no. 40 paper, and the filtrate was reduced in volume to a few milliliters in vacuo at 40 °C. This was rediluted with 200 mL of $CHCl_3$ and reduced in volume to about 5 mL. This volume was then filtered through a 0.5-μm MF millipore filter in a syringe filtration apparatus to eliminate any remaining silica gel. The product, 124 mg (0.21 mmol) of pure **4**, was precipitated as before with *n*-hexane (chromatographic yield 69%; overall synthetic yield 45%).

The product was pure by TLC and HPLC.[10,21] The EI mass spectrum of **4** showed *m/z* 386 (20%, the aglycon fragment, $C_{21}H_{22}O_7$) 356 (85%, M^+· - glucoside OH - CH_2 = O), and 325 (*m/z* 356 - CH_2OH·, stable even electron ion and base peak). The accurate masses of these ions were determined by mass spectrometry and were all accurate to within 10 parts per million of the suggested compositions. (There was not enough material available after biological testing for satisfactory elemental analysis of **4** or **3**.) The 470-MHz ^1H NMR data ($CDCl_3$) are reported in Tables I and II. The ^1H NMR was consistent with the proposed product. IR (KBr) verified the absence of the carbonyl group at 1775 cm^{-1}.

Synthesis of 3. All glassware was flame dried prior to use. Pyridine was distilled over calcium hydride prior to use. Tosyl chloride (TsCl) was purified before use. A solution of 26 mg/mL of the fresh TsCl in pyridine was made up. Pure, silica-free **4** (134 mg, 0.226 mmol) was added to pyridine (1.67 mL) in a 15-mL conical test tube with a small stirring bar. An equal volume of the TsCl/pyridine solution was added, and the reaction was stirred at 5 °C in a refrigerator. TsCl (43 mg, 0.226 mmol) was added at 48 h and again at 70 h. The reaction was stopped at 80 h by adding the mixture directly to 34 mL of 2 N HCl, which precipitated the products. Extraction with 170 mL of EtOAc was carried out immediately for 5 min, and the extract was washed with an equal volume of water. The EtOAc was then dried over sodium sulfate and reduced in volume in vacuo at 40 °C. The product was precipitated with *n*-hexane as before, yielding 85 mg (0.148 mmol) of crude product. Analytical TLC (EtOAc/MeOH, 20:3) showed four spots at R_f 0.23 (starting material), 0.46 (**3**), 0.51 (competing aglycon product, which is only apparent after addition of the 3rd equiv of TsCl), and 0.61 (minor). HPLC analysis (methanol/water, 55:45 or 70:30, μBondapak C_{18} column, 1.0 mL/min) showed about 50% of **3**, assuming a similar absorbance at 288 nm for the compounds.

The mixture was streaked in MeOH onto two 1000-μm TLC plates as before. The separation of the closely traveling product at R_f 0.51 (analytical TLC) was difficult to achieve. Enrichment was obtained, to about 80%, but smaller batches of the material (~25 mg, 0.043 mmol) were rechromatographed on 250-μm silica gel Redi-plates to achieve purification to 97%. These plates also seemed more activated. In each of the prep TLC steps isolation was by swirling in MeOH. Final purification to free the product from any silica gel was again done by filtration through a 0.5-μm MF-millipore filter. About 20 mg (0.035 mmol) of 97% purity material was collected for analysis and biological testing (overall synthetic yield 15%). The ether (**3**) and the competing product at R_f 0.51 were later well separated by analytical TLC on K-C18 reverse-phase plates, appearing at an R_f of 0.50 and 0.19, respectively. Thus, preparative reverse-phase TLC or HPLC would now be a preferred procedure.

The mass spectrum of **3** contained ions at *m/z* 574 (M^+·, 55%) and 368 (the aglycon fragment and base peak, $C_{21}H_{16}O_6$). High-resolution mass data: *m/z* 574 (calcd, 574.2050; found, 574.2019); 470-MHz ^1H NMR data are reported in Tables I and II. The ^1H NMR confirmed the dehydration to form the trans cyclic ether. IR (KBr) again confirmed the absence of the carbonyl group.

Acknowledgment. Research was supported by the Mayo Foundation and by a grant from the National Eagles Cancer Fund. We thank the Purdue University Biological Magnetic Resonance Lab, West Lafayette, IN, for cooperation and support in obtaining the 470-MHz proton NMR spectra. The work at Purdue was supported in part by the NIH Division of Research Resources (RR01077). We thank Dr. Michael Colvin of the Johns Hopkins Oncology Center, Baltimore, MD, for testing compounds in the mouse leukemia L1210 system in vivo. We thank Dr. Michael Lieber of the Department of Oncology, Mayo Clinic, Rochester, MN, for testing compounds in the L1210 in vitro colony-forming assay.

β-Carbolines: Synthesis and Neurochemical and Pharmacological Actions on Brain Benzodiazepine Receptors

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We have prepared a series of tetrahydro-β-carbolines (THβC), β-carbolines (β-C), and other nitrogen heterocycles and evaluated them in vitro with respect to their ability to bind to benzodiazepine receptors. The fully aromatic β-C's were more potent than their corresponding THβC derivatives. When substituents possessing a carbonyl (CO_2Me, $COCH_3$, CHO) were introduced at the β-C 3-position the in vitro potency was augmented. Alcohol substituents (CH_2OH, $CHOHCH_3$) demonstrated decreased in vitro potency. The importance of the carbonyl moiety was further demonstrated when β-carboline-3-carboxylic acid was shown to bind tighter to benzodiazepine receptors at lower pH. A lower pH increases the concentration of the acid and decreases the concentration of the anion. 3-(Hydroxymethyl)-β-carboline (**24**), 3-formyl-β-carboline (**25**) and 3-acetyl-β-carboline (**27**) were benzodiazepine antagonists in vivo. Methyl isoquinoline-3-carboxylate (**31a**) also had in vitro activity. The same structure–activity relationships seen in β-C's were also observed for isoquinolines.

The discovery of high affinity, saturable, and stereospecific receptors for benzodiazepines in the mammalian central nervous system has led to an intensive search for endogenous factors that physiologically regulate this receptor.[1,2] Although Nielsen and co-workers[3,4] originally

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[§] Laboratory of Medicinal Chemistry, NIADDK.
[⊥] NIMH.
[∥] Laboratory of Bioorganic Chemistry, NIADDK.

(1) Tallman, J.; Paul, S.; Skolnick, P.; Gallager, D. *Science* **1980**, *207*, 274.
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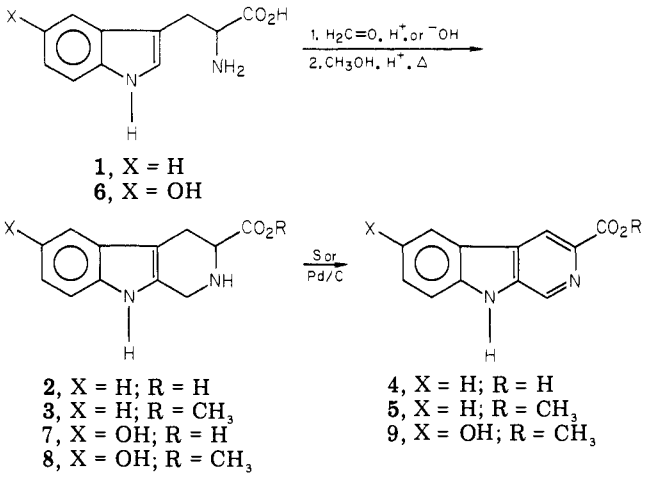
0022-2623/82/1825-1081$01.25/0

Scheme I



1, X = H
6, X = OH

2, X = H; R = H
3, X = H; R = CH_3
7, X = OH; R = H
8, X = OH; R = CH_3

4, X = H; R = H
5, X = H; R = CH_3
9, X = OH; R = CH_3

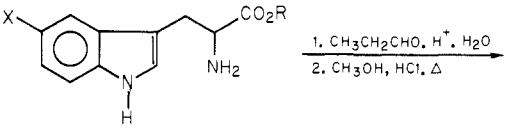
proposed β-carboline-3-carboxylic acid ethyl ester (β-CCE) as the endogenous factor, subsequent studies[5,6] have shown that this compound is probably formed during the isolation and extraction procedure. Nonetheless, the demonstration that certain β-carbolines potently inhibit [^{3}H]diazepam binding with a high affinity suggests this group of compounds may be useful both as tools for studying benzodiazepine receptors, as well as for the development of new therapeutic agents.

Recently, it has been demonstrated[7-9] that β-CCE (**5b**) antagonizes the anticonvulsant actions of diazepam (**65**), lowers the seizure threshold of the convulsant pentylenetetrazole (PTZ), and antagonizes the sedative actions of the benzodiazepine flurazepam. In addition, a closely related compound, 3-(hydroxymethyl)-β-carboline (3-HMC, **24**), which is approximately two orders of magnitude less potent than β-CCE in vitro, antagonizes both the anticonvulsant and "anxiolytic" actions of diazepam at doses comparable to those of β-CCE used in other studies.[10]

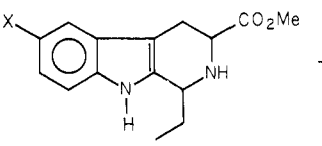
We now report on the synthesis and interaction of a series of β-C, THβC, and related heterocycles with brain benzodiazepine receptors in vitro. Behavioral studies support earlier observations, suggesting that these agents are antagonists of some of the pharmacological actions of benzodiazepines in vivo.

Chemistry. The preparation of the THβC's employed in this investigation was accomplished via the well-known Pictet–Spengler reaction[11] utilizing the appropriate 3-(aminoethyl)indole and aldehyde in either protic[12] or aprotic media.[13a,b] In general, conversion of the THβC

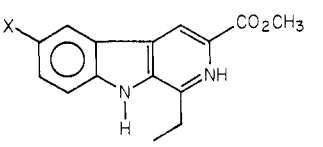
Scheme II



1, X = H; R = H
6, X = OH; R = H
10, X = H; R = CH_3
13, X = OH; R = CH_3

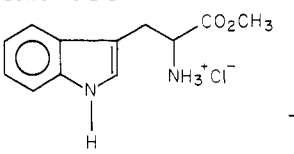


11a, X = H cis
b, X = H trans
14a, X = OH cis
b, X = OH trans

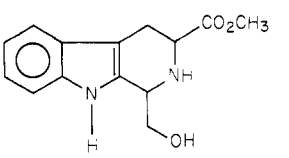


12, X = H
15, X = OH

Scheme III



10



17, cis
18, trans

to the corresponding β-C was carried out either by oxidation of the tetrahydro derivatives with sulfur[14] or treatment with Pd/C in refluxing cumene.[13b] The THβC **2** was prepared by condensation of tryptophan with formaldehyde according to the method of Snyder et al.[15] and was subsequently converted to the methyl ester **3** on heating in methanolic hydrogen chloride solution (Scheme I). This base was then subjected to oxidation with sulfur[14] in refluxing dioxane–xylene to provide methyl β-carboline-3-carboxylate (**5**, the methyl congener of the so-called γ-substance of Nielsen[3]). This sequence of reactions can be easily scaled up to provide gram quantities of **5** for further transformations.

In order to examine the effect of an aromatic hydroxy group on the in vitro binding of β-C's to the benzodiazepine receptor, a simple synthesis of hydroxy substituted β-C's

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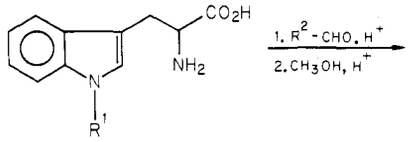
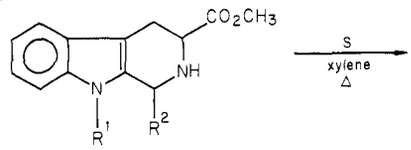
(15) Snyder, H. R.; Walker, H. G.; Werber, F. X. *J. Am. Chem. Soc.* **1959**, *71*, 527.

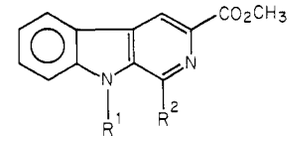
Scheme IV



20, $R^1 = CH_3$
1, $R^1 = H$



21, $R^1 = CH_3$; $R^2 = H$
22, $R^1 = CH_3$; $R^2 = Et$, trans
11a, $R^1 = H$; $R^2 = Et$, cis
b, $R^1 = H$; $R^2 = Et$, trans



5a, $R_1 = CH_3$; $R_2 = H$

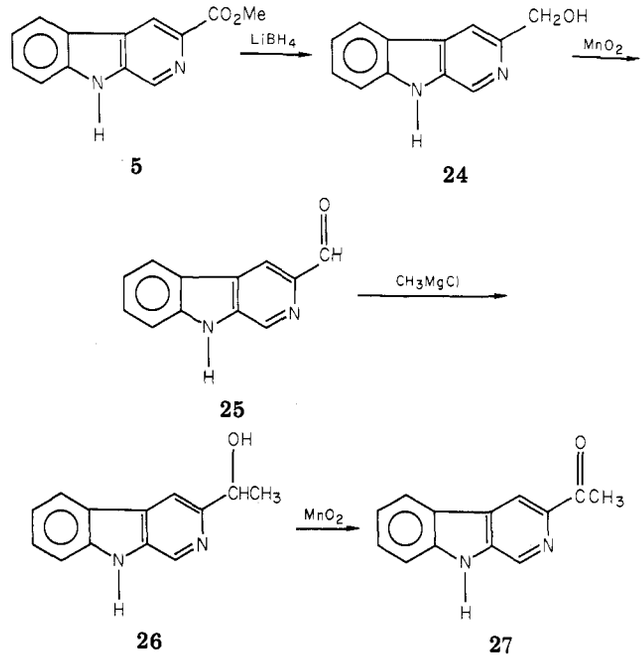
was necessary. This was accomplished by stirring 5-hydroxytryptophan (**6**) with formaldehyde, according to the method of Brossi,[16] to provide the hydroxy acid **7**; this material was esterified to give the desired methyl ester **8**. Conversion of the ester **8** to 6-hydroxy-3-carbomethoxy-β-carboline (**9**) was carried out by treatment of the tetrahydro derivative **8** with Pd/C, as illustrated in Scheme I.

Substitution of alkyl or aryl groups at the 1-position of β-C's would be expected to affect binding; consequently, a number of derivatives were synthesized with substituents ranging in size from hydroxymethyl to phenyl. The preparation of *cis*- and *trans*-1-ethyl-3-carbomethoxy-1,2,3,4-tetrahydro-β-carboline (**11a** and **11b**)[17] and the corresponding β-C **12**[18] have been reported elsewhere. The 6-hydroxy-1-ethyl derivative **14** was synthesized from 5-hydroxytryptophan and propionaldehyde, as illustrated in Scheme II, and converted to the 1-ethyl-6-hydroxy-3-carbomethoxy-β-carboline (**15**) on heating over Pd/C in dioxane. In addition, the 1-hydroxymethyl derivatives were prepared as a cis/trans mixture (**17** and **18**) by condensation of tryptophan methyl ester hydrochloride (**1**) with glycolaldehyde, as shown in Scheme III. The diastereomers were separated by fractional crystallization, and the relative stereochemistry in each (**17**, cis; **18**, trans) was assigned on the basis of their ^{13}C NMR spectra.[17]

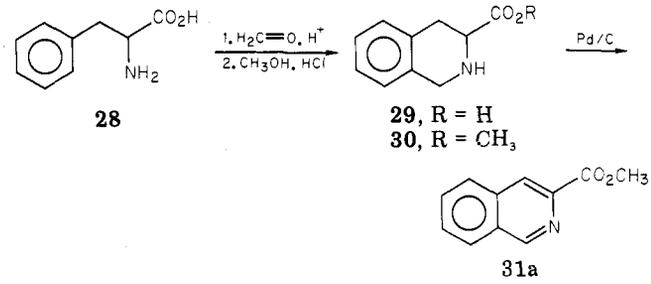
The construction of 9-methyl derivatives in order to examine the effect of this substitution pattern on binding was accomplished by simply substituting N_a-methyltryptophan (**20**) for **1** in the reaction sequence. As illustrated in Scheme IV, the 9-methyl derivative **20** was heated with formaldehyde, followed by esterification of the resulting acid, to provide the 9-methyl-3-carbomethoxy-1,2,3,4-tetrahydro-β-carboline (**21**). The *trans*-9-methyl-1-ethyl derivative **22** was prepared in similar fashion and reported elsewhere.[17]

For the most part, substitution at the 3-position of a β-C with a carbonyl group greatly augments in vitro binding to the benzodiazepine receptor, which has led us to prepare

Scheme V



Scheme VI



a number of 3-substituted β-C's, as illustrated in Scheme V. The conversion of 3-carbomethoxy-β-carboline (**5**) to the 3-hydroxymethyl derivative (3-HMC, **24**) can be accomplished in greater than 80% yield by treatment of **5** with lithium borohydride under conditions analogous to those used for the preparation of pyridindolol.[13b] The 3-formyl-β-carboline (**25**) was prepared for study simply by treating the 3-hydroxymethyl derivative **24** with activated manganese dioxide in acetonitrile.

The 3-formyl analogue **25** was converted to the secondary alcohol **26** on treatment of **25** with methylmagnesium chloride at 25 °C. In a manner analogous to the conversion of **24** to **25**, the alcohol **26** was cleanly converted (MnO_2) to 3-acetyl-β-carboline (**27**), as shown in Scheme V. In addition, the Pictet–Spengler reaction of phenylalanine[19] (**28**) with formaldehyde provided **29**, according to the method of Julian and Meyer et al.[20] (see Scheme VI). The acid **29** was esterified to give 3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline (**30**), and this material was subsequently converted to the desired 3-carbomethoxyisoquinoline (**31a**) on treatment with Pd/C.

Neurochemistry: Inhibition of [^{3}H]Diazepam Binding to Benzodiazepine Receptors by β-C, THβC,

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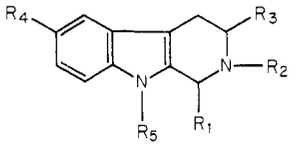
Table I. In Vitro Binding of Tetrahydro-β-carbolines



no.	R_1	R_2	R_3	R_4	R_5	K_i[a]	isomer
49	Ph	H	CO_2CH_3	H	H	54 μM	(±) cis
60	Ph	H	CO_2CH_3	H	H	174 μM	(±) trans
21	H	H	CO_2CH_3	H	CH_3	4.5 μM	
50	Ph	H	CO_2CH_3	H	CH_3	>100 μM	(±) trans
59	Ph	H	CH_2OH	H	H	6.11 μM	(±) trans
57	CH_3	H	CO_2CH_3	H	H	>50 μM	(±) cis
17	CH_2OH	H	CO_2CH_3	H	H	5 μM	(±) cis
18	CH_2OH	H	CO_2CH_3	H	H	510 nM	(±) trans
11a	C_2H_5	H	CO_2CH_3	H	H	>100 μM	(±) cis
11b	C_2H_5	H	CO_2CH_3	H	H	>100 μM	(±) trans
14a + 14b	C_2H_5	H	CO_2CH_3	OH	H	39 μM	(±) cis/trans
8	H	H	CO_2CH_3	OH	H	573 nM	(±)
14a	C_2H_5	H	CO_2CH_3	OH	H	>100 μM	(±) cis
61	H	H	H	OCH_3	H	>500 μM	
62	H	H	H	H	H	>100 μM	
63	H	CH_3	H	H	H	>100 μM	
64	H	H	H	OH	H	>100 μM	
55	3-pyridyl	H	CO_2CH_3	H	H	6.25 μM	(±) cis/trans
22	C_2H_5	H	CO_2CH_3	H	CH_3	>100 μM	(±) trans

[a] For inhibition of [^{3}H]diazepam binding to rat cerebral cortical membranes as described under Experimental Section.

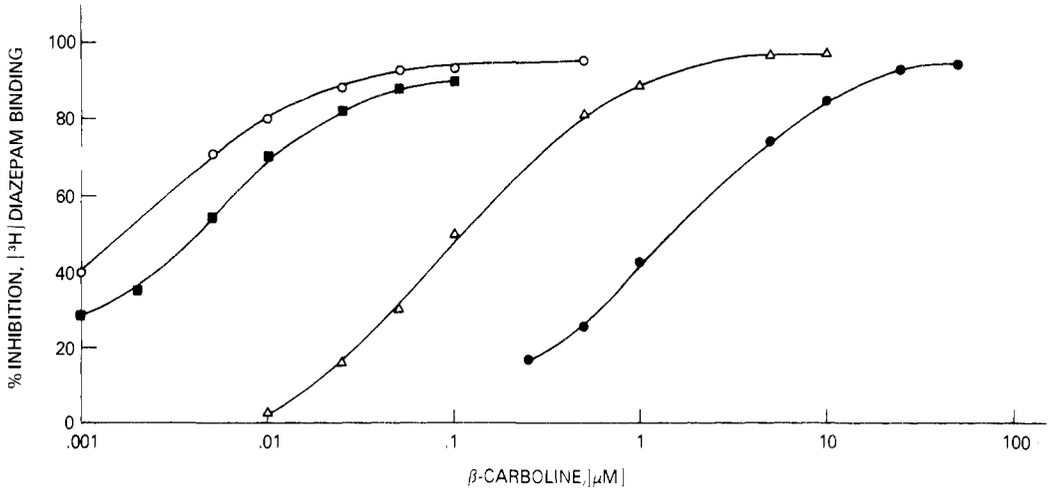


Figure 1. Inhibition of [^{3}H]diazepam binding by β-carbolines. Values are from representative experiments; each compound was assayed at least three times using not less than six concentrations of compound. Assays were performed and data calculated as described under Experimental Section. The K_i values in Table I were calculated as described in the text using graphically estimated IC_{50} values: (○), 3-carbomethoxy-β-carboline (**5**); (■), 6-hydroxy-3-carbomethoxy-β-carboline (**9**); (△), 3-formyl-β-carboline (**25**); (●) 3-(hydroxymethyl)-β-carboline (**24**).

and Related Heterocyclic Compounds. The inhibition of [^{3}H]diazepam binding by β-C, THβC, and related heterocyclic compounds (see Tables I–IV) was studied in thrice-washed membranes prepared from rat cerebral cortex with a radioligand concentration of approximately 2 nM. The inhibition of [^{3}H]diazepam binding was examined using six to eight concentrations of test compound; each concentration was assayed in triplicate. The percent inhibition of specific binding (see Experimental Section) was plotted vs. the concentration of compound tested; the IC_{50} (concentration of compound which inhibited binding by 50%) and K_i values were determined directly from the plot (Figure 1).

The naturally occurring alkaloids[21] harmane, 1-methyl-β-carboline (**42**), and norharmane (β-C, **43**) inhibited diazepam binding with K_i values of 12.4 and 1.6 μM, respectively. Introduction of substituents at the C-1 position in the fully aromatic (β-C) series resulted in a loss of activity, as demonstrated by the 8-fold decrease in potency of harmane (**42**) compared with norharmane (**43**), as illustrated in Table II. 1-Ethyl-β-carboline (**44**) did not significantly inhibit [^{3}H]diazepam binding even at 250 μM, the highest concentration tested. The 1-phenyl analogue **12a** (Table II) of **5** showed a reduction in affinity relative to **5** of more than three orders of magnitude. Introduction of substituents possessing a carbonyl oxygen at the 3-position significantly enhanced affinity for the benzodiazepine receptor. For example, the methyl, ethyl, and propyl esters of carboxylic acids **5**, **5b**, and **5c**, respectively, were approximately three orders of magnitude more potent than norharmane. Introduction of a methyl ester at the 3-position converts the completely inactive **44** to a compound with moderate affinity (**12**, $K_i \approx 7.5$ μM) for the benzodiazepine receptor. The potency of 3-substituted

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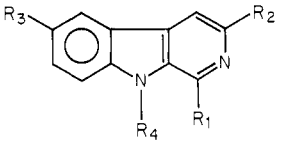
Table II. In Vitro Binding of β-Carbolines



no.	R_1	R_2	R_3	R_4	K_i[b]
15	C_2H_5	CO_2CH_3	OH	H	5.78 μM
12	C_2H_5	CO_2CH_3	H	H	7.54 μM
12a	C_6H_5	CO_2CH_3	H	H	3.89 μM
44	C_2H_3	H	H	H	250 μM
42	CH_3	H	H	H	12.4 μM
5	H	CO_2CH_3	H	H	1.05 nM
4	H	CO_2H	H	H	24 μM
27	H	$COCH_3$	H	H	58 nM
25	H	CHO	H	H	62.3 nM
9	H	CO_2CH_3	OH	H	2.66 nM
5b	H	$CO_2C_2H_5$	H	H	1.1 nM
5c	H	$CO_2C_3H_7$	H	H	1 nM[c]
5a	H	CO_2CH_3	H	CH_3	>50 μM
24	H	CH_2OH	H	H	1470 nM
26	H	$CHOH(CH_3)$	H	H	3160 nM
43	H	H	H	H	1620 nM
45 (α-carboline)					>250 μM
65[a] (diazepam)					5 nM

[a] Standard employed for data presented in Tables I–III. [b] For inhibition of [^{3}H]diazepam binding to rat cerebral corticol membranes as described under Experimental Section. [c] Reference 58.

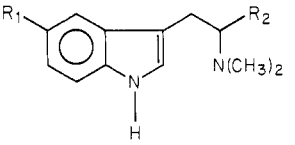
esters was not significantly altered by varying the chain length, since the potency of methyl β-carboline-3-carboxylate (**5**) is not markedly different than that of either the ethyl or propyl congeners.[4,6]

The carbonyl oxygen, rather than the ester function per se, may be of primary importance as a determinant of affinity for the benzodiazepine receptor in 3-substituted β-C, since both 3-formyl- (**25**) and 3-acetyl-β-carboline (**27**) had K_i values of approximately 60 nM, while the corresponding compounds which have been reduced to the primary and secondary alcohols **24** and **26** are 20- to 50-fold less potent (see Table II). The low affinity of β-carboline-3-carboxylic acid (**4**) may be due in part to the ionization of this compound at pH 7.4. This hypothesis has been supported by the observation that decreasing the pH of the incubation medium to 6.5 (resulting in a lower concentration of the 3-carboxylate anion) led to a significant increase in the potency of this compound. In contrast, the affinity of 3-formyl-β-carboline (**25**) was not significantly altered over the same pH range (unpublished observations).

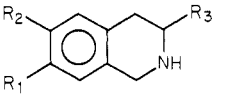
Introduction of a hydroxy group at the 6-position does not appear to markedly alter the potency of β-C (compare **5** with **9** and **12** with **15**), as illustrated in Table II. However, substitution at the 7-position results in an almost complete loss of activity, as in the naturally occurring alkaloid harmine (7-methoxy-1-methyl-β-carboline).[6] Methyl substitution at the N-9 position of 3-carbomethoxy-β-carboline also resulted in a dramatic loss in activity (compare **5** with **5a**).

THβC's were generally less potent than the corresponding fully aromatic structures, as shown in Table I. For example, THβC (**62**) did not inhibit [^{3}H]diazepam binding at concentrations up to 100 μM, while a K_i of 1.6 μM was obtained for the corresponding fully aromatic β-C **43**. As with the fully aromatic series, substitution at the C-1 position markedly reduced affinity for the benzodiazepine receptor (compare **21** and **50**). Substitution at the C-3 position, as in the fully aromatic series, dramatically increased the potency of THβC; the 3-carbomethoxy-1,2,3,4-tetrahydro-β-carbolines (**8** and **18**) had potencies of less than 1 μM, comparable to some clinically active benzodiazepines (see Table I).

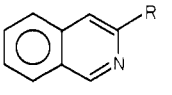
Table III. In Vitro Binding of Isoquinolines and Related Heterocycles



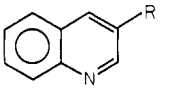
no.	R_1	R_2	K_i,[a] μM
32	OH	H	>100
33	H	H	>100
34	H	CO_2CH_3	>100



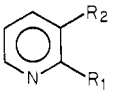
no.	R_1	R_2	R_3	K_i, μM
35	OH	OH	H	>100
36	OCH_3	OCH_3	H	>100
29	H	H	CO_2H	>100
30	H	H	CO_2CH_3	>100



no.	R	K_i, μM
31a	CO_2CH_3	13.2
31b	H	>100



no.	R	K_i, μM
37	CO_2CH_3	>100
38	CO_2H	>100
39	CHO	>100



no.	R_1	R_2	K_i, μM
40	CHO	H	>100
41a	H	CHO	>100
41b	$COCH_3$	H	>100

[a] For inhibition of [^{3}H]diazepam binding to rat cerebral cortical membranes as described under Experimental Section.

Table IV. Hill Coefficients of β-Carbolines

no.	compound	Hill coefficient[a]
4	β-carboline-3-carboxylic acid	0.72 ± 0.04 (3)
5	methyl β-carboline-3-carboxylate	0.70 (1)
5b	ethyl β-carboline-3-carboxylate	0.74 ± 0.07 (3)[b]
27	3-acetyl-β-carboline (**27**)	0.73 (2)
24	3-(hydroxymethyl)-β-carboline (**24**)	0.93 ± 0.09 (3)
25	3-formyl-β-carboline (**25**)	0.93 ± 0.07 (3)

[a] Values are the $\bar{x}$ (±SEM) with the number of determinations in parentheses. Hill Coefficients were calculated as described under Experimental Section. The data were obtained using membranes prepared from rat cerebral cortex using [^{3}H]diazepam (~2 nM) as a ligand. [b] Data from ref 4 using [^{3}H]flunitrazepam as a ligand.

Several heterocyclic compounds related to β-carbolines were also examined for their abilities to inhibit [^{3}H]diazepam binding. Substitution of a benzene nucleus for the indole system of a β-C or THβC yielded the corresponding isoquinoline or tetrahydroisoquinoline base (see Table III). Within the limited number of compounds examined, only methyl isoquinoline-3-carboxylate (**31a**) had any affinity for the benzodiazepine receptor, with a K_i value of approximately 13.2 μM. When the heterocyclic ring of the isoquinoline was saturated to yield methyl tetrahydroisoquinoline-3-carboxylate (**30**), an inactive compound resulted. Isoquinoline (**31b**) itself was inactive at concentrations up to 100 μM and inhibited binding by less than 30% at 500 μM. Quinoline derivatives substituted at the 3-position [e.g., quinoline-3-carboxylic acid (**38**)] were also inactive at concentrations up to 100 μM and inhibited binding by less than 30% at 500 μM (see Table III). Quinoline derivatives substituted at the 3-position [e.g., 3-quinolinecarboxaldehyde (**39**), methyl quinoline-3-carboxylate (**37**), and quinoline-3-carboxylic acid (**38**)] were also inactive at concentrations up to 100 μM. It is felt that the active isoquinoline **31a** is binding to the same "type" benzodiazepine receptor as the β-C **5**, since reversal of the juxtaposition of carbon and nitrogen atoms in **31a** (C=N) to N=C in the quinoline **37** resulted in almost complete loss of activity. The naturally occurring tetrahydroisoquinolines, 6,7-dihydroxy- and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**35** and **36**) were inactive at concentrations up to 100 μM. Pyridine derivatives containing a 2-substituent were also studied; both 2-acetylpyridine (**41b**) and 2-pyridinecarboxaldehyde (**40**) were inactive at concentrations up to 100 μM, as was 3-pyridinecarboxaldehyde (**41a**).

Pharmacological Results. Effects of β-C on the Anticonvulsant and Anxiolytic Actions of Diazepam. Several β-C derivatives that were examined for their abilities to inhibit [^{3}H]diazepam binding to benzodiazepine receptors in vitro were also examined for their actions (ip) on the anticonvulsant and anxiolytic properties of diazepam.

In order to assess the effects of β-C derivatives on the anticonvulsant actions of diazepam, a standard protocol was devised using doses of diazepam that were effective in protecting 80–90% of animals against a maximally effective dose of the chemical convulsant, pentylenetetrazole (PTZ).[10] The effects of these compounds on the "anxiolytic" actions of diazepam were assessed using a newly developed animal model that has been demonstrated to discriminate benzodiazepines and other clinically useful anxiolytics, such as meprobamate.[22,23] Furthermore, this model is capable of detecting a behavioral antagonism of diazepam by the purines inosine, 2′-deoxyinosine, and 2′-deoxyguanosine.[24]

The 3-HMC (**24**) was selected as a prototype β-C for study, since this compound has a moderately high affinity for the benzodiazepine receptor and may not be as rapidly metabolized as β-C's possessing an ester group at the 3-position. The 3-HMC antagonized the protective effects of diazepam against PTZ-induced seizures in a dose-dependent fashion (Figure 2). Maximally effective doses (ip) of 3-HMC reduced the ED_{90} of diazepam to the ED_{10}. No overt behavioral changes were observed in mice treated with 3-HMC alone (12.5 and 25 mg/kg). However, at 50 mg/kg, a decrease in spontaneous exploratory activity was observed (Figure 3).

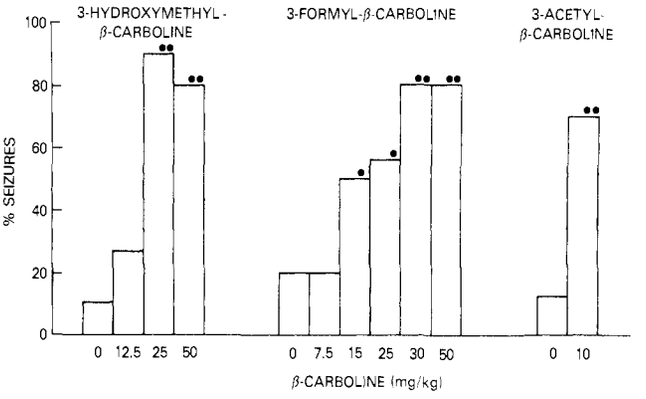


Figure 2. Disruption of the effects of diazepam on pentylenetetrazole-induced seizures by 3-(hydroxymethyl)-β-carboline (**24**). Groups of 9–20 mice were injected with sufficient diazepam to protect 80–90% against pentylenetetrazole (100 mg/kg, ip) induced seizures. Uninjected or vehicle-injected mice all had tonic–clonic convulsions when injected with this dose of pentylenetetrazole. Twenty minutes after administration of diazepam (1.5–2.5 mg/kg, ip), mice were administered either β-C or vehicle as described under Experimental Section: (●) $p < 0.05$, (● ●) $p < 0.01$ compared with mice treated with diazepam followed by vehicle.

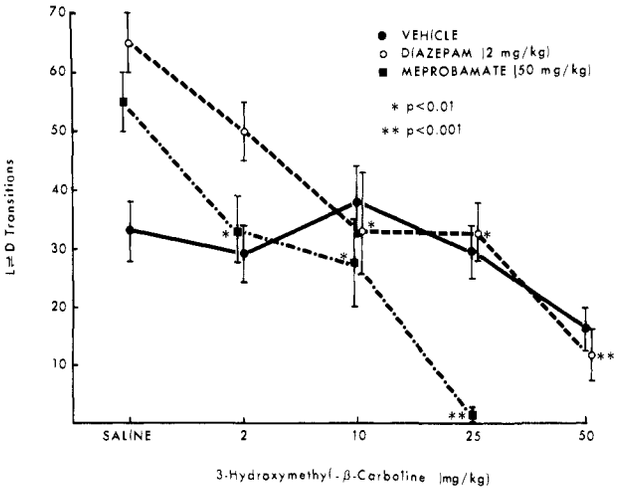


Figure 3. Disruption of the "anxiolytic" actions of meprobamate and diazepam by 3-(hydroxymethyl)-β-carboline (**24**). Mice were treated with either diazepam (2 mg/kg, ip) or meprobamate (50 mg/kg, ip), followed by either saline or **24** as described under Experimental Section. Both diazepam and meprobamate significantly increased ($p < 0.001$) the number of light ⇌ dark transitions (L ⇌ D) during the 10-min test period when compared with vehicle-injected mice.

3-Formyl-β-carboline (**25**), which is approximately 20-fold more potent than 3-HMC in vitro, was also examined for its ability to disrupt the anticonvulsant actions of diazepam. When an identical experimental protocol was used [with a 10-min interval between the injection of compound and PTZ (see Experimental Section)], 3-formyl-β-carboline (25 mg/kg) did not antagonize the anticonvulsant actions of diazepam. However, if the interval between injection of this compound and PTZ was decreased to 5 min, a dose-dependent (15–50 mg/kg) antagonism of the effects of diazepam resulted (Figure 2). 3-Acetyl-β-carboline (**27**, 10 mg/kg) also elicited a significant disruption of the anticonvulsant action of diazepam using a 10-min interval between compound and PTZ (Figure 2). Longer intervals between administration of β-C and PTZ were not examined.

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The 3-HMC (**24**) also antagonized diazepam (2 mg/kg) induced increases in light ⇌ dark transitions in a dose-dependent fashion, which reached statistical significance at a dose of 10 mg/kg (Figure 3). No effects of 3-HMC were observed over a dose range of 2–25 mg/kg when administered alone (Figure 3). However, at doses of 50 mg/kg a significant decrease in spontaneous exploratory activity was noted. 3-HMC was more potent in antagonizing a behaviorally equivalent dose of meprobamate; 2 mg/kg of 3-HMC completely abolished the increase in light ⇌ dark transitions elicited by 50 mg/kg of meprobamate (Figure 3).

Several other β-C's were examined in the same behavioral paradigm. 1-Ethyl-β-carboline (**44**) elicited profound sedation at doses as low as 10 mg/kg. Compounds that possess a prominant sedative action which results in a decrease in spontaneous exploratory activity are not suitable for testing in this paradigm. The sedative actions confound the interpretation of any test that includes a motor component (as do most types of "conflict" paradigms routinely used for screening anxiolytic compounds). Within 5 min following administration of α-carboline (**45**, 50–100 mg/kg), mice developed tremors (fine) and appeared profoundly sedated, rendering this compound unsuitable for testing in the behavioral paradigm employed.

Discussion

β-Carbolines, such as the naturally occurring harmala alkaloids, possess a number of behavioral and neurochemical actions. β-C's elicit hallucinations, tremors, convulsions, and sedation. Neurochemical actions of β-C include inhibition of monoamine oxidase, catecholamine uptake, and (Na, K)ATPase.[21]

The findings of Braestrup and co-workers[4,6] that β-CCE and related compounds are potent inhibitors of [^{3}H]diazepam binding to brain benzodiazepine receptors resulted in an interest in these compounds both as endogenous ligands of the benzodiazepine receptor and as pharmacological agents. Another β-C, harmane, has also been isolated in small amounts from the arcuate nucleus of the rat.[25,26] Rommelspacher et al.[27] have suggested that harmane may be a physiologically relevant ligand of the benzodiazepine receptor in vivo. However, neither the potency, concentration, nor neuroanatomic localization of harmane in mammalian brain makes this hypothesis tenable. Furthermore, Peura et al.[28] have recently reported that attempts to measure the corresponding tetrahydro derivative of harmane (1-methyltetrahydro-β-carboline) by the GC/MS technique of Shoemaker et al.[29] and Bidder et al.[30] for the identification of harmane lead to the artifactual formation of harmane via oxidation of the tetrahydro compound at the rather high separator temperatures used.[26,29,30] Thus, the reported identification of harmane as an in vivo constituent of rat brain[26,29] and human blood platelets[30] must also await further clarification.

Several THβC's have been isolated from rat brain,[31-35] adrenal gland,[35] urine,[31,33] and blood.[33,36-38] However, none of the known endogenous THβC's are effective inhibitors of [^{3}H]diazepam binding in vitro[27] (Table I). Heterocyclic derivatives, such as methyl isoquinoline-3-carboxylate (**31a**), are relatively potent (K_i = 10 μM) inhibitors of [^{3}H]diazepam binding (see Table III), and it is possible that as yet unidentified compounds with similar structures may be important for the physiological regulation of this receptor.

It is now clear that β-C can antagonize the anxiolytic, anticonvulsant, and sedative properties of benzodiazepines, such as diazepam and flurazepam.[7-9] β-CCE (**5b**), which has a high affinity for the benzodiazepine receptor in vitro, had been reported to be effective in vivo only if administered intravenously.[7,9] Another report[8] demonstrated that this compound is effective if administered intraperitoneally, but the action is short lived, and the doses needed to antagonize the pharmacological actions of benzodiazepines are *not* consonant with the in vitro potency reported for the compound. We have demonstrated that 3-HMC, which is approximately two orders of magnitude less potent than β-CCE in vitro, is an effective antagonist of the anticonvulsant and anxiolytic actions of diazepam when administered intraperitoneally at doses comparable to those reported for intravenous administration of β-CCE. This apparent discrepancy may well be due to a pharmacokinetic difference between these compounds, since hydrolysis of β-CCE (which could occur both peripherally and centrally) would result in formation of the less potent β-carboline-3-carboxylic acid (**4**). The 3-HMC may also be oxidized to **4** in vivo, but it is likely this oxidation occurs less rapidly than the hydrolysis of β-CCE. 3-Formyl-β-carboline (**25**), which is at an intermediate oxidation state between 3-HMC and β-carboline-3-carboxylic acid, is *ineffective* in disrupting the anticonvulsant actions of diazepam when administered 10 min prior to PTZ. However, if this interval is shortened to 5 min, 3-formyl-β-carboline is as potent an antagonist as 3-HMC (Figure 2). Since 3-formyl-β-carboline is approximately 20-fold more potent than 3-HMC in vitro, this finding supports the hypothesis that degradation of these compounds to a common pharmacologically inert product (β-carboline-3-carboxylic acid) would terminate the actions of both compounds.

Substitution at the 3-position of the β-C molecule appears to be important for increasing affinity at the benzodiazepine receptor (see Table II). However, the presence of a carbonyl oxygen at this position is most critical for very high affinity binding (<100 nM). 3-HMC was only slightly more potent than norharmane, but 3-formyl-β-carboline was about 20-fold more active than 3-HMC. The ester derivatives 3-methyl-, 3-ethyl-, and 3-propyl-β-carboline are 50- to 100-fold more potent than either 3-acetyl- or 3-formyl-β-carboline. It is possible that the other oxygen atom in the ester function may also contribute

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significantly to hydrogen bonding. The relatively low potency of β-carboline-3-carboxylic acid may be partially explained by the realization that this compound exists principally as the carboxylate anion at physiological pH as mentioned above. Planar molecules had higher potencies than nonplanar analogues, since β-C's were generally more potent than THβC analogues, which exist in a twist-chair conformation.[17] The complete three-ring skeleton is essential for optimum binding to the benzodiazepine receptor, although a two-ring planar structure with a carbonyl oxygen at the 3-position (e.g., **31a**) did retain some affinity for the receptor. The lack of activity of pyridine-3-carboxaldehyde (**41**) and other substituted pyridines suggests that the indole portion present in β-C or a nonnitrogenous ring (e.g., present in isoquinolines) or some other planar substitution is necessary for high affinity at the benzodiazepine receptor.

Modification of both β-C and THβC at the 1-position resulted in a loss of potency. A small substituent (e.g., methyl) resulted in a small reduction in potency, larger substituents resulted in more dramatic losses in potencies, especially in the rigid β-C molecule. THβC could accommodate a larger substitution at the 1-position (e.g., phenyl) without as great a reduction in potency.

Several reports have appeared recently[4,6,39] which indicate that heterogenous populations of benzodiazepine receptors are present in the central nervous system and that β-carboline-3-carboxylic esters, such as **5b**, may interact selectively with one of these subpopulations.[40-44] Analysis of the displacement of [^{3}H]diazepam from benzodiazepine receptors by several of the newly synthesized β-C's **24** and **25** revealed that there is no apparent relationship between the substituent at the 3-position and the Hill coefficient. These coefficients are interpreted as the ability of these compounds to selectively interact with one subpopulation of receptor (Table IV). For example, both **24** and **25** have Hill coefficients of approximately 1, while both **4** and **5** have Hill coefficients of less than 1 (~0.7). The significance of these observations for antagonism of the pharmacological actions of diazepam is unknown. However, it is tempting to speculate that there could be differences in the potency of these compounds in antagonizing certain of the pharmacological actions of diazepam, e.g., anxiolytic vs. sedative actions. This hypothesis is currently under investigation.

β-C's have been shown to possess a number of behavioral actions. The present report suggests that several of these actions are *not* mediated directly via interaction with benzodiazepine receptors. For example, 1-ethyl-β-carboline (**44**), which does not inhibit binding effectively in vitro at concentrations up to 250 μM, produces sedation in animals at doses as low as 10 mg/kg, while 3-HMC (**24**) with a moderate affinity ($K_i \approx 1470$ nM) for the benzodiazepine receptor does not reduce spontaneous exploratory activity at doses below 50 mg/kg. α-Carboline (**45**), which did not inhibit [^{3}H]diazepam binding in vitro, produced behavioral changes, including tremors and a rigidity resembling catatonia (unpublished observations). Recent reports suggest that the tremors and convulsions elicited by harmane and harmaline may be due to the interaction of these compounds with benzodiazepine receptors,[45,46] since both drugs displace [^{3}H]diazepam from benzodiazepine receptors, and diazepam is effective in reducing tremors induced by these agents.[46,47] However, it is quite possible diazepam may prevent or reduce the tremors induced by these agents indirectly, analogous to the ability of diazepam to prevent seizures induced by a wide variety of convulsants, some of which (e.g., strychnine) do not directly affect the benzodiazepine receptor. Furthermore, there does not appear to be a relationship between the ability of β-C's to elicit tremors and their affinities for the benzodiazepine receptor.

Imidazobenzodiazepines, such as RO-15-1788, antagonize some of the pharmacological actions of benzodiazepines[48a] but do not antagonize the depressant actions of meprobamate. This observation suggests that there are differences in the mechanisms by which β-C's such as 3-HMC (**24**) and compounds such as RO-15-1788 exert their antagonistic actions. This hypothesis is further supported by the recent observation that the benzodiazepine "antagonists" RO-15-1788 and CGS-8216 are even more potent than diazepam in blocking methyl β-carboline-3-carboxylate induced seizures.[48b] In addition, these observations may have significant implications as to the mechanism of action of compounds such as meprobamate. These hypotheses are currently under investigation.

Studies described here demonstrate that β-C's and related heterocycles are useful tools for probing the molecular pharmacology of the benzodiazepine receptor and may be potentially valuable as benzodiazepine antagonists. Further work is necessary to determine if a β-carboline, or a molecule of related structure, plays a physiological role in the regulation of the benzodiazepine receptor.

Experimental Section

Chemistry. Microanalyses were performed on a F&M Scientific Corp. Model 185 CHN analyzer. Melting points were taken on a Thomas-Hoover melting point apparatus; they are uncorrected. NMR spectra were recorded on Varian T-60 and CFT-20 spectrometers. IR spectra were taken on a Beckman Acculab-1 instrument. Electron-ionization (EI) mass spectra were recorded on a Hitachi RMU-6E spectrometer, and chemical-ionization (CI) mass spectra were obtained using a Finnigan GC/MS.

Analytical TLC plates used were E. Merck Brinkman UV active silica gel or alumina on plastic. Activated MnO_2 was prepared by the method of Mancera.[48c] Silica gel 60 and aluminum oxide for chromatography were purchased from EM Laboratories and J. T. Baker, respectively. The TLC plates were developed with the spray reagent ceric ammonium sulfate in 50% sulfuric acid. DL-Tryptophan, tryptamine hydrochloride, quinoline-3-carboxylic acid (**38**), 5-hydroxytryptophan, glycolaldehyde, quinoline-3-carboxaldehyde (**39**), methylmagnesium chloride, phenylalanine, isoquinoline, pyridine-2-carboxaldehyde (**40**), pyridine-3-carboxaldehyde (**41**), and lithium borohydride were purchased from Aldrich Chemical Co. The palladium on carbon catalyst was obtained from Pfaltz and Bauer.

The syntheses of 1-ethyl-β-carboline (**44**),[18] *cis*- and *trans*-1-ethyl-3-carbomethoxy-1,2,3,4-tetrahydro-β-carboline (**11a,b**),[17] *trans*-9-methyl-1-phenyl-3-carbomethoxy-1,2,3,4-tetrahydro-β-

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carboline (**50**),[17] 1-(3-pyridyl)-3-carbomethoxy-1,2,3,4-tetrahydro-β-carboline (**55**),[17] *cis*-1-methyl-3-carbomethoxy-1,2,3,4-tetrahydro-β-carboline (**57**),[17] *trans*-1-phenyl-3-(hydroxymethyl)-1,2,3,4-tetrahydro-β-carboline (**59**),[17] *trans*-1-phenyl-3-carbomethoxy-1,2,3,4-tetrahydro-β-carboline (**60**),[13b] and *trans*-9-methyl-1-ethyl-3-carbomethoxy-1,2,3,4-tetrahydro-β-carboline (**22**)[7] have been reported elsewhere.

N-Methyltryptamine and 3,4-dimethoxyphenylethylamine were gifts from Professor Fred Benington and Dr. Richard Morin of the Neurosciences Program at the University of Alabama in Birmingham, Birmingham, AL.

THβC (**62**), 2-methyltetrahydro-β-carboline (**63**), 6-methoxytetrahydro-β-carboline (**61**), and 6-hydroxytetrahydro-β-carboline (**64**) were prepared via the method of Ho and Walker.[49] 6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (**36**) and 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline hydrobromide (**35**) were prepared according to the method of Cohen et al.,[50] as modified by Barker et al.[51] The preparation of 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**2**) was achieved by the method of Synder et al.[15]

3-Carbomethoxy-1,2,3,4-tetrahydro-β-carboline (3). The acid **2**[15] (31.16 g, 0.144 mol) was dissolved in saturated methanolic HCl solution (500 mL), and the mixture was held at reflux under nitrogen for 6 h. The solvent was removed under reduced pressure, and aq. NH_3 (300 mL, 14%) was added to the residue. The aqueous alkaline suspension was extracted with $CHCl_3$ (2 × 300 mL) and EtOAc (3 × 300 mL). The organic layers were combined and dried (Na_2SO_4). The solvent was evaporated to provide an oil, which was crystallized from EtOAc to provide **3** (18.8 g, 57%), mp 184–187 °C (lit.[52] mp 186 °C).

3-Carbomethoxy-β-carboline (5). A solution of **3** (21 g, 91.3 mmol) in dioxane (1 L) and xylene (1 L) was treated with sulfur (25 g), and the mixture was held at reflux for 4 days. The mixture was then refluxed for an additional 2 days with sulfur (10 g) added at the beginning of each day. The solvent was removed under reduced pressure to provide a brown residue. A fine suspension of this residue was made in HCl (1 N, 400 mL). The suspension was filtered and the precipitate was washed with H_2O (400 mL). The combined aqueous fractions were extracted with C_6H_6 (3 × 100 mL) and then basified with concentrated aqueous NH_3 (100 mL) to yield a thick, cream-colored precipitate, which was filtered and dried for 12 h under vacuum at 40 °C to yield **5** (20 g, 97%), mp 245 °C (lit.[53] mp 243 °C).

β-Carboline-3-carboxylic Acid (4). The ester **5** (100 mg, 0.44 mmol) was added to an aqueous NaOH solution (1 N, 20 mL), and the suspension that resulted was stirred at 60 °C for 1 h. The solution was then neutralized to pH 7 with 37% HCl to afford a solid, which was filtered to provide **4** (60 mg, 64%), mp 295 °C (lit.[54] mp 309–310 °C).

3-(Hydroxymethyl)-β-carboline (24). A fine suspension of **5** (7 g, 31 mmol) in THF (900 mL) was treated with $LiBH_4$ (3.4 g, 155 mmol), and the mixture was stirred at room temperature for 9 h. The reaction was cooled, treated with H_2O (100 mL), and stirred overnight. The solvent was evaporated in vacuo, and H_2O (600 mL) was added. The aqueous suspension was extracted with CH_2Cl_2 (1 L) and with EtOAc (2 × 800 mL). The organic extracts were combined, and the solvent was evaporated in vacuo. The residue was chromatographed on silica gel and eluted with an EtOAc/MeOH gradient to yield 3-(hydroxymethyl)-β-carboline (**24**; 5 g, 82%), mp 228–230 °C (lit.[55] mp 225–228 °C).

β-Carboline-3-carboxaldehyde (25). To a solution of **24** (1 g, 5 mmol) in CH_3CN (900 mL) was added activated MnO_2 (3 g). The suspension was refluxed for 4.5 h and then cooled and filtered through Celite. The filtrate was passed through silica gel (100 g). The silica gel was washed with EtOAc (400 mL), and the solvent was removed under reduced pressure. EtOAc was added to the residue to provide **25** (700 mg, 70%): mp 273–277 °C; IR (KBr) 2600–3300, 1695 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.10–7.70 (m, 3 H), 8.20–8.70 (m, 1 H), 8.40 (d of d, 1 H, J_1 = 8 Hz, J_2 = 1 Hz), 8.75 (s, 1 H), 10.10 (s, 1 H); MS, *m/e* 196.0637 ($C_{12}H_8N_2O$ requires 196.0636).

3-(1-Hydroxyethyl)-β-carboline (26). CH_3MgCl (2.8 M in THF, 5.24 mL, 14.7 mmol) was added to dry THF (50 mL). A solution of **25** (480 mg, 2.45 mmol) in dry THF (100 mL) was then added dropwise at 25 °C. After the solution was stirred at 25 °C for 2.5 h, a saturated aqueous solution of NH_4Cl (40 mL) was added, and the solution was allowed to stir for an additional 20 min. The mixture was extracted with EtOAc (3 × 100 mL), and the combined extracts were dried (Na_2SO_4), followed by removal of the solvent under reduced pressure. Et_2O was added to the residue, and crystalline **26** was obtained (500 mg, 96%): mp 175–177 °C; IR (KBr) 2100–3700 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.55 (d, 3 H, J = 8 Hz), 4.85 (q, 1 H, J = 8 Hz), 6.90–7.60 (m, 4 H), 7.80–8.30 (m, 2 H), 8.70 (s, 1 H); MS, *m/e* 212.0939 ($C_{13}H_{12}N_2O$ requires 212.0949).

3-Acetyl-β-carboline (27). To a solution of **26** (500 mg, 2.36 mmol) in CH_3CN (500 mL) was added activated manganese dioxide (1.5 g). The mixture was refluxed for 1.5 h and then filtered initially through Celite, followed by silica gel (50 g). The filtrate was evaporated under reduced pressure, and the residue was crystallized from Et_2O. The yellow crystals were filtered and then dried under vacuum for 12 h at 50 °C to furnish **27** (400 mg, 80%): mp 234–235 °C; IR (KBr) 1720 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.75 (s, 3 H), 7.10–7.80 (m, 3 H), 8.45 (d of d, 1 H, J_1 = 9 Hz, J_2 = 1 Hz), 8.85 (s, 1 H), 9.00 (s, 1 H); MS (CI, CH_4), *m/e* 211 (M + 1, 100). Anal. ($C_{13}H_{10}N_2O$) C, H, N.

9-Methyl-3-carbomethoxy-1,2,3,4-tetrahydro-β-carboline (21). To a solution of 1-methyltryptophan (**20**; 2.5 g) in aqueous NaOH (0.1 N, 100 mL), was added formaldehyde (1.4 mL, 37%), and the mixture was stirred at 37 °C for 48 h. AcOH (1.0 mL) was added to afford a precipitate, which was filtered, dried (2 g), and dissolved in saturated methanolic HCl solution (300 mL). The mixture was held at reflux for 6 h, and the solvent was removed under reduced pressure. The HCl salt that remained was converted to the free base on treatment with 14% aqueous NH_3 (100 mL), and the aqueous solution extracted with CH_2Cl_2 (2 × 75 mL). The organic layers were combined and dried (K_2CO_3). The solvent was removed under reduced pressure to provide ester **21** as an oil (1.6 g, 57%): IR (film) 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.95 (1 H, s, NH), 2.80 (2 H, m), 3.20 (3 H, s), 3.50 (2 H, m), 3.65 (3 H, s), 6.90–7.40 (4 H, m); MS, *m/e* 244.1207 ($C_{14}H_{16}N_2O_2$ requires 244.1212).

9-Methyl-3-carbomethoxy-β-carboline (5a). To a solution of **21** (500 mg) in dioxane (20 mL) and xylene (200 mL), sulfur (2 g) was added. The mixture that resulted was heated to reflux for 24 h, after which the solvent was removed under pressure. To the residue, hydrochloric acid (1 N, 200 mL) was added, and a fine suspension resulted, which was subsequently filtered. The filtrate was basified with concentrated aqueous NH_3 and extracted with EtOAc (3 × 100 mL). The organic extracts were evaporated to provide a solid, which was crystallized from MeOH to furnish **5a** (300 mg, 60%): mp 200–201 °C; IR (KBr) 1710 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.00 (s, 3 H), 4.05 (s, 3 H), 7.20–7.80 (m, 3 H), 8.40 (d, 1 H, J = 7 Hz), 8.90 (s, 1 H), 9.10 (s, 1 H); MS (CI, CH_4), *m/e* 241 (M +·1). Anal. ($C_{14}H_{12}N_2O_2$) C, H, N.

3-Carbomethoxy-6-hydroxy-1,2,3,4-tetrahydro-β-carboline (8). Acid **7** (1.0 g, 0.0043 mol) was converted to the ester **8** under conditions analogous to the conversion of **2** to **3**. The ester **8** was purified by washing through a short column of Al_2O_3 (7% CH_3OH in CH_2Cl_2). The solid that resulted was crystallized from EtOAc to afford pure ester **8** [0.91 g (86%)]: mp 130–133 °C; IR (KBr) 3300 (br), 1730 (s) cm^{-1}; NMR (Me_2SO-d_6) δ 2.81 (m, 2 H), 3.68 (m, 1 H; s, 3 H), 3.95 (s, 2 H), 6.58 (d of d, 1 H, J_{meta} = 2, J_{ortho} = 8.5 Hz), 6.73 (d, 1 H, J_{meta} = 2 Hz), 7.08 (d, 1 H, J_{ortho} = 8.5 Hz); MS (CI/NH_3) *m/e* 247 (M + 1, 100). Anal. ($C_{13}H_{14}N_2O_2$) C, H, N.

3-Carbomethoxy-6-hydroxy-β-carboline (9). Solid **8** (0.5 g, 0.002 mol) was dissolved in a solution of cumene (50 mL) and diglyme (50 mL), after which Pd/C (0.4 g, 5%) was added and the mixture refluxed for 3 days. The catalyst was then removed

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by filtration, and the solvent was removed under reduced pressure. Chromatography of the residue on alumina (CH_3OH/CH_2Cl_2 gradient elution) afforded pure **9** (0.21 g, 43% yield): mp 258–262 °C dec; IR (KBr) 3300 (br), 1730 (s) cm^{-1}; NMR (Me_2SO-d_6) δ 3.94 (s, 3 H), 7.20–7.90 (m, 3 H), 8.94 (s, 1 H), 9.10 (s, 1 H); MS (CI/CH_4), m/e 243 (M + 1, 100). Anal. ($C_{13}H_{10}N_2O_3$) C, H; N: calcd, 11.56; found, 11.00.

***cis*- and *trans*-1-(Hydroxymethyl)-3-carbomethoxy-1,2,3,4-tetrahydro-β-carboline (17 and 18).** Tryptophan methyl ester hydrochloride (**10**; 3 g, 11.8 mmol) and glycolaldehyde (850 mg, 14.1 mmol) were heated to reflux in methanol–water (7:3) for 15 h under N_2. The solvent was removed under reduced pressure, and the residue was crystallized from EtOAc to provide *cis*-**17**·HCl (1.2 g, 34%), mp 198 °C. The hydrochloride was basified with 14% aqueous NH_3, and the solution was extracted with CH_2Cl_2 (3 × 200 mL). Removal of solvent gave *cis*-**17** as the free base: mp 179–180 °C; IR (KBr) 3600–2600, 1735 cm^{-1}; ^1H NMR ($CDCl_3$, Me_2SO-d_6) δ 2.40–3.30 (m, 4 H), 3.70 (s, 3 H), 3.50–3.90 (m, 2 H), 4.00–4.30 (m, 1 H), 4.50–5.90 (m, 1 H), 6.80–7.40 (m, 4 H), 10.20 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) 25.43, 51.57, 54.58, 55.73, 63.49, 106.88, 111.06, 117.24, 118.37, 120.56, 126.68, 134.16, 136.14, 173.14 ppm; MS (CI, NH_3), m/e 261 (M + 1, 100). Anal. ($C_{14}H_{16}N_2O_3$) C, H, N.

The mother liquor which contained a mixture of the cis and trans isomers was concentrated under reduced pressure and then basified with 14% aqueous NH_3. Extraction with CH_2Cl_2 (3 × 100 mL), followed by chromatography on alumina, gave *trans*-**18** (1.7 g, 48%): mp 125–129 °C; IR (KBr) 3600–2600, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.50–2.80 (m, 2 H), 2.80–3.10 (m, 2 H), 3.50–3.68 (m, 2 H), 3.68 (s, 3 H), 3.68–4.30 (m, 2 H), 6.90–7.50 (m, 4 H), 8.10 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) 24.43, 51.34, 51.98, 52.19, 63.44, 106.07, 110.90, 117.30, 118.20, 120.50, 126.53, 130.30, 136.00, 173.56 ppm; MS, m/e 260.1158 ($C_{14}H_{16}N_2O_3$ requires 260.1161).

***cis*-1-Ethyl-3-carbomethoxy-6-hydroxy-1,2,3,4-tetrahydro-β-carboline (14a).** To a suspension of 5-hydroxytryptophan (**6**; 1 g, 4.5 mmol) in aqueous sulfuric acid (30 mL, 0.005 M) was added propionaldehyde (1.2 g, 20.7 mmol), and the mixture was stirred at 25 °C overnight. The white precipitate that formed was filtered, dried, and dissolved in saturated methanolic HCl (60 mL). The solution that resulted was refluxed under nitrogen for 7 h. The solvent volume was reduced, after which EtOAc was added, and the reaction was kept in the freezer overnight. The resulting crystalline material was filtered and dried to yield **14a**·HCl (820 mg, 58%); the cis isomer was isolated as the hydrochloride salt, mp 259 °C. The salt was converted to the free base on treatment with 14% aqueous NH_3, and the solution was extracted with EtOAc to give the free base **14a**: mp 118–121 °C; IR (KBr) 1740 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.98 (t, 3 H, J = 6 Hz), 1.45–2.20 (m, 2 H), 2.40–2.60 (m, 1 H), 2.60–2.80 (m, 2 H), 3.4–3.6 (m, 1 H), 3.7 (s, 3 H, OMe), 3.8–4.1 (m, 1 H), 6.3–6.7 (m, 2 H), 7.0 (d, 1 H, J = 8 Hz), 10.3 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) 173.08, 150.13, 136.73, 130.36, 127.33, 110.92, 110.28, 105.43, 101.59, 56.00, 53.26, 51.35, 26.26, 25.46, 9.22 ppm; MS, m/e 274.1295 ($C_{15}H_{18}N_2O_3$ requires 274.1317).

The mother liquor was basified with aqueous ammonia (14%), and the alkaline solution was extracted with ethyl acetate. The solvent was evaporated under reduced pressure to provide an oil, which contained both the cis (**14a**) and trans (**14b**) isomers of **14**. The trans isomer was not isolated; however, a ^{13}C NMR spectrum (Me_2SO-d_6) of the mixture showed signals at 52.32, 51.05, and 50.87 ppm for the trans isomer, in addition to those for the cis isomer.

1-Ethyl-3-carbomethoxy-6-hydroxy-β-carboline (15). To a solution of the tetrahydro-β-carboline **14a** (520 mg, 192 mmol) in dioxane (50 mL) was added 10% Pd/C (520 mg), and the mixture was refluxed for 3 days. The suspension was initially filtered through Celite, followed by filtration through silica gel to yield **15** (250 mg, 48%): mp 254–257 °C; IR (KBr) 3600–2800, 1700 cm^{-1}; ^1H NMR ($CDCl_3$–Me_2SO-d_6) δ 1.40 (t, 3 H), 3.20 (q, 2 H), 3.95 (s, 3 H), 7.10 (d of d, 1 H, J_1 = 9 Hz, J_2 = 3 Hz), 8.60 (s, 1 H), 10.80 (s, 1 H); MS, m/e 270.0988 ($C_{15}H_{14}N_2O_3$ requires 270.1004).

1-Phenyl-3-carbomethoxy-β-carboline (12a). The cis isomer **49**[17] (0.9 g) and 10% Pd/C (40 mg) were added to xylene (50 mL) and refluxed for 43 h. The hot solution was filtered over Celite. The Celite bed was washed with hot xylene. While the solution was cooling, pale yellow crystals precipitated. The precipitate was filtered and air-dried to give **12a** (0.71 g, 80%): mp 253 °C; IR (KBr) 3310, 1710 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.10 (s, 3 H), 7.20–8.60 (m, 9 H), 9.05 (s, 1 H); MS (CI/CH_4), m/e 303 (M + 1, 100). Anal. ($C_{19}H_{14}N_2O_2$) C, H, N.

3-Carbomethoxy-1,2,3,4-tetrahydroisoquinoline (30). The 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**29**;[20a] 1 g) was converted to ester **30** under conditions analogous to the conversion of **2** to **3**. After workup, removal of solvent under reduced pressure gave the air-sensitive base **30**, which was treated in MeOH with dry HCl gas to afford **30**·HCl (0.74 g, 69%).[20b]

3-Carbomethoxyisoquinoline (31a). To a solution of **30** (1.0 g, 5.2 mmol) in xylene (150 mL) was added 10% Pd/C (0.8 g). The mixture was refluxed for 8 h, at which time starting material was not detectable by TLC. The catalyst was filtered from the reaction through Celite, and the solvent was removed under reduced pressure. The solid that resulted was crystallized from ethanol to provide 0.71 g (73%) of 3-carbomethoxyisoquinoline (**31a**): mp 70–71 °C; IR (KBr) 1725 cm^{-1}; NMR ($CDCl_3$) δ 4.10 (3 H, s), 7.30–8.30 (4 H, m), 8.60 (1 H, s), 9.90 (1 H, s); MS, m/e 187.0620. ($C_{11}H_9NO_2$ requires 187.0633).

3-Carbomethoxyquinoline (37). Quinoline-3-carboxylic acid (**38**; 0.6 g) was converted to the ester (**37**) under conditions analogous to the conversion of **2** to **3**. The solid that resulted was crystallized from ethanol to provide ester **37** (56%): mp 70–74 °C; IR (KBr) 1712 cm^{-1}; NMR ($CDCl_3$) δ 4.00 (s, 3 H), 7.20–8.40 (m, 4 H), 8.70 (d, 1 H, J = 2 Hz), 9.40 (s, br, 1 H); MS (CI/CH_4), m/e 188 (M + 1, 68%). Anal. ($C_9H_9NO_2$) H, N; C: calcd, 70.59; found, 69.63.

Pharmacology. Male Sprague–Dawley rats (175–225 g; Taconic Farms, Germantown, NY) and NIH general purpose or Swiss-Webster mice (20–25 g) (Veterinary Resources Branch, NIH, Bethesda, MD) were used in these studies. All substances tested in vitro were dissolved in either distilled water or 0.1 N HCl. Concentrated stock solutions were then diluted with distilled water to the appropriate concentrations. For in vitro studies, compounds were generally added in a volume of 37.5 μL and did not significantly affect the pH of the incubation mixture. Compounds tested in vivo were dissolved in a phosphate-buffered saline (pH 7.2) or sufficient 1 N HCl (usually less than 10% of the total volume) to dissolve the compound and diluted with phosphate-buffered saline. Mice were injected intraperitoneally with a volume of 0.1 mL. Diazepam (Hoffman LaRoche, Nutley, NJ) and meprobamate (Wallace Laboratories, Cranbury, NJ) were dissolved in a vehicle containing 0.5 mL of ethanol, 1 mL of propylene glycol, and 1 mL of phosphate-buffered saline. The concentrated drug solution was then diluted 1:10 with phosphate-buffered saline. Drugs were prepared daily and diluted immediately prior to use. Norharmane (**43**), α-carboline (**45**), and harmane (**42**) were supplied by Dr. John Daly, NIH. Other chemicals were purchased from standard commercial sources. [^{3}H]Diazepam (sp act. 87.6 Ci/mmol) was obtained from New England Nuclear, Boston, MA.

Measurement of [^{3}H]Diazepam Binding to Brain Benzodiazepine Receptors. [^{3}H]Diazepam binding to rat cerebral cortical membranes was accomplished using a modification of a previously described method.[56] In brief, rats were killed by decapitation, and the cerebral cortex was removed. Tissue was distributed in 100 volumes of Tris-HCl buffer (50 mM, pH 7.4) with a Polytron (15 s, setting 6, Brinkmann Instruments, Westbury, NY) and centrifuged (4 °C) for 20 min at 20000g. Tissue was resuspended in an equal volume of buffer and recentrifuged. This procedure was repeated until the tissue was washed a total of three times. The tissue was then resuspended in 100 volumes of buffer (protein concentrations: 0.5–0.6 mg/mL), and 1 mL was added to 0.4 mL of buffer and 0.0375 mL of test compound. Incubations (4 °C) were initiated by addition of [^{3}H]diazepam (2 nM) and terminated after 30 min by addition of 5 mL of ice-cold Tris buffer, followed by filtration on Whatman GF/B under vacuum, and an additional washing of the filter with 5 mL of buffer. Filters were suspended in 10 mL of Hydrofluor (National Diagnostics, Summerville, NJ) and then shaken vig-

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orously for 30 min, and the radioactivity was measured in a Packard B-2450 liquid scintillation counter. Nonspecific binding was determined by substituting nonradioactive diazepam (final concentration 3 μM) or clonazepam (final concentration 1 μM) for the test compound. Nonspecific binding was less than 10% of total binding under these conditions. Specific binding was defined as the difference in binding in the presence and absence of the large excess of nonradioactive benzodiazepine. Data were expressed as percent inhibition of specific binding, and IC_{50} values were estimated from semilogarithmic plots (see Figure 1). Inhibitory constants of compounds under study were calculated by the equation $K_i = IC_{50}/1 + [L]/K_D$, where [L] = ligand concentration (~2 nM), and the K_D for [^{3}H]diazepam was estimated to be 5.6 ± 0.34 nM in thrice-washed cerebral cortical membranes.[57] Hill coefficients for compounds under study were estimated using least mean squares regression analysis with values obtained from inhibition curves as described.[58]

Antagonism of the Anticonvulsant Effects of Diazepam. Mice were injected with sufficient diazepam (1.5-5.5 mg/kg, ip) to protect 80-90% against tonic-clonic convulsions induced by pentylenetetrazole (100 mg/kg, ip). This dose of PTZ elicits convulsions in 100% of mice pretreated with vehicle. Mice were injected with diazepam and 20 min later injected (ip) with either β-C or vehicle. Ten-minutes later, the animals were challenged with PTZ. In some studies with 3-formyl-β-carboline (**25**), the interval between administration of compound and PTZ was decreased to 5 min. Animals were scored as protected by diazepam if no appearance of seizures was noted for 10 min after injection of PTZ.

Antagonism of the Anxiolytic Actions of Diazepam and Meprobamate. Mice were treated with diazepam (2 mg/kg, ip) or meprobamate (50 mg/kg, ip) 20 min prior to injection with either vehicle or test compound. The number of light ⇌ dark transitions was measured in a chamber that was partitioned so that two-thirds of the chamber was illuminated and one-third was dark. A series of photocells records the number of light ⇌ dark transitions, which represent spontaneous exploratory activity, during a 10-min test period beginning 10 min after injection of the test compound or vehicle.[22]

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(±)-*cis*-2-Acetoxycyclobutyltrimethylammonium Iodide: A Semirigid Analogue of Acetylcholine

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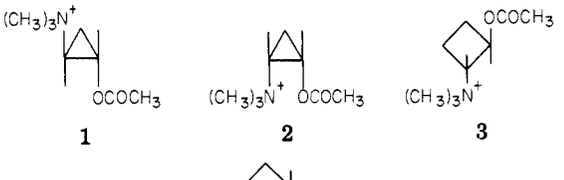
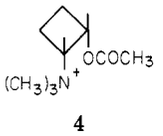
The title compound was prepared to complete a series of small ring (cyclopropane, cyclobutane) cis/trans 1,2-disubstituted semirigid congeners of acetylcholine. A multistep synthetic sequence, beginning with *cis*-cyclobutane-1,2-dicarboxylic anhydride, permitted unequivocal preparation of the (±)-cis target compound 4. The geometry of 4 was confirmed by comparison with an authentic sample of the (±)-trans isomer. The cis and trans isomers were equipotent as muscarinic agonists, but they were much weaker than acetyl-β-methylcholine.

Prior communications from this laboratory have described the synthesis[1] and the remarkably high muscarinic effects[2] of (1*S*,2*S*)-*trans*-2-acetoxycyclopropyltrimethylammonium iodide ("*trans*-ACTM", **1**). The enantiomer



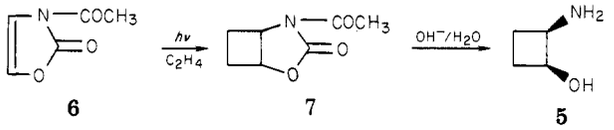
1 **2** **3**



4

1*R*,2*R* of **1** had only $^1/_{200}$ the muscarinic potency of **1**, and like **1**, it had almost no nicotinic activity. In contrast, (±)-*cis*-2-acetoxycyclopropyltrimethylammonium **2** was virtually inert in both nicotinic and muscarinic assays (one-twentieth as potent in a muscarinic assay and approximately equipotent in a nicotinic assay compared to (1*R*,2*R*)-*trans*-ACTM, the less active enantiomer). Subsequently,[3] the (±)-*trans*-cyclobutane analogue **3** of *trans*-ACTM was found to possess muscarinic activity, but the potency was decidedly less than that of (±)-*trans*-ACTM. The present work describes the synthesis and biological evaluation of (±)-*cis*-2-acetoxycyclobutyltrimethylammonium iodide (**4**) for completion of the series of cis and trans isomers of cyclopropane- and cyclobutane-derived congeners of acetylcholine.

Scheme I. Synthesis of *cis*-2-Aminocyclobutanol [a]



6 **7** **5**

[a] See ref 4.

Chemistry. Hartmann et al.[4] have reported the synthesis of (±)-*cis*-2-aminocyclobutanol (**5**) (Scheme I), and this compound would be an ideal precursor to the target species **4**. In the present study, all attempts to prepare adequate amounts of (±)-4-acetyl-2-oxa-4-azabicyclo-

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