and assayed for adenylate cyclase to determine if basal activity was elevated.

Effects of Compounds on Light Emission. The procedures were as previously described,[8] except that drugs were dissolved in insect saline containing no calcium and 20 mM manganese chloride. These salt conditions increased the specificity of the assay by blocking any potential effects of drugs on endogenous release of octopamine from nerve terminals.

Determination of Irreversible Binding of ^{3}H-NC-5Z. Washed light-organ membranes (0.04 mL of 100 mg wet wt/mL) were added to 0.16 mL of a mixture containing (final concentration): 80 mM Tris maleate, pH 7.4; 10 mM theophylline; 8 mM $MgCl_2$; 0.5 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; 2 mM ATP; and 0.1 mM GTP. Displacing agents were added and the mixture was incubated at 4 °C for 15 min, after which ^{3}H-NC-5Z (17 Ci/mmol) was added to a final concentration of 1 μM and the incubation continued for an additional 60 min. The mixture was then transferred to quartz minicuvettes (1-mm path length, total volume 0.25 mL) and photolyzed for 15 min at a distance of 2 cm from a bank of three 15-W Sylvania GTE germicidal lamps (G15T8). Cuvettes were turned over every 2 min during the exposure and, following photolysis, the contents of the cuvettes were transferred to tubes containing 10 mL of 6 mM Tris maleate, pH 7.4. The tubes were centrifuged at 120 000*g* for 30 min, the pellet was resuspended in 10 mL of buffer and washed twice more. The final pellet was solubilized in 1 mL of Protosol (New England Nuclear) for 15 h at 30 °C, transferred to a scintillation vial, and neutralized with 1 mL of 1N HCl. Eighteen milliliters of Aquasol-2 (New England Nuclear) was then added and the radioactivity was measured by scintillation counting in a Serle Delta 300. In some experiments, membranes (along with 0.2 mL of 0.63% bovine serum albumin was carrier protein) were washed, instead, by five cycles of precipitation with 7.5% cold trichloroacetic acid and solubilization of the centrifuged pellet with 0.2 mL of 1 M NaOH.

Acknowledgment. We wish to thank Edward J. Hunnicutt and Dr. Kenneth Kirshenbaum for technical assistance. This work was supported by USDA 8600090 and 88-37153-3617 and by grants from the Upjohn Company and the Katharine Daniels Research Fund.

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Synthesis and Benzodiazepine Receptor Affinities of Rigid Analogues of 3-Carboxy-β-carbolines: Demonstration That the Benzodiazepine Receptor Recognizes Preferentially the s-Cis Conformation of the 3-Carboxy Group

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1*H*-Indolo[3',2':4,5]pyrido[3,2-*b*]-2-penten-5-olide (**6**) and 1*H*,5*H*-indolo[3',2'-*c*]-6,7-dihydro-2-pyridone (**7**), rigid analogues of methyl 4-ethyl-β-carboline-3-carboxylate (**8**) and *N*-methyl-4-ethyl-β-carboline-3-carboxamide (**9**), respectively, were synthesized and their in vitro binding affinities to the central type benzodiazepine receptors were compared. The IC_{50} values of **6** and **8** were approximately equivalent (42 and 27 nM, respectively). The amide derivative **9**, for which theoretical energy calculations indicate that the s-trans carbonyl conformation is the preferred one, displayed very low affinity ($IC_{50} > 10^4$ nM). However, when the carbonyl group of **9** was forced to adopt the s-cis conformation as in lactam **7**, binding to the benzodiazepine receptor was largely restored (IC_{50} = 150 nM), indicating that the s-cis carboxy conformation at C-3 of β-carbolines is preferentially recognized by this receptor. In vivo, compound **6** showed neither convulsant, proconvulsant, nor anticonvulsant activity in mice. Moreover, **6** did not antagonize methyl β-carboline-3-carboxylate induced convulsions in mice. This lack of activity of **6** was attributed to its inability to cross the blood–brain barrier since no significant displacement of [^{3}H]Ro 15-1788 from mouse brain benzodiazepine receptors by **6** could be observed in vivo.

β-Carbolines possessing a carboxyl group at the 3-position [for example, ethyl β-carboline-3-carboxylate (β-CCE, **1**; see Chart I)], are known to bind with high affinity to the central benzodiazepine receptors (BZR)[1,2] and have been proposed as endogenous ligands of these receptors.[1,3] Moreover, structure–activity studies in this series have shown that a carboxyl group implicated in an ester linkage [e.g., **1**, IC_{50} (concentration of ligand inhibiting 50% of tritiated benzodiazepine binding) = 7 nM][1] demonstrates a higher affinity for the BZR than one that is part of an amide linkage [e.g., FG 7142 (**2**); IC_{50} = 657 nM].[4,5] On the basis of the X-ray crystal structures of a homologous ester methyl β-carboline-3-carboxylate (β-CCM, **3**)[6,7] and amide [*N*-ethyl-β-carboline-3-carboxamide (β-CEA, **4**)],[8] it has been proposed by Codding that one possible reason for this observed difference in binding affinities of the

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(5) The IC_{50} value of FG7142 (compound **2**) which we report in Table II (197 nM) differs somewhat from the literature value[4] (657 nM). This may be due to minor differences in the in vitro procedures used. However, all the values of Table II were determined by ourselves under exactly the same experimental conditions and are consistently reproducible.
(6) Muir, A. K. S.; Codding, P. W. *Can J. Chem.* **1985**, *63*, 2752.

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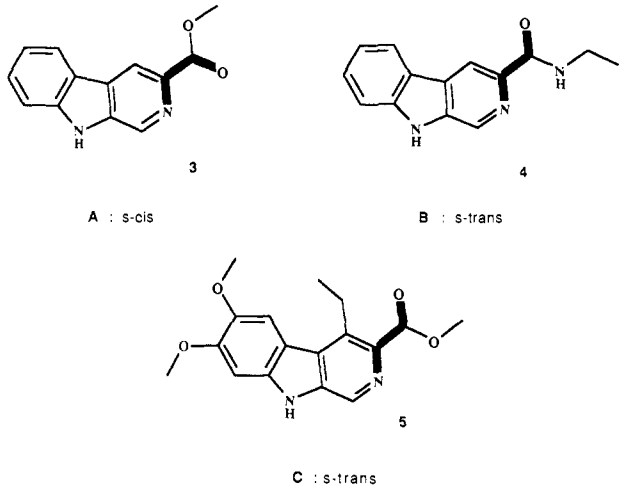
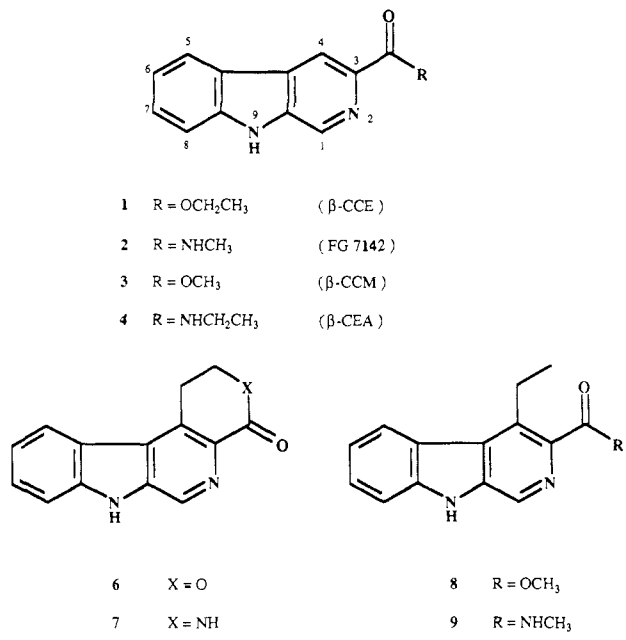


Figure 1. Conformations of the C-3 side chains of various 3-carboxy-β-carbolines as determined by X-ray crystallography: (A) β-CCM (3) (refs 5 and 6); (B) β-CEA (4) (ref 8); (C) DMCM (5) (ref 9).

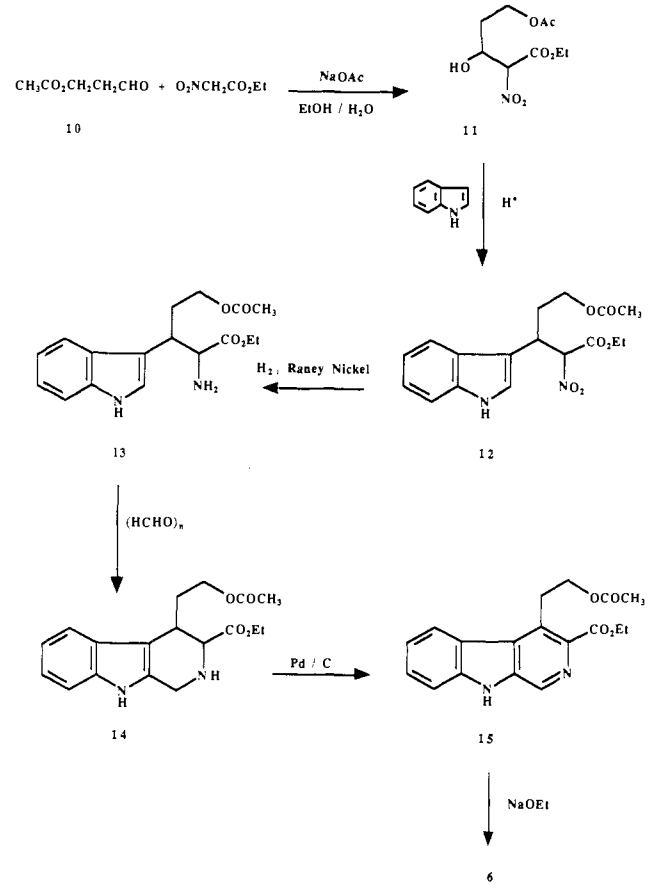
Chart I



esters and amides resides in the different conformations of the respective carbonyl groups apparently favored by each type of molecule. The carbonyl group of β-CCM (3) in the solid state adopts a conformation in which its oxygen atom is cis to the pyridine nitrogen (Figure 1A) while the carbonyl group of β-CEA (4) favors the opposite, trans conformation (Figure 1B). Preferential recognition of the s-cis conformation by the BZR would then explain the higher binding affinity of β-CCM compared to β-CEA.

This argument is however tempered by the fact that some high-affinity esters [e.g., methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM, 5), Figure 1C] possess an s-trans conformation in the crystalline state.[9-11]

Scheme I



Assuming that only one conformation of the carbonyl group is recognized by the BZR, identical for both β-CCM and DMCM, then it is obvious that the X-ray crystal structure of one of these molecules does not reflect the real nature of the ligand–receptor interaction. Extension of conformational preferences of compounds in the solid state to those in solution may not always be justified; a normally stable conformation of a moiety may be forced into a less stable one by reason of the energy imparted by its interaction with a receptor.

In an effort to resolve this question as to which conformation of the carbonyl group of 3-carboxy-β-carbolines is recognized by the BZR, we have synthesized β-carbolines in which this critical functionality is forced to adopt a rigid fixed s-cis conformation as part of either a cyclic ester (lactone 6) or cyclic amide (lactam 7) linkage.

The affinities of 6 and 7 for the BZR were determined and compared to the affinities of the structurally related but conformationally free 4-ethyl ester and amide ana-

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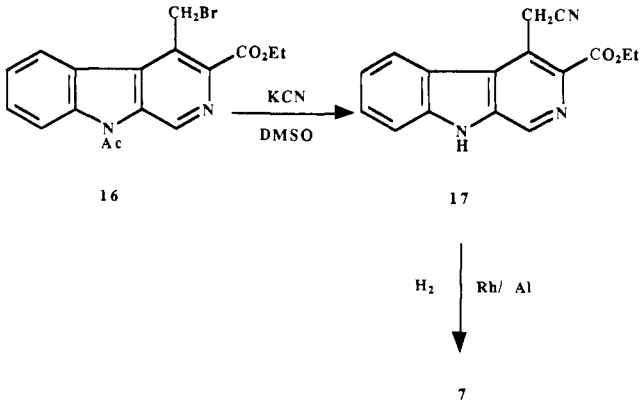
(11) A referee has pointed out that the high affinity of DMCM for the benzodiazepine receptor as determined in vitro with rat hippocampal membranes (IC_{50} = 1.1 nM using [^{3}H]DMCM as radioligand;[26] IC_{50} = 6.6 nM using [^{3}H]flunitrazepam[27]) would tend to indicate that the trans conformation of the carbonyl group, which this compound adopts in the crystalline state (Figure 1), is actually that recognized preferentially by the receptor. However, our results with compound 8 show that this is unlikely. This compound, which represents DMCM without its dimethoxy groups, demonstrates a reduced affinity for the benzodiazepine receptor (IC_{50} = 27 nM using [^{3}H]-flunitrazepam as radioligand, Table II) compared to both DMCM and β-CCM, precisely as a result of this compound's preference for the s-trans conformation, as our calculations show. The high affinity of DMCM is thus more likely due to the presence of the dimethoxy groups than to its favored s-trans carbonyl conformation.

Scheme II



logues, **8** and **9**, respectively. The most stable conformers of these 4-substituted and 4-unsubstituted β-carbolines were also determined by theoretical calculations using an energy minimization program. The results strongly suggest that the s-cis conformation of the carbonyl group (Figure 1A) or one approaching this position is indeed preferentially recognized by the BZR.

Chemistry. The lactone **6** has reportedly been synthesized in low yield via microbial hydroxylation of 4-ethyl-β-CCE (**8**, R = OEt)[12] while 5-membered lactones and lactams analogous to **6** and **7** have been produced as side products in the reactions of 4-(bromomethyl)-β-CCE (**16**)[13] with potassium hydroxide and primary amines, respectively. No binding data for these compounds were given.

We chose to synthesize the six-membered lactone **6** since this rigid structure disposes the carbonyl group and the adjoining oxygen atom in a geometry most closely resembling that observed in the crystal structure of β-CCM (**3**) (see below). The methodology of Neef and co-workers[13] developed for the synthesis of 4-substituted β-carboline-3-carboxylic esters was utilized to produce the precursor **15** (Scheme I). Thus, condensation of ethyl nitroacetate with 2-formylethyl acetate (**10**)[14] gave the unstable β-hydroxy ester adduct **11**, which was reacted directly with indole in toluene–acetic acid, yielding the addition product **12**. Reduction of the nitro group of **12** with Raney nickel in ethanol followed by Pictet–Spengler-type cyclization of the resulting tryptophan derivative **13** with paraformaldehyde (Sandrin procedure)[15] led to the 1,2,3,4-tetrahydro-β-carboline **14**. This compound was subsequently dehydrogenated to the fully aromatic β-carboline **15** by heating under reflux in xylene in the presence of palladium on charcoal. When a solution of β-carboline **15** in ethanol was treated with a catalytic quantity of sodium, the desired lactone **6** precipitated almost quantitatively.

The δ-lactam **7** was prepared according to Scheme II. The protected 4-(bromomethyl) derivative of β-CCE (**16**)[13] reacted cleanly with potassium cyanide in dimethyl sulfoxide to give the 4-(cyanomethyl)-β-carboline **17**. Hydrogenation of the cyano group using rhodium on alumina as catalyst led, in one step, to the lactam **7**.

The *N*-methylamide **9** was prepared by treatment of 4-ethyl-β-CCE (**8**, R = OEt)[13] with methylamine in a sealed tube at room temperature for 7 days.

Table I. Calculated[a] Energies and Torsion Angles of the Various 3-Carboxy-β-carbolines

no.	C=O conformer	*E*, kcal/mol[b] (±0.1)[d]	α, deg[c] (±1°)[d]
3	s-trans	37.4	0
3	s-cis	41.6	0
2	s-trans	41.6	0
2	s-cis	51.0	0
8	s-trans	41.7	±161
8	s-cis	47.9	±35
9	s-trans	46.9	±172
9	s-cis	59.4	±32
6	s-cis	43.8	±16
7	s-cis	51.7	±11

[a] By using the program MACROMODEL.[16] [b] Lowest energy after minimization. [c] Torsion angle formed by $-N_2-C_3-C=O$. [d] Estimated standard error.

Results and Discussion

Energy Calculations. The MACROMODEL molecular graphics program was used to calculate the energies (*E*) and the torsion angles (α, formed by $N_2-C_3-C=O$) of the most stable conformations of the C-3 ester and amide side chains of both the unsubstituted (compounds **3** and **2**, respectively) and 4-ethyl-substituted β-carbolines (compounds **8** and **9**, respectively) (Table I).[16] The results show that, for the ester derivatives **3** and **8**, the s-trans conformers are always the most stable (i.e., lowest energy). Moreover, whereas for the nonsubstituted ester **3** the energy difference between the s-trans and s-cis conformers is relatively small (ΔE = 4.2 kcal), that for the 4-substituted ester **8** is somewhat higher (ΔE = 6.3 kcal), and this regardless of the conformation adopted by the 4-ethyl group (not shown).[11] Moreover, the carbonyl group of **8** is forced out of the plane of the heterocycle (α = ±161°), no doubt due to the proximity of the sterically bulky ethyl moiety.

In the case of the amides **2** and **9**, the s-trans conformers are again the most stable. In contrast to the esters, however, a much higher energy difference (10–12 kcal) is seen between the s-trans amide and its s-cis conformer. As with the esters, the 4-ethyl substituent has, as a principle effect, an increase in the energy difference between the s-cis and s-trans conformers and a forcing of the carbonyl group outside the plane of the heterocycle.

It is interesting to note that this theoretical approach to molecular geometry reproduces, in the case of the 4-unsubstituted amide derivative **2**, almost exactly the crystal structure of the *N*-ethyl analogue of **2** (compound **4**) as determined by X-ray diffraction.[8] On the other hand, the calculated conformational preference of the carbonyl group of β-CCM (**3**) (s-trans) is the opposite of that determined by X-ray crystallography (s-cis).[6,7] However, as others have inferred,[10,17] our theoretical calculations show that the energy differences between the cis and trans esters are sufficiently small that both forms can be expected to exist in solution in approximately equal amounts.

Energy minimizations of the rigid structures **6** and **7** show a maximum deflection of the carbonyl group of, respectively, ±16° and ±10.8° from the plane of the carboline heterocycle, which is more than that of the unsubstituted s-cis carbolines **3** and **2** (α = 0°) but considerably less than

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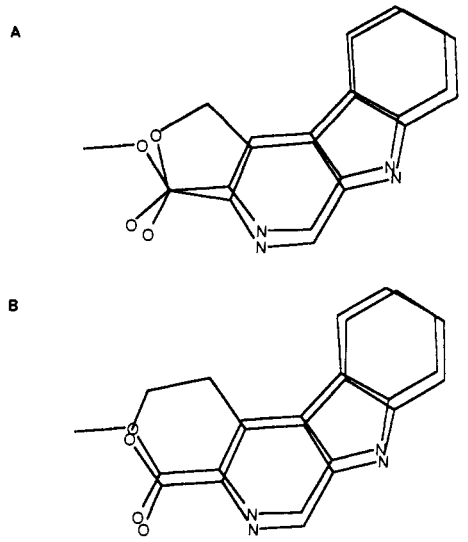

Figure 2. Computer graphics generated superpositions of β-CCM (3) with (A) a five-membered lactone and (B) the six-membered lactone 6.

Table II. Benzodiazepine Receptor Affinities of the Rigid and Nonrigid β-Carboline Derivatives

no.	IC_{50}, nM[a]	no.	IC_{50}, nM
3	3.5	2	197[b]
8	27	9	>10000
6	42	7	150

[a] Concentration of compound required to inhibit 50% of [^{3}H]-flunitrazepam specific binding to in vitro preparations of rat cerebral cortex membranes at 0 °C as determined by the technique of ref 18; average of triplicate determinations. [b] Reference 5.

in the s-cis conformations of the substituted analogues 8 and 9 (>30°). Superposition of the minimized structures of the lactone 6 and of β-CCM (3) indicates a good spatial correlation between the respective carbonyl groups and their attached oxygen atoms (Figure 2). Superposition of β-CCM with the five-membered lactone (not synthesized) shows that the critical —O—C=O geometry of the latter is somewhat distorted with respect to that of the freely rotating ester, though in this lactone, the carbonyl group is now entirely in the plane of the carboline ring. The six-membered lactone 6 thus appears as the better compromise in reproducing the lowest energy conformation of β-CCM (3).

In Vitro Binding Experiments. The affinities of compounds 2, 3, and 6–9 for the BZR, as reflected by their ability to inhibit [^{3}H]flunitrazepam binding to rat brain cortical membranes in vitro, were determined by the method of Rehavi et al.[18] and are given in Table II. In the ester series (columns 1 and 2) it can be seen that introduction of the ethyl group in position 4 of β-CCM to give compound 8 leads to an almost 8-fold decrease in affinity. This can be attributed either to some steric repulsion between the ethyl moiety and the receptor, to electronic effects of the ethyl group on the heterocycle, and/or to the greater energy required in this series to transform the more stable s-trans carbonyl into the presumably higher affinity s-cis conformation (Table I).[11] The rigid analogue of 8, the lactone 6, in which the carbonyl group is forced to maintain an s-cis geometry, in fact shows an affinity almost identical with that of 8, the steric volume of both molecules being also similar. This result strongly suggests that the BZR does in fact preferentially recognize the s-cis carbonyl conformation of 3-carboxy-β-carbolines. This s-cis conformation of the carbonyl group would place the oxygen atom of the latter in the most favored position for efficient interaction with a postulated hydrogen bond acceptor site of the BZR, essential for β-carboline (and other inverse agonist) binding.[19]

Even more telling in this respect are the binding values obtained with the amide derivatives (Table II, columns 3 and 4). As has been observed by others,[4] the 3-(*N*-methylcarboxamide) derivative 2 binds much more weakly than the ester 3 to the BZR.[5] As has been suggested by Codding[8] and as our calculations indicate, the barrier to rotation of the s-trans carbonyl of the amide 2 to its less stable s-cis conformation may be sufficiently high (~10 kcal) that relatively few of these molecules are found in this latter geometry. Binding of 2 with the BZR, which apparently recognizes preferentially the s-cis carbonyl conformation, would then be diminished, as is observed. Although the receptor can be expected to supply energy to effect such an unfavorable conformational change, the energy required (>10 kcal/mol) is at the upper limit of that provided by, for example, hydrogen bond formation between the receptor and the β-carboline (1–7 kcal/mol).[20]

It is thus not surprising that the 4-ethyl analogue of the amide 2, that is, compound 9, displays practically no affinity for the BZR. This molecule combines the steric impedement of the 4-ethyl moiety, already observed to decrease the affinity of the ester derivatives, with an increased energy barrier for trans → cis rotation (almost 13 kcal, Table I). This latter feature is in fact preeminent in inhibiting the binding of 9 to the BZR. This is dramatically demonstrated with the lactam 7, whose steric requirements are basically the same as those of 9 but whose carbonyl bond is now forced into the receptor-favored s-cis conformation, with the result that the affinity of 7 is now comparable to that of the unsubstituted amide 2.

Comparison of the in vitro binding values of 6 and 7, which differ only with respect to the atom attached to their respective carbonyl groups, suggests that the BZR prefers a hydrogen-accepting atom (oxygen) in this position rather than a hydrogen-donating atom (nitrogen). Alternatively, differing contributions of the oxygen and nitrogen atoms to the double-bond character of the adjacent carbonyl moiety could also explain the enhanced binding of 6 as compared to 7, as could differences in their lipid solubilities.

Of the two rigid compounds 6 and 7, the more soluble and higher affinity lactone 6 was chosen for in vivo studies in mice. This compound, administered in Tween 80 at doses of 5 and 20 mg/kg, ip, showed no convulsive or tremorogenic activity by itself, nor were the animals sedated. The anticonvulsant activity of 6 was then investigated in pentylenetetrazole (PTZ) (70 mg/kg) and β-CCM (10 mg/kg) treated mice. Again, compound 6, at doses of 5 or 10 mg/kg, was completely without effect, being unable to inhibit the convulsions consistently produced in 70–80% of mice by these two agents. Lactone 6 was also unable to potentiate the action of subconvulsive doses of PTZ (30 mg/kg) and β-CCM (5 mg/kg).[21]

Because lactone 6 displayed no observable in vivo convulsive, anticonvulsive, or antagonist activity despite a

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good in vitro affinity for the benzodiazepine receptor, we investigated the ability of compounds **6** and **7** to inhibit the binding of [^{3}H]Ro 15-1788 in vivo in mice. Thus, according to a procedure that we have previously described,[22] the lactone **6** or the lactam **7** was administered (20 mg/kg in Tween 80, ip) in mice followed, 30 min later, by [^{3}H]Ro 15-1788. After 3 min, the mice were sacrificed, and after dissection, the radioactivity retained in the cortex, cerebellum, and hippocampus was determined. The inhibition of [^{3}H]Ro 15-1788 by compounds **6** and **7** was found to be practically insignificant (between 5 and 10%) in the three brain regions studied. In contrast, β-CCM (**3**) displaces 50% of specifically bound [^{3}H]Ro 15-1788 at as little as 0.72 mg/kg in mouse cerebellum under the same conditions.[22b] Thus, the lack of in vivo activity displayed by **6** may be explained by its slow or incomplete penetration into the brain, the result of unfavorable pharmacokinetic or pharmacodynamic factors.

In conclusion, the present study demonstrates that the BZR recognizes, preferentially, the s-cis conformation of 3-carboxy-β-carbolines or one approaching this geometry. This result thus corroborates several recently proposed benzodiazepine receptor ligand binding models[10,19,23] in which this particular conformation was only tacitly assumed. The effect of a fixed carbonyl conformation on the in vivo activity of β-carbolines is currently being investigated via the synthesis and pharmacological testing of more lipophilic analogues of **6** and **7**.

Experimental Section

Chemistry. Melting points were determined on a Büchi apparatus and are uncorrected. IR spectra were taken in Nujol or neat with a Perkin-Elmer 297 instrument. Proton NMR spectra were determined on Varian T-60, Bruker WP 80, or Bruker WP 200-MHz instruments. Chemical shifts are given as δ values with reference to Me_4Si as internal standard. Thin-layer chromatography was performed on Merck silica gel 60 plates with fluorescent indicator. The plates were visualized with UV light (254 and 366 nm). Merck silica gel (230–400 mesh) was used for all column chromatography. Electron impact (EI) mass spectra were done on an AEI MS-50 spectrometer. Chemical ionization (CI) and fast atomic bombardment (FAB) mass spectra were obtained respectively on a modified[24] AEI MS-9 and on a Kratos MS-80 spectrometer. High-performance liquid chromatography (HPLC) was performed on a Waters 6000 A instrument equipped with a 10 × 250 mm column of Spherisorb ODS2 (5-μm) and a UV440 detector. Elemental analyses were performed at the ICSN, CNRS, Gif-sur-Yvette, France.

(2*RS*,3*RS*)-Ethyl 5-Acetoxy-3-hydroxy-2-nitropentanoate (11). To a solution of 2-formylethyl acetate (**10**)[14] (17.2 mmol) in ethanol (15 mL) containing sodium acetate (0.2 mmol) in water (1.5 mL) at 0 °C was added dropwise ethyl nitroacetate (17.2 mmol) under a nitrogen atmosphere. The reaction mixture was stirred at 10 °C for 1 h and allowed to stand at room temperature overnight. The ethanol was then removed in vacuo, and the residue was extracted with ether (40 mL). The extract was washed with saturated aqueous sodium chloride, dried over anhydrous sodium sulfate, and concentrated to leave 3.5 g of an oily residue (**11**), which was used without further purification in the following steps: IR (neat) 1560 (NO_2), 1740 (O—C=O), 3500 cm^{-1} (CH–OH); EIMS, m/z 232 ($MH^+ - H_2O$), 207 ($MH^+ - CH_3CO$); NMR ($CDCl_3$) δ 1.28 (3 H, t, CH_3CH_2O), 1.28 (2 H, d of t, CH_2CH_2O), 2.02 (3 H, s, CH_3CO), 3.62 (1 H, bs, OH), 4.12 (5 H, m, CH_3CH_2O, $CHOHCH_2$, CH_2OAc), 5.33 (1 H, m, NO_2CHCO_2Et).

(2*RS*,3*RS*)-Ethyl 5-Acetoxy-3-(indol-3-yl)-2-nitropentanoate (12). A solution of indole (50 mg; 0.43 mmol), hydroxynitroester **11** (314 mg) (containing 1/3 of unreactive ethyl nitroacetate by NMR), toluene (5 mL), and glacial acetic acid (0.5 mL) was heated under reflux at 110 °C under a nitrogen atmosphere for 2 h. After this period another 314 mg of hydroxynitroester **11** was added, and the reaction mixture was heated under reflux for a further 2 h. Evaporation of the solvents in vacuo gave a brown syrup. Traces of acetic acid were removed by repeated codistillation with toluene in vacuo. The residue was chromatographed on silica gel by using 1:1 toluene–ethanol as developer to yield 58 mg (39%, based on indole) of **12** as a yellow oil: IR ($CHCl_3$) 1560 cm^{-1} (NO_2); EIMS, m/z 348 (M^+), 301 ($M^+ - HNO_2$); NMR ($CDCl_3$) δ 0.90 [1.8 H, t, J = 7 Hz, R (or S), CH_3CH_2O], 1.27 [1.2 H, t, J = 7 Hz, S (or R), CH_3CH_2O], 2.00 (3 H, s, CH_3CO), 2.20 (2 H, m, H-4), 3.77–4.53 (5 H, m, H-3, H-5, OCH_2CH_3), 5.57 [1 H, t, J = 10 Hz (R and S), H-2], 6.87 (3 H, m, Ar), 7.40 (1 H, d, J = 8 Hz, Ar), 7.67 (1 H, d, J = 7 Hz, Ar), 8.33 (1 H, bs, NH indole, D_2O exchangeable). Anal. ($C_{17}H_{20}N_2O_6$) C, H, N.

(2*RS*,3*RS*)-Ethyl 5-Acetoxy-3-(indol-3-yl)-2-aminopentanoate (13). A vigorously stirred mixture of indole **12** (240 mg; 0.69 mmol), ethanol (35 mL), and Raney nickel (1 g) was hydrogenated at atmospheric pressure for 4 h. The nickel was then removed by filtration over Celite and washed with hot ethanol (250 mL). Evaporation of the filtrate in vacuo afforded a yellow syrup which was chromatographed on a silica gel column using dichloromethane–ethanol (7:0.5) as developer to give 107 mg (48%) of the desired amine **13**: IR ($CHCl_3$) 3400 cm^{-1} (NH_2); EIMS, m/z 318 (M^+), 245 ($M^+ - CO_2Et$, $M^+ - CH_2OAc$), 216 ($M^+ - NH_2CHCO_2Et$); NMR ($CDCl_3$) δ 1.13 [1.8 H, t, J = 7 Hz, R (or S), CH_3CH_2O], 1.33 [1.2 H, t, J = 7 Hz, S (or R), CH_3CH_2O], 2.13 (2 H, s, NH_2, D_2O exchangeable), 2.15 (3 H, s, CH_3CO), 2.15 (2 H, m, H-4), 3.53 (1 H, m, H-3), 3.80 (1 H, d, H-2), 4.00 (4 H, m, H-5, OCH_2CH_3), 8.20–8.60 (5 H, m, Ar), 9.33 (1 H, bs, NH indole, D_2O exchangeable); HRMS, m/z calcd for $C_{17}H_{22}N_2O_4$ 318.1558, found 318.1569.

Ethyl 4-(Acetoxyethyl)-β-carboline-3-carboxylate (15). A mixture of amine **13** (570 mg; 1.79 mmol) and paraformaldehyde (65 mg; 0.72 mmol) in toluene (75 mL) was heated under reflux under a nitrogen atmosphere for 3 h. The water formed was removed by means of a Dean–Stark apparatus. The reaction mixture was then filtered to remove excess solid paraformaldehyde, and the toluene was evaporated in vacuo. The resulting crude ethyl 4-(acetoxyethyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (**14**) (HRMS, m/z calcd for $C_{18}H_{22}N_2O_4$ 330.1593, found 330.1586) was dissolved in anhydrous xylene (150 mL), and 10% palladium on charcoal catalyst (300 mg) was added. The mixture was heated under reflux with vigorous stirring for 2.5 h until no tetrahydro-β-carboline **14** remained, as indicated by TLC using toluene–ethanol (9:2) as developer. The catalyst was removed by filtration over Celite and washed with warm xylene (500 mL). Evaporation of the filtrate gave an oily residue which was purified by chromatography on silica gel with chloroform–ethyl acetate (1:1) as developer, yielding 230 mg (39% in two steps) of crystalline β-carboline **15**: mp 190–191 °C; EIMS, m/z 326 (M^+); NMR ($CDCl_3$) δ 1.28 (3 H, t, J = 7.5 Hz, OCH_2CH_3), 1.73 (3 H, s, CH_3CO), 3.75 (2 H, t, J = 7 Hz, CH_2CH_2OAc), 4.40 (2 H, q, J = 7.5 Hz, OCH_2CH_3), 4.54 (2 H, t, J = 7 Hz, CH_2CH_2OAc), 7.33 (1 H, t, J = 7 Hz, Ar), 7.57 (2 H, m, Ar), 8.38 (1 H, d, J = 8 Hz, Ar), 8.90 (1 H, s, Ar), 10.28 (1 H, s, NH indole, D_2O exchangeable). Anal. ($C_{18}H_{18}N_2O_4$) C, H, N.

1*H*-Indolo[3′,2′:4,5]pyrido[3,2-*b*]-2-penten-5-olide (6). To a stirring solution of sodium (2.5 mg; 0.1 mmol) in ethanol (80 mL) was added the β-carboline **15** (320 mg; 0.98 mmol). After 23 h at room temperature, the white solid that precipitated was collected by filtration and recrystallized from dichloromethane–ethanol to give the desired lactone **6** (200 mg; 86%) as a white powder: mp 315–318 °C dec; EIMS, m/z 238 (M^+); NMR (Me_2SO-d_6) δ 3.82 (2 H, t, J = 6 Hz, CH_2CH_2O), 4.73 (2 H, t, J = 6 Hz, CH_2CH_2O), 7.43 (1 H, t, J = 8 Hz, Ar), 7.70 (1 H, t, J = 8 Hz, Ar), 7.78 (1 H, d, J = 8 Hz, Ar), 8.33 (1 H, d, J = 8 Hz, Ar), 9.03 (1 H, s, Ar), 12.28 (1 H, bs, NH indole, D_2O exchangeable). Anal. ($C_{14}H_{10}N_2O_2$) C, H, N.

Ethyl 4-(Cyanomethyl)-β-carboline-3-carboxylate (17). To a suspension of potassium cyanide (5 mg; 0.077 mmol) in an-

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hydrous dimethyl sulfoxide (2 mL) was added dropwise ethyl 9-acetyl-4-(bromomethyl)-β-carboline-3-carboxylate (**16**) (prepared as described in ref 13) (20 mg; 0.053 mmol) dissolved in 1 mL of DMSO. The stirring mixture was heated at 70 °C for 40 min and then allowed to stand at room temperature for 1.5 h until no starting material remained, as indicated by TLC. After evaporation of the solvent in vacuo, the residue was extracted with dichloromethane (5 mL), and the organic solution was washed with saturated aqueous sodium chloride, dried over sodium sulfate, and concentrated to leave a yellow solid (13 mg) which was recrystallized from dichloromethane–hexane to yield **17** (11 mg; 74%) as pale yellow needles: mp 183–187 °C dec; CIMS (C_4H_{10}), m/e 280 (M + 1, 100); NMR ($CDCl_3$) δ 1.47 (3 H, t, J = 7 Hz, OCH_2CH_3), 4.60 (2 H, q, J = 7 Hz, OCH_2CH_3), 4.88 (2 H, s, CH_2CN), 7.47 (1 H, m, Ar), 7.65 (2 H, d, J = 5 Hz, Ar), 8.35 (1 H, d, J = 8 Hz, Ar), 9.03 (1 H, s, Ar), 9.88 (1 H, bs, NH indole); HRMS, m/z calcd for $C_{16}H_{13}N_3O_2$ 279.0950, found 279.0979.

1*H*,5*H*-Indolo[3′,2′:4,5]pyrido[2,3-*c*]-6,7-dihydro-2-pyridone (7). A mixture of 4-(cyanomethyl)-β-carboline **17** (20 mg; 0.072 mmol), 10% ethanolic ammonia (10 mL), and 5% rhodium on alumina (10 mg) was hydrogenated on a Parr apparatus at 45 psi for 15 h. The catalyst was removed by filtration over a glass filter and washed with warm ethanol (100 mL), and the filtrate was evaporated to give 14 mg of crude product. This material was purified by high-performance liquid chromatography on a reversed-phase silica gel column using acetonitrile–water–triethylamine (28:72:0.1, flow rate = 3 mL/min) as developer, affording, with a retention time of 12 min, the desired lactam **7** (9 mg; 53%) as a white powder: mp >300 °C; FABMS (Gly, DMF^+), 238 (M + 1); NMR (Me_2SO-d_6) δ 3.67 (4 H, s, CH_2CH_2), 7.39 (1 H, t, J = 8 Hz, Ar), 7.66 (1 H, t, J = 8 Hz, Ar), 7.76 (1 H, t, J = 8 Hz, Ar), 7.99 (1 H, s, NH amide, D_2O exchangeable), 8.31 (1 H, d, J = 8 Hz, Ar), 8.94 (1 H, s, Ar), 12.07 (1 H, bs, NH indole, D_2O exchangeable); HRMS, m/z calcd for $C_{14}H_{11}N_3O$ 237.0902, found 237.0906.

***N*-Methyl-4-ethyl-β-carboline-3-carboxamide (9).** Into a tube cooled to −78 °C was introduced ethyl 4-ethyl-β-carboline-3-carboxylate (51 mg; 0.19 mmol) (prepared as describe in ref 13) and liquid methylamine (3 mL). The ampule was sealed and allowed to stand at room temperature for 1 week. The tube was then opened, and the mixture was poured into cold ethanol (10 mL). Evaporation of the solvents gave a white solid which was recrystallized from ethanol–hexane to afford the desired β-carbolinecarboxamide **9** (20 mg; 42%) as colorless needles: mp 216–217 °C; EIMS, m/z 253 (M^+); NMR (Me_2SO-d_6) δ 1.52 (3 H, t, J = 7 Hz, CH_3CH_2), 2.97 (3 H, d, J = 4 Hz, $NHCH_3$), 3.80 (2 H, q, J = 7 Hz, CH_3CH_2), 7.48 (1 H, t, J = 8 Hz, Ar), 7.80 (2 H, m, Ar), 8.42 (1 H, d, J = 8 Hz, Ar), 8.72 (1 H, d, J = 4 Hz, NH amide), 8.92 (1 H, s, Ar), 11.80 (1 H, bs, NH indole). Anal. ($C_{15}H_{15}N_3O \cdot {}^1/_4H_2O$) C, H, N.

Biological Methods. In Vitro Benzodiazepine Receptor Binding Assays. The technique described by Rehavi et al.[18] was used. Briefly, male Sprague-Dawley rats were decapitated, the brains were excised, and the cortex was dissected. Each cortex was homogenized in 5 mL of ice-cold Tris-HCl (50 mM, pH 7.4) (corresponding to 6 mL/g of tissue) with a Polytron. The homogenate was centrifuged a first time at 460g for 3 min, and the supernatant was recentrifuged at 22400g for 20 min. The resulting supernatant was discarded, and each pellet was resuspended in 3 mL of buffer and centrifuged again at 22400g for 20 min. The resulting pellet was again suspended in 3 mL of buffer, rehomogenized, divided into 1-mL fractions, and stored at −20 °C for at least 24 h before use. For the inhibition studies, the thawed membrane preparations were diluted with 20 volumes of ice-cold buffer, and 900-μL aliquots containing approximately 60 μg of protein, as determined by the Lowry method,[25] were incubated at 0 °C for 60 min with 50 μL of [^{3}H]flunitrazepam (65 Ci/mmol, CEA, final concentration 1 nM) and 50 μL of varying concentrations of the test compound ranging from 10^{-5} to 5×10^{-10} M (final concentrations for a total volume of 1 mL). Nonspecific binding was measured in the presence of 1 μM nonradioactive flunitrazepam and represented 10–15% of the total binding. Incubations were terminated by adding 3 mL of cold buffer to each incubation tube, filtering immediately through Whatman GF/B glass fiber filters, and washing each filter four times with 3 mL of ice-cold buffer. These operations were performed in less than 15 s per sample. Radioactivity retained on the filters was counted in 10 mL of Aquasol (NEN) scintillation solution with an LKB Wallac 1215 Rackbeta 2 counter. Each value was determined in triplicate. IC_{50} values (the concentration of ligand inhibiting 50% of flunitrazepam binding) were determined by Hofstee analysis. Results are given in Table II.

In Vivo Studies. Drugs. Pentylenetetrazole (PTZ) (M. Richard Laboratories, Sauzet, France) was dissolved in saline. Methyl β-carboline-3-carboxylate (β-CCM), synthesized by one of us (R.H.D.), was dissolved in 0.1 N HCl (1 mg in 100 μL) and diluted to volume with saline. [^{3}H]Ro 15-1788 (87 Ci/mmol) was from Du Pont NEN (Boston, MA). The test drugs were used as a suspension in Tween 80 and saline. Unless otherwise stated, all drugs were administered subcutaneously (sc) at a dose volume of 0.05 mL/10 g of body weight.

Convulsion Studies with Compound 6. The convulsant activity of compound **6** was studied by treating male Swiss mice (25 g) with 5 and 20 mg/kg, ip, of **6** (lots of five mice). The mice were observed for 30 min for convulsions, indications of myoclonic activity, tremors, or other behavioral anomalies. To test for anticonvulsant activity, mice (10 per experiment) were treated with compound **6** (5 and 10 mg/kg, ip) before administration of PTZ (70 mg/kg) or β-CCM (10 mg/kg), 10 and 5 min later, respectively. The same protocol using subconvulsive doses of PTZ (30 mg/kg) or β-CCM (5 mg/kg) was used to test for proconvulsant properties of **6** (10 mg/kg). For each series of experiments, parallel controls were run with animals injected with saline and Tween only.

In Vivo Inhibition of [^{3}H]Ro 15-1788 Binding. Inhibition of the in vivo binding of [^{3}H]Ro 15-1788 to mouse brain benzodiazepine receptors was performed as previously described.[22] Briefly, mice were treated with the test compound (**6** or **7**, 20 mg/kg, sc) 30 min before the iv administration of [^{3}H]Ro 15–1788 (50 μCi/kg). After 3 min, the mice were decapitated, and their brains were rapidly excised and dissected on ice. Cortex from one hemisphere, cerebellum, and hippocampus were homogenized in approximately 30 volumes (3 mL for cerebellum and cortex and 1.5 mL for hippocampus) of ice-cold Tris-HCl (50 mM, pH 7.4) using a Kinematica Polytron (type PT 10/35) for 5 s at half speed. Aliquots of the homogenates (600 μL) were immediately filtered through Whatman glass fiber filters (GF/B), and the filters were rinsed with two 5-mL portions of ice-cold Tris-HCl buffer. Membrane-bound radioactivity was retained on the filters and was counted by liquid scintillation in an LKB Rackbeta 2 counter after addition of 10 mL of aqueous counting scintillant (Amersham, Les Ulis, France). Total binding values were obtained from mice treated only with [^{3}H]Ro 15-1788. Nonspecific binding values were determined from mice injected with diazepam (10 mg/kg) 60 min before decapitation.

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