
2	$CO_2C_2H_5$	–	106–107	Et_2O–hexane		−16.3 (1.05, $CHCl_3$)	C	0.130 ± 0.040
3	$CO_2(CH_2)_2CH_3$	–	79–80	petroleum ether	83	−17.7 (0.92, $CHCl_3$)	C	0.191 ± 0.046
4	$CO_2CH(CH_3)_2$	HCl	205–207	MeOH–Et_2O	38	−61.4 (0.92, H_2O)	A	0.211 ± 0.059
5	$CO_2C_6H_5$	HCl	179–180	MeOH–Et_2O	45	−116.0 (0.95, MeOH)	A	0.112 ± 0.036
6	$CO_2CH_2C_6H_5$	$(CHCO_2H_2)_2$	181–183	MeOH–Et_2O	63	−10.2 (2.19, MeOH)	B	0.257 ± 0.014
7	$CO_2(CH_2)_2C_6H_5$	$(CHCO_2H)_2$	168–170	MeOH–Et_2O	70	−7.43 (1.975, MeOH)	B	0.248 ± 0.058
8	$CO_2(CH_2)_3C_6H_5$	$(CHCO_2H)_2$	156–158	MeOH–Et_2O	50	−28.4 (2.195, MeOH)	A	0.139 ± 0.024
9	$CO_2CH_2CH{=}CHC_6H_5$	HCl	138–139	MeOH–Et_2O	58	−19.4 (0.165, MeOH)	A	0.371 ± 0.015
10	CO_2H[a]	–						195 ± 22.6
11	$CONHCH_3$	HCl	213–215	MeOH–Et_2O	41	−40.9 (0.088, MeOH)	–	3.18 ± 0.644
12	CH_2OH	–	96–97	CH_2Cl_2–Hex	31	−36.6 (0.96, $CHCl_3$)	–	0.561 ± 0.149
13	CH_2OCOCH_3	HCl	240–242	MeOH–Et_2O	82	−8.3 (0.12, MeOH)	–	0.272 ± 0.047
14	$CO_2(CH_2)_2$–C6H4–NO_2	HCl	118–121	MeOH–Et_2O	66	−32.2 (0.118, MeOH)	B	0.601 ± 0.028
15	$CO_2(CH_2)_2$–C6H4–Cl	HCl	160–161	MeOH–Et_2O	31	−38.6 (0.145, MeOH)	A	0.271 ± 0.012
16	$CO_2(CH_2)_2$–C6H4–NH_2	HCl	231–239	MeOH–Et_2O	87	−35.7 (0.118, MeOH)	–	0.072 ± 0.007
17	$CO_2(CH_2)_2$–C6H4–NCS	HCl	182–184	MeOH–Et_2O	46	−31.4 (0.175, MeOH)	–	0.196 ± 0.014
18	$CO_2(CH_2)_2$–C6H4–N_3	HCl	170–173	MeOH–Et_2O	65	−40.9 (0.11, MeOH)	–	0.227 ± 0.019
19	$CO_2(CH_2)_2$–C6H4–$NHCOCH_2Br$	HCl	142–146	MeOH–Et_2O	71	−52.8 (0.125, MeOH)	–	0.061 ± 0.006
20	$CO_2(CH_2)_2$–C6H4–$NHCO(CH_2)_2CO_2Et$	HCl	115–119	MeOH–Et_2O	58	−63.8 (0.08, MeOH)	–	0.086 ± 0.004
21	H[b]							5.18 ± 1.16

[a] Reference 14. [b] Reference 13.

ochemistry of substitution on the cocaine skeleton, particularly at C-2, had a profound effect on binding affinity at the dopamine transporter. Thus the IC_{50} value for inhibition of [^{3}H]-**23** binding by (*R*)-cocaine (C-2 substituent in the β-position) is 1/160th that of (*R*)-pseudococaine (C-2 substituent in the α-position), and the IC_{50} value of (*R*)-WIN 35065-2 (C-2 carbomethoxy is β) is 1/45th that of the analogous (*R*)-WIN 35140 (C-2 carbomethoxy is α). Furthermore, it has been shown that although stereochemical changes at C-3 had only slight effects on binding affinity,[9] replacement of the benzoyl group at C-3 could have marked effects on activity.[10-12,18-20] It had also been reported that whereas (*R*)-ecgonine methyl ester (C-3 substituent = β-OH) is only 1/60th as active as (*R*)-cocaine,[16] WIN 35065-2 (C-3 substituent = β-phenyl) is approximately 4 times more potent than (*R*)-cocaine.[10]

Few effects of substitition at C-2 have been reported. Factors ranging from 50 to 200 have been reported for the decrease in potency to displace [^{3}H]cocaine[17] and [^{3}H]-mazindol[16] upon replacement of the carbomethoxy group of cocaine by a hydrogen; replacement of the carbomethoxy group by a carboxyl is reported to result in 16–1000-fold decrease in activity vis-a-vis the same radioligands.[16,21] Our results confirm and extend these observations. In our hands, removal of the carbomethoxy group to give compound **21** decreased the potency to displace [^{3}H]-**23** by a factor of 50,[13] reminiscent in magnitude to the effect of epimerization at C-2.[9] In other words, replacement of the carbomethoxy group at the C-2β position by a hydrogen reduces the activity by a factor in the range of 50–200, whether an α-carbomethoxy group is present or not. This observation emphasizes the previously noted[16,17] requirement for a 2β-ester (or equivalent) function for high affinity to the receptor and suggests the presence of specific hydrogen bond donating residues in the receptor, for which the 2β moiety serves as a hydrogen-bond acceptor. An additional effect of the 2β-substituent may be to distort the 8-azabicyclo[3.2.1]octane skeleton by flattening of the piperidine ring, particularly at the 8-aza end, to relieve the steric strain between the 2β-substituent and the aza bridge. The effect of this flattening on binding affinity will require additional studies.

(17) Reith, M. E. A.; Meisler, B. E.; Sershen, H.; Lajtha, A. Structural Requirements for Cocaine Congeners to Interact with Dopamine and Serotonin Uptake Sites in Mouse Brain and to Induce Stereotyped Behavior. *Biochem. Pharmacol.* **1986**, *35*, 1123–1129.

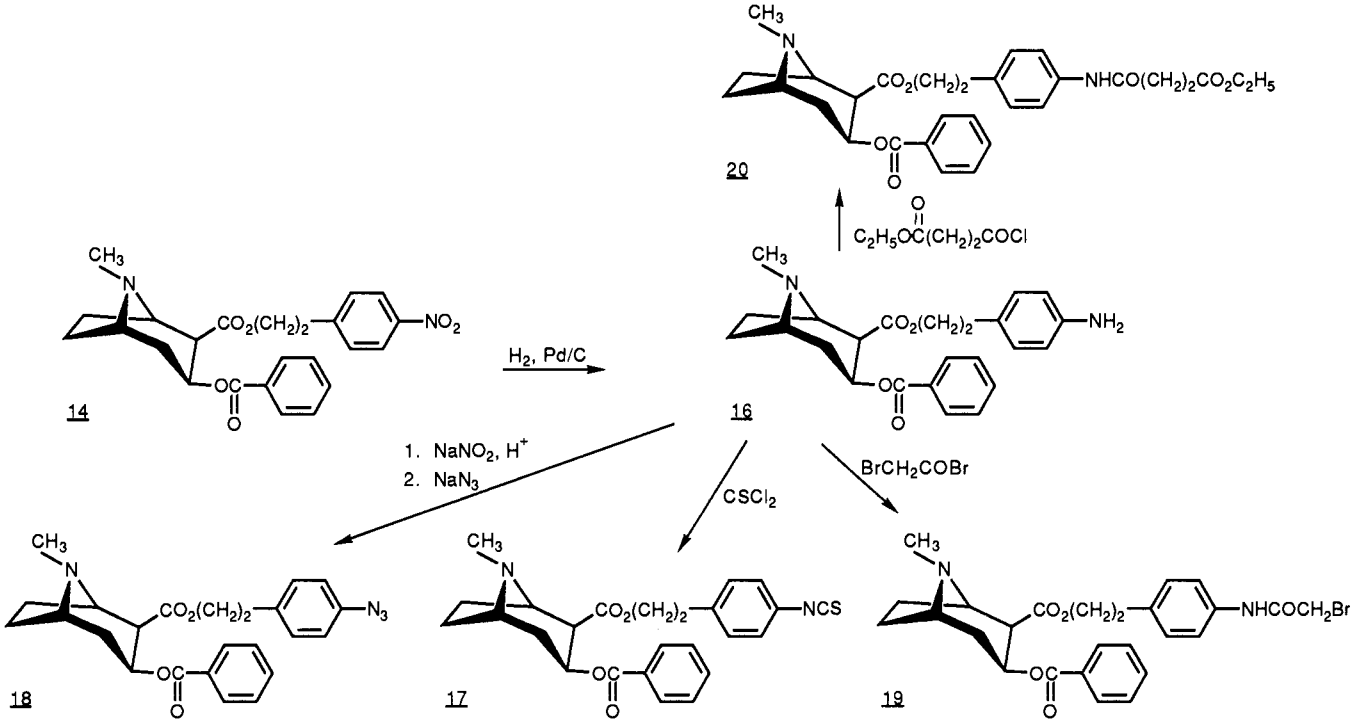
(18) Clarke, R. L.; Daum, S. J.; Gambino, A. J.; Aceto, M. D.; Pearl, J.; Levitt, M.; Cumiskey, W. R.; Bogado, E. F. Compounds Affecting the Central Nervous System. 4. 3β-Phenyltropane-2-carboxylic Esters and Analogues. *J. Med. Chem.* **1973**, *16*, 1260–1267.

(19) Kline, R. H., Jr.; Wright, J.; Fox, K. M.; Eldefrawi, M. E. Synthesis of 3-Arylecgonine Analogues as Inhibitors of Cocaine Binding and Dopamine Uptake. *J. Med. Chem.* **1990**, *33*, 2024–2027.

(20) Kline, R. H., Jr.; Wright, J.; Eshleman, A. J.; Fox, K. M.; Eldefrawi, M. E. Synthesis of 3-Carbamoylecgonine Methyl Ester Analogues as Inhibitors of Cocaine Binding and Dopamine Uptake. *J. Med. Chem.* **1991**, *34*, 702–705.

(21) Madras, B. K.; Fahey, M. A.; Bergman, J.; Canfield, D. R.; Spealman, R. D. Effects of Cocaine and Related Drugs in Nonhuman Primates. I. [^{3}H]Cocaine Binding Sites in Caudate-Putamen. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 131–141.

Scheme II



Conversion of the 2β-ester function of cocaine to a carboxylic acid (**10**) reduces the potency to displace [^{3}H]-**23** 2000-fold compared to (*R*)-cocaine. Similar reduction in potency is reported for displacement of (*R*)-[^{3}H]cocaine[21] and [^{3}H]mazindol.[16] This large effect on activity is undoubtedly related to the zwitterionic character of benzoylecgonine (**10**). Thus the positive charge at the 8-aza position has been shown to decrease potency by a factor of about 100, as in cocaine methiodide vs cocaine.[13] If benzoylecgonine (**10**) were entirely zwitterionic, the decrease in affinity due to the positive charge may be as large as that observed for cocaine methiodide, in which the piperidine is expected to be flattened. Since the decrease in activity observed for benzoylecgonine is larger than that observed for cocaine methiodide, ring-puckering may be a contributing factor. A less flattened, puckered, chairlike conformation of the piperidine ring would be the result of the attractive electrostatic interaction between the protonated 8-aza group and the carboxylate anion, which is expected to counteract, at least in part, the steric effect of the C-2β substituent (see above). An additional factor which may contribute to the reduced binding capacity of **10** is that the carboxyl group can adopt an orientation which does not contribute to, or even interferes with, binding.

Replacement of the methyl group in the carbomethoxy functionality by bulky groups (e.g. i-Pr, **4**), by long lipophilic groups (phenylpropyl, **8**) or by an aromatic group (**5**) changes the activity only by factors in the range of 0.6–6. These small effects suggest that in addition to the remarkable tolerance of the alkoxy group in the C-2β ester to substitution, the presence of aromatic groups enhances potency. Thus, the small steric effect which is apparent in the series of ethyl (**2**), propyl (**3**), and isopropyl (**4**) esters is offset in the phenyl (**5**) ester. Similarly, electron-rich aromatic groups in the phenethyl esters (e.g. **16**), lead to higher potency relative to the alkyl esters (e.g. **3**) while electron-poor aromatic analogues (e.g. **14**) have reduced potency. An even more important feature associated with good activity is the ability of the C-2β group to accept two hydrogen bonds. This is manifested in the reduced activity, relative to cocaine, of the amide **11**. However, the magnitude of the reduction (300-fold) suggests that additional factors such as have been discussed for benzoylecgonine (**10**) may be involved. The contribution of the carboxylate oxygens to the binding component associated with the C-2β substituent is also supported by the activities of the alcohol **12** and its acetate **13**. Thus, the 5-fold reduction in activity resulting from replacement of the carbomethoxy group in cocaine by a methylenehydroxy group (**12**) can be attributed to the loss of one of the hydrogen bond accepting oxygens. The partial (2-fold) restoration of activity by addition of a second oxygen, as in the acetate **13**, is consistent with this hydrogen bond acceptor model; conformational factors may also be involved.

The tolerance of the 2β-position of cocaine to substitution recommends its modification to provide irreversible ligands. Thus, if the para position of the phenylethyl ester moiety is not involved in binding, it could provide a useful locus for a chemically or photochemically active residue. For example, the azido compound **18** may act as a photoaffinity ligand, and the isothiocyanate derivative **17** should be capable of acylating bionucleophiles in the vicinity of the receptor. Both **17** and **18** could be prepared in radioactive form. Furthermore, an isothiocyanate such as **17** could be attached to a resin and utilized in affinity chromatography to isolate the protein associated with the cocaine receptor at the dopamine transporter.

Conclusions

The pattern of activity of C-2β-substituted cocaine analogues supports the need for a 2β-substituent. While loss of hydrogen bonding acceptor atoms and decreased electron density in proximity to C-2 reduces the affinity to displace radioligands such as [^{3}H]-**23**, the C-2β position exhibits high tolerance for steric variations. This class of analogues may be useful as irreversible chemical probes and in isolation and purification of the receptor.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary tube apparatus. All optical rotations were determined at the sodium D line using a Rudolph Research Autopol III Polarimeter

(1-dm cell). NMR spectra were recorded on a Bruker WM-250 spectrometer using tetramethylsilane as an internal standard. Thin-layer chromatography was carried out on Whatman silica gel 60 TLC plates using $CHCl_3$–MeOH–concentrated NH_4OH (40:9:1) unless otherwise noted. Visualization was accomplished under UV or in an iodine chamber. Microanalyses were carried out by Atlantic Microlab, Inc. [^{3}H]-3β-(*p*-Fluorophenyl)tropane-2β-carboxylic acid methyl ester (**23**) with specific activity 83.1 Ci/mmol was purchased from Dupont-New England Nuclear (Boston, MA).

Preparation of 3β-(Benzoyloxy)-8-methyl-8-azabicyclo-[3.2.1]octane-2β-carboxylic Acid Esters (2–9, 14, 15). The esters **2–9**, **14**, and **15** were prepared following methods A–C as shown in Table I. The physical parameters, recrystallization solvent, and yields are also in Table I.

Method A. A suspension of benzoylecgonine (**10**) in thionyl chloride (4 mL/mmol) at 0 °C was stirred for 4 h to obtain a clear yellow solution which was diluted with dry toluene (3 mL/mmol of **10**) and evaporated under reduced pressure. The residue was taken up in $CHCl_3$ (3 mL/mmol of **10**) and stirred with Et_3N (2.2 equiv) and the corresponding alcohol (1.1 equiv) at 0 °C for 4 h. The reaction mixture was further diluted with $CHCl_3$ (5 mL/mmol), washed with H_2O, and dried over Na_2SO_4. The residue, after removal of the solvents, was purified by chromatography on a silica gel (230–400 mesh) column.

Method B. A solution of benzoylecgonine (**10**) and *N,N*-carbonyldiimidazole (1 equiv) in CH_2Cl_2 (4 mL/mmol of **10**) was stirred at room temperature for 6 h. The solvent was removed under reduced pressure, and the resulting residue was taken up in acetone (3 mL/mmol of **10**) and heated under reflux with the corresponding alcohol (1.1 equiv) for 3 h. The residue obtained after removal of the solvent was purified by chromatography on a silica gel (230–400 mesh) column.

Method C. A solution of **10** in the appropriate alcohol (25 mL/mmol) was saturated with dry hydrogen chloride at 0 °C and was stirred for 48 h. The reaction was concentrated under reduced pressure and partitioned between CH_2Cl_2 and 20% NH_4OH solution. The organic fraction was washed with H_2O and dried over Na_2SO_4. The residue, after removal of the solvent, was purified on a silica gel (230–400 mesh) column eluting with 10% MeOH–CH_2Cl_2.

3β-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic acid ethyl ester (2) (method C):[22] ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3, CCH_3), 1.78 (m, 2), 1.89 (m, 1), 2.18 (m, 2), 2.23 (s, 3, NCH_3), 2.45 (m, 1), 3.05 (m, 1), 3.32 (m, 1), 3.62 (m, 1), 4.20 (m, 2, OCH_2), 3.24 (m, 1, H-3), 7.41–8.01 (m, 5, ArH). Anal. ($C_{18}H_{23}NO_4$): C, H, N.

3β-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic acid propyl ester (3) (method C):[23] ^{1}H NMR ($CDCl_3$) δ 0.94 (t, 3, CCH_3), 1.60 (m, 2, CCH_2CH_3), 1.68 (m, 2), 1.90 (m, 1), 2.23 (s, 3, NCH_3), 2.48 (m, 1), 3.05 (m, 1), 3.28 (m, 1), 3.55 (m, 1), 4.25 (m, 2, OCH_2), 5.21 (m, 1, H-3), 7.30–8.05 (m, 5, ArH). Anal. ($C_{19}H_{25}NO_4$): C, H, N.

3β-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic Acid Isopropyl Ester (4) Hydrochloride (Method A). Elution with 10% MeOH–CH_2Cl_2 gave pure **4** free base, which was converted to the hydrogen chloride salt: ^{1}H NMR ($CDCl_3$–CD_3OD, 500 MHz) δ 0.87 and 1.16 (2 d, due to chemical shift nonequivalence, 6, $CHCH_3$), 2.23 (m, 2), 2.39 (m, 1), 2.47 (m, 2), 2.92 (s, 3, NCH_3), 3.56 (m, 1), 4.10 (m, 1), 4.20 (m, 1), 5.02 (m, 1, $OCH(CH_3)_2$), 5.51 (m, 1, H-3), 7.49 (m, 2, ArH), 7.65 (m, 1, ArH), 7.99 (m, 2, ArH). Anal. ($C_{19}H_{25}NO_4$·1.5HCl·H_2O): C, H, Cl, N.

3β-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic Acid Phenyl Ester (5) Hydrochloride (Method A). The pure base was isolated using 10% MeOH–CH_2Cl_2 as the eluent. It was converted to the hydrogen chloride salt: ^{1}H NMR (CD_3OD) δ 2.25 (m, 3), 2.85 (m, 2), 2.80 (m, 1), 3.03 (s, 3, NCH_3), 3.73 (m, 1), 4.28 (m, 1), 4.50 (m, 1), 5.53 (m, 1, H-3), 6.95 (d, 2, ArH), 7.25 (m, 3, ArH), 7.54 (m, 2, ArH), 7.58 (m, 1, ArH), 8.05 (d, 2, ArH). Anal. ($C_{22}H_{23}NO_4$·HCl): C, H, N.

3β-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic Acid Benzyl Ester (6) Fumarate (Method B). The pure sample obtained on elution with $CHCl_3$–MeOH–NH_4OH (190:9:1) was converted to the fumarate salt: ^{1}H NMR (CD_3OD) δ 1.55 (m, 4), 2.64 (m, 1), 2.81 (s, 3, NCH_3), 3.04 (m, 1), 3.51 (m, 1), 3.62 (m, 1), 5.51 (d, 2, OCH_2), 5.65 (m, 1, H-3), 6.25 (s, 2, olefinic), 6.64 (d, 2, ArH), 6.67 (m, 5, ArH), 6.87 (t, 1, ArH), 7.34 (d, 2, ArH). Anal. ($C_{23}H_{25}NO_4$·$C_4H_4O_4$): C, H, N.

3β-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic Acid 2-Phenylethyl Ester (7) Fumarate (Method B). The free base was obtained by elution with $CHCl_3$–MeOH–NH_4OH (190:9:1). The fumarate salt had ^{1}H NMR (CD_3OD) δ 1.65 (m, 4), 2.56 (s, 3, NCH_3), 2.64 (m, 1), 3.04 (m, 1), 3.45 (t, 2, OCH_2), 3.51 (m, 1), 3.62 (m, 1), 4.95 (m, 1, H-3), 6.21 (s, 2, olefinic), 6.53 (d, 2, ArH), 6.66 (m, 5, ArH), 6.84 (t, 1, ArH), 7.32 (d, 2, ArH). Anal. ($C_{24}H_{27}NO_4$·$C_4H_4O_4$): C, H, N.

3β-(Benzoyloxy)-8-azabicyclo[3.2.1]octane-2β-carboxylic Acid 3-Phenylpropyl Ester (8) Fumarate (Method A). The sample was eluted with 10% MeOH–CH_2Cl_2 and was converted to a fumarate salt: ^{1}H NMR ($CDCl_3$–CD_3OD) δ 1.12 (m, 2), 1.56 (m, 6), 2.48 (s, 3, NCH_3), 2.68 (m, 1), 3.05 (d, 1), 3.5 (m, 1), 4.95 (m, 1, H-3), 6.10 (s, 2, olefinic), 6.45–7.43 (m, 10, ArH). Anal. ($C_{25}H_{29}NO$·$C_4H_4O_4$): C, H, N.

3β-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic Acid Cinnamyl Ester (9) Hydrochloride (Method A). The sample was eluted with 5% MeOH–CH_2Cl_2: ^{1}H NMR of **9**·HCl ($CDCl_3$) δ 1.31 (m, 1), 1.70–2.18 (m, 5), 2.22 (s, 3, NCH_3), 2.46 (m, 1), 3.07 (m, 1, H-2), 3.25 (m, 1, H-5), 3.62 (m, 1, H-1), 4.80 (m, 2), 3.25 (m, 1, H-3), 6.27 (m, 1, CH_2CH), 6.45 (d, 1, $ArCH{=}CH$), 7.29 (m, 1, ArH), 7.46 (t, 1, ArH), 8.02 (d, 2, ArH). Anal. ($C_{25}H_{27}NO_4$·HCl·0.5H_2O): C, H, N.

3β-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic Acid *N*-Methylamide (11) Hydrochloride. A solution of 3β-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid (**10**) (200 mg, 0.691 mmol) and CDI (162 mg, 0.899 mmol) in dried CH_2Cl_2 (15 mL) was stirred at room temperature for 1 h, treated with $MeNH_2$ (g) for 10 min, and stirred for 3 h. The reaction mixture was evaporated to dryness, and the residue was suspended in H_2O and extracted with Et_2O. The organic layer was washed with brine then H_2O, dried over $MgSO_4$, and concentrated to give an oil which was converted to the HCl salt with HCl–Et_2O to yield 96 mg (41%) of **11**·HCl: ^{1}H NMR (CD_3OD) δ 2.16–2.56 (m, 6), 2.73 (s, 3, NCH_3), 2.85 (s, 3, $CONHCH_3$), 3.19 (m, 1, H-2), 4.01 (m, 1, H-5), 4.16 (m, 1, H-1), 5.57 (m, 1, H-3), 7.46–7.66 (m, 3, ArH), 7.94 (d, 2, ArH). Anal. ($C_{17}H_{22}N_2O_3$·HCl·1.25H_2O): C, H, N.

3β-(Benzoyloxy)-2β-(hydroxymethyl)-8-methyl-8-azabicyclo[3.2.1]octane (12). To a stirred suspension of **10** (1.45 g, 5 mmol) in freshly distilled THF (75 mL) and 0 °C was added dropwise diborane–THF complex (18 mL, 18 mmol) over a period of 15 min. After stirring at 0 °C for another 2 h and at room temperature for 1 h, excess diborane was carefully destroyed by the addition of MeOH. The mixture was acidified to pH 1 with 6 N HCl and concentrated by evaporation. The solution was basified with 6 N NH_4OH and extracted with CH_2Cl_2. The concentrated extract was dried (Na_2SO_4) and evaporated. The residue was purified by chromatography on silica gel, eluting with 10% MeOH–CH_2Cl_2. The fractions containing the product were pooled, evaporated, and crystallized from CH_2Cl_2–petroleum ether to give 0.428 g (31%) of **12**: ^{1}H NMR ($CDCl_3$) δ 1.8 (m, 2), 2.05 (m, 2), 2.12 (m, 2), 2.27 (s, 3, NCH_3), 3.31 (m, 1), 3.48 (m, 1), 3.99 (dd, 2, 2H, CH_2O), 5.35 (m, 1, H-3), 7.42 (m, 2, ArH), 7.55 (m, 1, ArH), 8.07 (m, 1, ArH). Anal. ($C_{16}H_{21}NO_3$): C, H, N.

3β-(Benzoyloxy)-2β-(acetoxymethyl)-8-methyl-8-azabicyclo[3.2.1]octane (13) Hydrochloride. To a stirred solution of **12** (155 mg, 0.55 mmol) and Et_3N (0.2 mL, 1.4 mmol) in CH_2Cl_2 (5 mL) at room temperature was added dropwise acetic anhydride (67 mg, 1.2 mmol). After 3 h, the mixture was treated with H_2O (2 mL). The organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 (2 × 5 mL). The combined organic extract was washed with H_2O and dried (Na_2SO_4). Removal of the solvent gave **13** as a waxy solid: ^{1}H NMR ($CDCl_3$) δ 1.66 (m, 3), 1.75 (s, 3, $COCH_3$), 1.90 (m, 1), 2.07 (m, 2), 2.20 (s, 3, NCH_3),

(22) The ester **2** has been previously identified by MS: Smith, R. M. Ethyl Esters of Arylhydroxy- and Arylhydroxymethoxy Cocaines in the Urines of Simultaneous Cocaine and Ethanol Users. *J. Anal. Toxicol.* **1984**, *8*, 38–42.

(23) The ester **3** has been previously identified by MS: von-Minden, D. L.; D'Amato, N. A. Simultaneous Determination of Cocaine and Benzoylecgonine in Urine by Gas–Liquid Chromatography. *Anal. Chem.* **1977**, *49*, 1974–1977.

2.38 (m, 1), 4.39 (m, 2), 5.31 (m, 1, H-3), 7.45 (m, 2, ArH), 7.55 (m, 1, ArH), 8.04 (m, 2, ArH). The free base was converted to the HCl salt and recrystallized from MeOH–Et_2O to give 158 mg (82%) of 13·HCl. Anal. ($C_{18}H_{23}NO_4$·HCl): C, H, N.

3β-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic Acid (p-Nitrophenyl)ethyl Ester (14) Hydrochloride (Method B). The column was eluted with hexane–Et_2O (4:1) to afford the free base 14, which was converted to its hydrochloride salt: ^1H NMR (CD_3OD) δ 2.16–2.50 (m, 6), 2.73 (m, 2), 2.91 (s, 3, NCH_3), 3.64 (dd, 1, H-2), 4.06 (m, 1, H-5), 4.21 (m, 2), 4.50 (m, 1), 5.60 (m, 1, H-3), 7.25 (d, 2, ArH), 7.49 (t, 2, ArH), 7.64 (t, 1, ArH), 7.89 (d, 2, ArH), 8.01 (d, 2, ArH). Anal. ($C_{24}H_{26}N_2O_6$·HCl·1.5H_2O): C, H, N.

3β-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic Acid (p-Chlorophenyl)ethyl Ester (15) Hydrochloride (Method A). The column was eluted with hexane–Et_2O (4:1) to give the free base which was converted to the hydrogen chloride salt: ^1H NMR ($CDCl_3$) δ 1.78 (m, 3), 2.07 (m, 2), 2.13 (s, 3, NCH_3), 2.41 (m, 1), 2.90 (t, 2), 2.98 (m, 1, H-2), 3.26 (m, 1, H-5), 3.45 (m, 1, H-1), 4.31 (m, 2), 5.22 (m, 1, H-3), 7.15 (d, 2, ArH), 7.22 (d, 2, ArH), 8.02 (d, 2, ArH). Anal. ($C_{24}H_{26}ClNO_4$·HCl·0.25H_2O): C, H, N.

3β-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic Acid (p-Aminophenyl)ethyl Ester (16) Hydrochloride. A solution of 14·HCl (1.13 g, 0.002 mol) in MeOH (70 mL) was reduced over PtO_2 (280 mg) at 50 psi of H_2 for 4 h. Evaporation of the solvent after removal of the catalyst gave pure 16·HCl: ^1H NMR (CD_3OD) δ 2.12–2.68 (m, 8), 2.91 (s, 3, NCH_3), 3.63 (dd, 1, H-2), 4.07 (m, 1, H-5), 4.19 (m, 2), 4.36 (m, 1), 5.62 (m, 1, H-3), 7.22 (dd, 4, ArH), 7.54 (t, 2, ArH), 7.64 (t, 1, ArH), 8.01 (d, 2, ArH). Anal. ($C_{24}H_{28}N_2O_4$·HCl·1.5H_2O): C, H, N.

3β-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic Acid (p-Isothiocyanatophenyl)ethyl Ester (17) Hydrochloride. To a rapidly stirred solution of 3β-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic acid (p-aminophenyl)ethyl ester (16) hydrochloride (50 mg, 0.106 mmol) and $NaHCO_3$ (55 mg, 0.655 mmol) in H_2O–THF (2 mL, 2:1) was added a fresh solution of thiophosgene (11.2 μL, 1.43 μmol) in THF (1 mL) at 0 °C. After 5 h at room temperature, TLC indicated that the reaction was complete. The organic layer was separated, diluted to 20 mL with $CHCl_3$, and washed once with water. After drying over $MgSO_4$, the solvent was evaporated to afford 3β-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic acid (p-isothiocyanatophenyl)ethyl ester as a viscous oil which was converted to the HCl salt to give 23 mg (46%) of 17·HCl as a solid: ^1H NMR ($CDCl_3$) δ 1.52–1.84 (m, 3), 2.0 (m, 2), 2.06 (s, 3, NCH_3), 2.32 (m, 1), 2.84 (t, 2), 2.92 (m, 1, H-2), 3.20 (m, 1, H-5), 3.37 (m, 1, H-1), 4.26 (m, 2), 5.13 (m, 1, H-3), 7.14 (dd, 4, J = 8.5, ArH), 7.33 (t, 2, J = 7.7, ArH), 7.45 (t, 1, J = 7.7, ArH), 7.92, (d, 2H, J = 7.7, ArH). Anal. ($C_{25}H_{26}N_2O_4S$·HCl·1.75H_2O): C, H, N.

3β-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic Acid (p-Azidophenyl)ethyl Ester (18) Hydrochloride. To a solution of 3β-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic acid (p-aminophenyl)ethyl ester (16) hydrochloride (50 mg, 0.106 mmol) in 3 M AcOH (0.5 mL) was added an aqueous solution of $NaNO_2$ (10 mg, 0.146 mmol in 0.3 mL of H_2O) at 0 °C. After 30 min at this temperature, a solution of NaN_3 (9.79 mg, 0.1507 mmol) in H_2O (0.3 mL) was added dropwise and stirred for 30 min at 0 °C and then 30 min at room temperature. After removal of the solvents under reduced pressure, the residue was dissolved in $CHCl_3$ and washed with H_2O. The organic layer was dried over $MgSO_4$ and concentrated to give an oil which was converted to the HCl salt (32 mg, 65%) of 3β-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic acid (p-azidophenyl)ethyl ester (18) hydrochloride as a pale yellow solid: ^1H NMR (250 MHz, $CDCl_3$) δ 1.31 (m, 1), 1.65–1.88 (m, 3), 2.09 (m, 1), 2.14 (s, 3, NCH_3), 2.42 (m, 1), 2.91 (t, 2), 3.0 (m, 1, H-2), 3.27 (m, 1, H-5), 3.48 (m, 1, H-1), 4.35 (m, 2), 5.22 (m, 1, H-3), 6.91 (d, 2, ArH), 7.20 (d, 1, J = 8.5, ArH), 7.41 (t, 2, J = 7.7, ArH), 7.52 (t, 1, J = 7.7, ArH), 7.02 (d, 2, J = 7.7, ArH). Anal. ($C_{24}H_{26}N_4O_4$·HCl·1.75H_2O): C, H, N.

3β-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic Acid [p-(Bromoacetamido)phenyl]ethyl Ester (19) Hydrochloride. To a solution of 3β-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic acid (p-aminophenyl)ethyl ester (16) hydrochloride (90 mg, 0.19 mmol) in dry 1,2-dichloroethane was added dropwise bromoacetyl bromide (54 μL, 620 μmol) at 0 °C under N_2. Stirring was continued for 24 h, allowing the mixture to come to room temperature. After the solvent was removed on a rotary evaporator, the residue was diluted with H_2O and basified with concentrated NH_4OH. The mixture was extracted with Et_2O and washed with H_2O. After drying over $MgSO_4$, the solvent was evaporated under reduced pressure and the residue was purified by flash chromatography (silica gel, hexane–Et_2O, 4:1) to give 88 mg of 3β-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic acid [p-(bromoacetamido)phenyl]ethyl ester (19) hydrochloride: ^1H NMR ($CDCl_3$) δ 1.31 (m, 1), 1.65–1.88 (m, 3), 2.09 (m, 1), 2.14 (s, 3, NCH_3), 2.42 (m, 1), 2.91 (t, 2), 3.0 (m, 1, H-2), 3.27 (m, 1, H-5), 3.48 (m, 1, H-1), 4.35 (m, 2), 5.22 (m, 1, H-3), 6.91 (d, 2, J = 8.5, ArH), 7.20 (d, 1, J = 8.5, ArH), 7.50 (m, 2, ArH), 7.64 (m, 1, ArH), 7.96 (t, 2, J = 7.7, ArH). Anal. ($C_{26}H_{29}BrN_2O_5$·HCl·2.5H_2O): C, H, N.

3β-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic Acid [p-(Ethylsuccinamido)phenyl]ethyl Ester (20) Hydrochloride. To a solution of 16·HCl (47 mg, 0.10 mmol) in dry 1,2-dichloroethane was added dropwise ethylsuccinamyl chloride (49 μL, 345 μmol) at 0 °C under NH_4OH. Stirring was continued for 4 h at room temperature. After removal of the solvent on a rotary evaporator, the residue was diluted with H_2O and basified with concentrated NH_4OH. The mixture was extracted with $CHCl_3$, and the extract was washed with H_2O and brine. After the extract was dried over $MgSO_4$, the solvent was evaporated to an oil which was treated with HCl/Et_2O to yield 38 mg (58%) of 3β-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2β-carboxylic acid [p-(ethylsuccinamido)phenyl]ethyl ester (20) hydrochloride: ^1H NMR (250 MHz, $CDCl_3$) δ 1.31 (m, 1), 1.65–1.88 (m, 3), 2.09 (m, 1), 2.14 (s, 3, NCH_3), 2.42 (m, 1), 2.91 (t, 2, J = 6.6), 3.0 (m, 1, H-2), 3.27 (m, 1, H-5), 3.48 (m, 1, H-1), 4.35 (m, 2), 5.22 (m, 1, H-3), 6.91 (d, 2, ArH), 7.20 (d, 1, ArH), 7.41 (t, 2, ArH), 7.52 (t, 1, ArH), 7.02 (d, 2, ArH). Anal. ($C_{30}H_{37}N_2O_7$·HCl·1.5H_2O) C, N; H: calcd, 6.88; found, 7.29.

Biological. [^{3}H]-23 Radioligand Binding. Rat striata from male Sprague–Dawley rats (250–350 g) were rapidly dissected, frozen, and stored at −70 °C until used. The frozen rat striata were homogenized in 20 volumes of 10 mM phosphate buffer (pH 7.4) containing 0.32 M sucrose using a polytron (setting 6) for 10 s. The homogenate was centrifuged for 10 min at 40000g, the resulting pellet was washed in buffer, recentrifuged, and resuspended to a tissue concentration of 1.0 mg/mL. Binding assays were carried out in a total volume of 0.5 mL containing 0.5 nM [^{3}H]-23. The suspensions were incubated for 2 h on ice. Incubations were terminated by filtration with three 5-mL washes through Whatman GF/B filters previously soaked in 0.05% polyethylenimine. Radioactivity was counted in 5 mL of scintillation cocktail at an efficiency of 50–55%. Nonspecific binding of [^{3}H]-23 was defined by the presence of 30 μM (−)-cocaine. Under these conditions nonspecific binding was approximately 5–8% of total binding. IC_{50} values were determined from competition curves of 10–12 points utilizing the curve-fitting program EBDA.[24] Mean values and standard errors were calculated from 3–4 assays for each test drug.

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(24) Biosoft Software, Ferguson, MO.