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Molecular Structure of 3-(Methoxycarbonyl) Amino-β-carboline, a Selective Antagonist of the Sedative Effects of Diazepam

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SUMMARY

The X-ray crystal structure of 3-(methoxycarbonyl) amino-β-carboline, a selective antagonist of the sedative effects of diazepam having a high affinity for the benzodiazepine receptor, has been determined. The results were compared with structural information obtrained from this compound, both in the solid state and in dilute solution, by use of Fourier transform infrared spectroscopy. Its X-ray structure was also compared with those of two other active β-carbolines, methyl β-carboline-3-carboxylate and *N*-ethyl-3-carbamoyl-β-carboline. The crystal packing characteristics of 3-(methoxycarbonyl) amino-β-carboline differ from those of these two β-carbolines in both the pattern of intermolecular hydrogen bonding and the quality of their π-π stacking interactions. The relevance this may have to the selective activity of 3-(methoxycarbonyl) amino-β-carboline is discussed.

The discovery of central receptors for 1,4-benzodiazepines (1, 2) which apparently mediate their anticonvulsant, anxiolytic, sedative, and muscle relaxant properties has greatly aided in the elucidation of the mechanism of action of these compounds. Briefly, the benzodiazepine receptor has been shown to be linked to the neuroinhibitory, postsynaptic GABA-A receptor, in turn coupled to a chloride channel. Binding of a benzodiazepine to its receptor would have the effect of increasing the efficacy of GABA such that the chloride channel is opened, resulting in inhibition of neuronal activity (3).

In vitro benzodiazepine receptor binding assays using radioactive ligands have permitted the discovery of compounds which, although apparently unrelated structurally to benzodiazepines, exhibit equivalent or superior binding affinities for the receptor. Among these are the derivatives of β-carboline-3-carboxylic acid (4–7). Interestingly enough, these β-carbolines often demonstrate *in vivo* pharmacological effects quite different from those of the benzodiazepines. For instance, β-CCM (Scheme 1, *1*) is highly convulsant in various species tested (8–12) as well as being anxiogenic in mice (13), whereas β-CCE (*2*) is proconvulsant in baboons (14) and rats (15). The term "inverse agonist" has been applied to these compounds having properties opposite to those of benzodiazepines (16). Conversely, β-CCP (*3*) antagonizes the convulsant effects of β-CCM as well as the anticonvulsant effects of diazepam and, thus, pharmacologically resembles the benzodiazepine antagonist, Ro 15-1788 (*4*) (11). Recently, it has been shown that suitable substituents on both the A and C rings of β-CCE provide compounds having benzodiazepine-like properties. Thus, ZK 91296 (*5*) and ZK 93423 (*6*) are, respectively, a partial benzodiazepine agonist (17) and a full benzodiazepine agonist (18, 19).

The inverse agonists also exert their properties via GABAergic mechanisms. In this case, it has been hypothesized that they provoke a change in the benzodiazepine receptor conformation different from that provoked by agonists such that the efficacy of GABA is *decreased* and the chloride ion flow is diminished (20). Following this reasoning, antagonists would by themselves produce no net change in the "resting" conformation of the benzodiazepine receptor and would thus neither increase nor decrease GABA transmission (16, 21). At the present time, however, the exact differences in the mode of binding of β-carbolines and benzodiazepines with the receptor which could give rise to the required conformational changes of the latter are less well understood.

We recently reported (22) the synthesis of β-CMC (*7*). This compound was shown to selectively antagonize the sedative effects of diazepam in mice while leaving the anxiolytic and anticonvulsant properties of the latter unaffected. β-CMC was also found to antagonize the convulsant properties of β-CCM. In order to explain this unique pharmacological profile of β-CMC, we decided to examine its X-ray structure for comparison with the known X-ray structures of other β-carbolines. The information so obtained was also compared to the structural data obtained for β-CMC, both solid and in solution, by means

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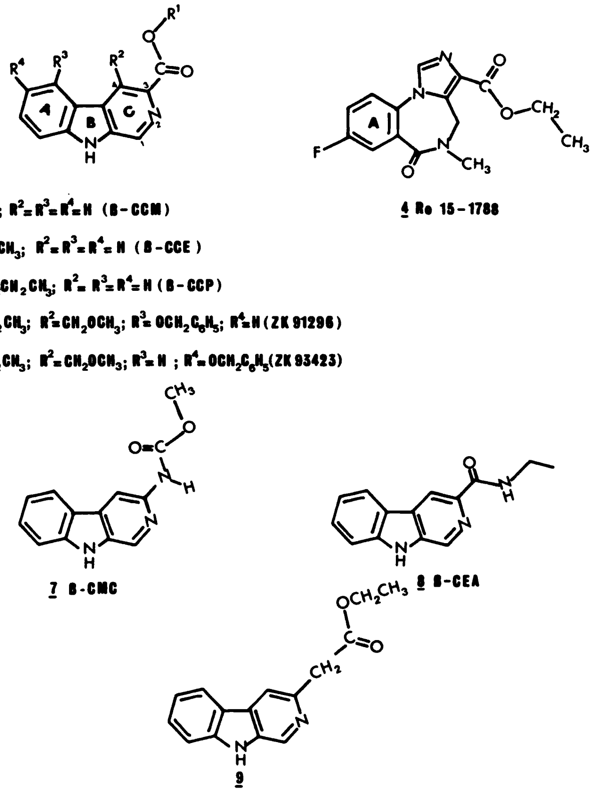
ABBREVIATIONS: GABA, γ-aminobutyric acid; β-CCM, methyl β-carboline-3-carboxylate; β-CCE, ethyl β-carboline-3-carboxylate; β-CCP, propyl β-carboline-3-carboxylate; β-CMC, 3-(methoxycarbonyl) amino-β-carboline; β-CEA, *N*-ethyl-3-carbamoyl-β-carboline; FTIR, Fourier transform infrared.



SCHEME 1

of FTIR spectroscopy. Herein, we report the results of this study. These results are discussed in the light of previously proposed benzodiazepine receptor binding models.

Materials and Methods

A sample of β-CMC was crystallized from toluene. Crystals are monoclinic. The space group is $P2_1/n$ with four molecules in the unit cell of dimensions: $a = 8.242(2)$, $b = 13.841(3)$, $c = 10.600(2)$ Å, $\beta = 102.27(2)°$, volume = 1182 Å^3, $\mu = 6.9$ cm^{-1}.

A total of 4010 intensity data was collected on a Philips PW 1100 diffractometer, using the monochromated Cu$_\alpha$K ($\lambda = 1.5418$ Å) and the $\theta - 2\theta$ scan method up to $\theta = 68°$. From 2008 unique reflections, 1709 with $I > 3\sigma(I)$ were considered as observed and kept in the refinement calculations; absorption was ignored. The final R and ωR values were, respectively, 0.045 and 0.052.

The structure was solved by direct methods with the program DEVIN (23) and refined by a least squares method with the program SHELX 76 (24), minimizing the function $\Sigma\omega(F_o - |F_c|)^2$, the weighting scheme being $\omega = 1/\sigma^2(F_o) + 0.0028(F_o)^2$ at the final stage. Nonhydrogen atoms were assigned anisotropic thermal parameters.[1] All hydrogen atoms were located on a difference map and their coordinates refined with an isotropic temperature factor equivalent to that of the bonded atom.

Atomic parameters are given in Table 1.

The infrared spectra were determined on a Brüker IFS 85 Fourier transform spectrometer equipped with an Aspect 2000 data station. Spectra of solid samples were obtained in KBr pellets and recorded at a resolution of 0.5 cm^{-1} with a mirror velocity of 0.198 cm/sec. Spectra of solution samples of β-CMC were measured in a variable path cell (1–6 mm) with KBr windows in dideuteriodichloromethane. A resolution of 2 cm^{-1} was obtained with a mirror velocity of 0.198 cm/sec. The concentration of β-CMC was less than 5.10^{-3} M. All spectra were recorded at room temperature.

TABLE 1

The fractional atomic coordinates (× 10^4) for the carbon, nitrogen, oxygen, and (× 10^3) hydrogen atoms of β-CMC

	X		Y		Z	
C1	585	(2)[a]	2008	(1)	−1027	(2)
N2	795	(1)	1158	(1)	−418	(1)
C3	2341	(2)	881	(1)	160	(1)
C4	3752	(2)	1432	(1)	173	(1)
C5	6337	(2)	3243	(1)	−295	(2)
C6	6979	(2)	4086	(1)	−682	(2)
C7	5945	(3)	4777	(1)	−1413	(2)
C8	4270	(3)	4651	(1)	−1776	(2)
N9	1983	(2)	3497	(1)	−1627	(1)
C10	1922	(2)	2607	(1)	−1059	(2)
C11	3527	(2)	2324	(1)	−442	(1)
C12	4625	(2)	3094	(1)	−649	(1)
C13	3607	(2)	3797	(1)	−1385	(2)
N14	2386	(2)	−46	(1)	707	(1)
C15	3596	(2)	−395	(1)	1658	(1)
O16	4860	(1)	8	(1)	2180	(1)
O17	3211	(1)	−1294	(1)	1966	(1)
C18	4319	(3)	−1734	(2)	3045	(2)
H1	−65		219		−144	
H4	482		122		55	
H5	712		274		25	
H6	814		419		−44	
H7	633		541		−165	
H8	355		514		−224	
H9	103		381		−203	
H14	141		−36		53	
H18A	547		−170		295	
H18B	398		−244		308	
H18C	434		−136		382	

[a] Values in parentheses, estimated standard deviations.

Results

The molecular structure of β-CMC (*7*) is shown in perspective in Fig. 1. The three fused rings appear strictly coplanar, with no deviation greater than 0.010 Å. The same planarity has been observed in other β-carboline structures studied (25–28). The methyl carbamate side-chain at C(3) is slightly twisted from the mean plane of the ring system, with torsion angles N(2)-C(3)-N(14)-C(15) of −158.2° and C(4)-C(3)-N(14)-C(15) of 24.3°. The result is that, for the enantiomer shown in Fig. 1, N(14) and H(14) are below the plane of the ring (deviations of −0.071 Å and −0.183 Å, respectively), whereas the carbonyl group points above this plane (deviations of +0.310 [C(15)] and +0.732 Å [0(16)]). The methoxy group lies slightly below the carbonyl group (deviations of +0.147 [0(17)] and +0.618 Å [C(18)]) but almost parallel to it [torsion angle 0(16)-C(15)-0(17)-C(18) = 5.4°]. This deviation of the C(3) side-chain of β-CMC away from the plane defined by the β-carboline ring structure is considerably more pronounced than in β-CCM, in which the N(2)-C(3)-C = 0 torsion angles are, according to Bertolasi *et al.* (27), 6.2° and −3.8° in the two independent molecules in the asymmetric unit. Approximately the same values (−5.3° and 3.2°) were also found by Muir and Codding (28) for β-CCM.

The carbonyl group being almost coplanar with the ring, intramolecular π-π interactions are maximized in β-CCM. The same is true for the C(3) side-chain of β-CEA (*8*) [torsion angle N(2)-C(3)-C = 0 = 179.9°] (26) although, in this case, the carbonyl group is *trans* to the pyridinyl nitrogen atom, whereas

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[1] The observed and calculated structure factors, bond distances, torsion angles, and anisotropic thermal parameters for compound 7 may be obtained from the authors upon request.

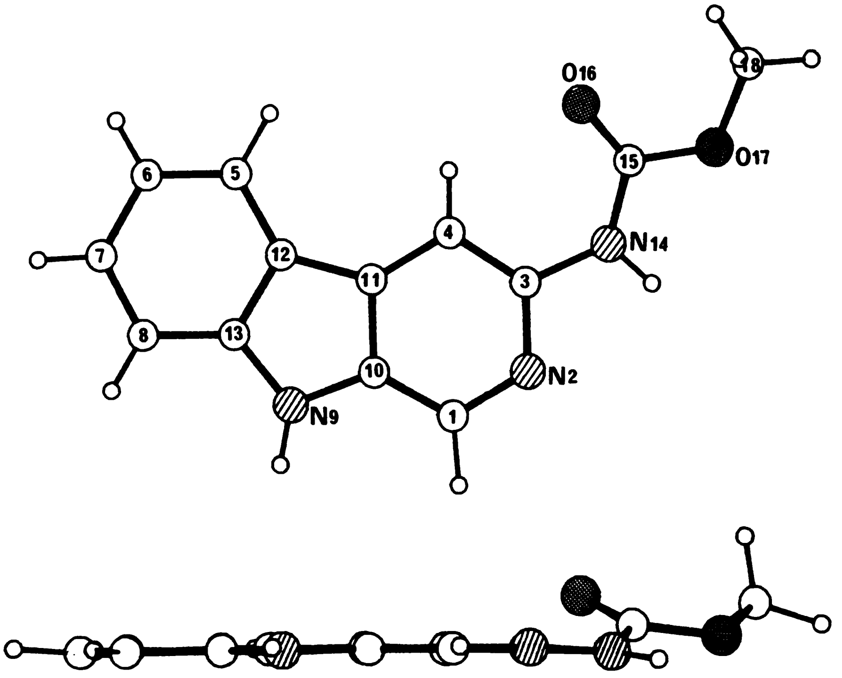

Fig. 1. Two orthogonal perspective views of β-CMC (7).

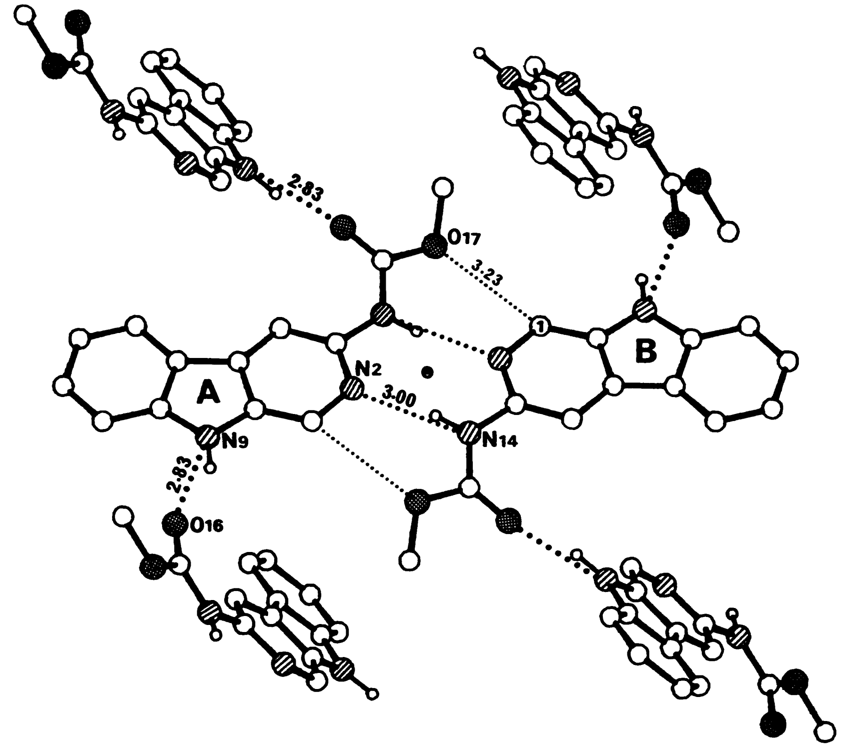

Fig. 2. Packing of β-CMC, showing the dimer. Only nitrogen-bonded hydrogen atoms are shown.

in β-CCM it is *cis*. Overall, then, the side-chain of β-CMC adopts an extended conformation with H(14) *cis* with respect to N(2), the oxygen atom of the carbonyl group [O(16)] being forced into a position *trans* to N(2).

The packing characteristics of β-CMC are shown in Fig. 2. In the crystalline state, β-CMC forms coplanar dimers, held together by two symmetrically related hydrogen-bonding interactions formed by N(2) and H(14) [N(2) . . . H—N(14) angle = 171°; N(2) . . . N(14) = 3.00 Å; N(2) . . . H(14) = 2.11 Å). Each molecule of the dimer is also hydrogen-bonded to two other molecules via its carbonyl oxygen atom O(16) (bonded to the indolic amine of a neighboring molecule) and its indolic amine hydrogen H(9) (bonded to the carbonyl oxygen of another) [N(9)—H(9) . . . 0(16) angle = 151°; N(9) . . . O(16) = 2.83 Å; H(9) . . . 0(16) = 1.99 Å).

In addition, the short H(1) . . . 0(17) distance (2.41 Å, C(1)—H(1) . . . 0(17) angle = 134°) can be considered attractive according to Taylor and Kennard (29). Drawing on a survey of a large number of X-ray structures, these authors speculated that short C—H . . . 0 bonds, particularly favored by adjacent nitrogen atoms (as in the β-carbolines), were indicative of hydrogen bonding. The H(1) . . . O(17) distance and angle in β-CMC fall just within the observed limits of attractive interactions. However, these short C—H . . . O bonds may be simply due to efficient crystal packing rather than to actual hydrogen bonding.

Thus, the crystalline environment of each molecule of β-CMC is such that it is involved in four different attractive interactions: two are used to maintain the coplanar, dimeric structure [H(A14) . . . N(B2), N(A2) . . . H(B14)], whereas the other two hydrogen bonds [0(16) . . . H(9), H(9) . . . O(16)] lead to the formation of a repeating chevron pattern with two adjacent molecules, each of the latter lying cross-wise from the "dimeric" molecules with a dihedral angle of 53°. No π-π stacking interactions are evident in β-CMC, in contrast to the crystal structure of β-CEA, in which the carboxamide π-system was found to be stacked over the π-system of the aromatic A-ring of the β-carboline (26).

The frequencies of absorption of the carbonyl and NH groups of β-CMC (solid and in solution) in the infrared are given in Table 2. In the solid phase, β-CMC shows two NH bands at 3388 and 3196 cm^{-1}, the one at higher frequency being attributed, on the basis of literature data (30), to hydrogen-bonded indolic NH, whereas the lower frequency band is assigned to the carbamate NH. The extremely low frequency of absorption of the latter is indicative of its involvement in strong hydrogen-bonding interactions: a value of 3295 cm^{-1} is found in the literature as the lowest limit of absorption of hydrogen-bonded carbamate-type NH (30, 31). This absorption of the carbamate NH can be associated with an N—H . . . N hydrogen bond, according to Sutherland (32). This, of course, is amply demonstrated by the X-ray crystal structure above. Moreover, the presence of an important amine-carbonyl hydrogen bond is indicated by a strong, wide absorption in the 2960- to 1950-cm^{-1} region (31).

The multiple absorptions of the carbonyl group of β-CMC in the solid state are also typical of hydrogen-bonding interactions for this group (33). Again, the low frequency carbonyl band at 1685 cm^{-1} observed for β-CMC is lower than that generally reported as the lowest limit of hydrogen-bonded carbonyl absorptions of the carbamate type in the solid phase (1692 cm^{-1}). This reflects the involvement of the carbonyl in a strong hydrogen bond.

The solution concentrations of β-CMC in dideuteriodichloromethane are sufficiently dilute (5.10^{-3} M) that inter-solute hydrogen-bonding no longer intervenes. Thus, both the indolic and carbamate NH absorptions (3459 and 3426 cm^{-1}, respectively) are found at frequencies considerably higher than those of their hydrogen-bonded counterparts in the solid state. Both values are well within the literature limits for these types of bonds (33, 34). More informative are the two carbonyl bonds at 1732 and 1717 cm^{-1} observed in the dilute solution FTIR spectrum of β-CMC. According to Oki and Nakanishi (35) these two absorptions correspond to the presence of both *cis* and *trans* conformers of the carbamate side-chain of β-CMC (Fig. 3). These authors were also able to demonstrate, using a variety of techniques that: 1) the absorption at higher wave number corresponds to the *cis* rotamer about the C—O bond (Fig. 3b) of the carbamate, whereas the *trans* rotamer (Fig. 3a) gives rise to the lower band; and 2) the *cis* conformer is the more favored one. Thus, at least in dilute dideuteriodichloromethane solution, β-CMC apparently also favors this *cis* conformation, the relative intensity of the higher frequency band being twice that of the lower frequency (*trans*) band. This, of course, is the reverse of the situation observed in the X-ray structure of crystalline β-CMC, where the carbamate side-chain displays exclusively the less stable *trans* conformation.

TABLE 2

Carbonyl and amine stretching frequencies of β-CMC in the solid state and in solution as determined by FTIR spectroscopy

	ν C═O[a]	ν N—H
Solid[b]	1755 (0.6)[c]	3388 (indole)
	1731 (1.0)	3196 (carbamate)
	1685 (0.5)	2960-1950 (N—H . . . O═C)
Solution[d]	1732 (1.0)	3459 (indole)
	1717 (0.5)	3426 (carbamate)

[a] Frequency of vibration in cm^{-1}.
[b] KBr pellet.
[c] Relative intensity.
[d] In dideuteriodichloromethane, $c = 5.10^{-3}$ M.

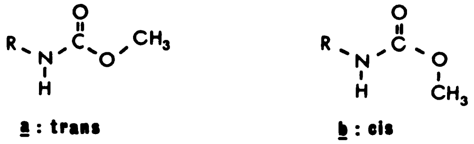

Fig. 3. The two possible planar conformations of β-CMC with respect to the carbonyl-oxygen bond (*R* = 3-β-carbolino). The *trans* conformer (a) is that found in the crystalline state while the *cis* conformer (b) predominates in dilute solution. With respect to the carbonyl-nitrogen bond, only the most stable *trans* conformer is shown.

Unfortunately, the limited solubility of β-CMC in nonpolar organic solvents did not permit the measurement of infrared spectra at higher concentrations, so that intermolecular hydrogen-bonding effects in solution could not be observed. Measurement of the FTIR spectrum of β-CMC in aqueous media, under conditions closer to those found physiologically, were also precluded by its insolubility.

Discussion

The *in vitro* benzodiazepine receptor affinities, as reflected by their IC_{50} values,[2] of the three active β-carbolines whose X-ray structures have so far been determined decrease in the order β-CCM > β-CMC > β-CEA (1.1, 71, and 246 nM, respectively) (4, 6, 22). Structure-activity studies of the β-carbolines have generally shown that a carbonyl group attached to C(3) of the β-carboline skeleton is necessary to obtain optimum binding affinity for the receptor (4). It is thus surprising that β-CMC, whose carbonyl group is no longer directly attached to the aromatic ring, should have a higher affinity than the ethylamide derivative β-CEA,[3] especially since the side-chains of these two molecules are, in fact, comparable in length, both being composed of a linear chain of five atoms terminating in a hydrophobic methyl group.

Probably the most important structural feature of β-CMC with regard to its pharmacological activity is the presence of the amine group at C-3. The C(3)-N(14) distance (1.40 Å) in β-CMC is indicative of a delocalization of the non-bonded electrons of the nitrogen atom into the π-system of the aromatic

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[2] The literature values for the IC_{50} values of these three compounds, quoted from several sources, were verified in our own laboratory. Thus, following the procedure of Ref. 22, the concentrations of compound required to inhibit 50% of [^{3}H]flunitrazepam binding (IC_{50} value) to *in vitro* preparations of rat cerebral cortex membranes after 60 min incubation at 0° in Tris-buffered medium (pH 7.4) were found to be: β-CCM, 2.2 nM; β-CMC, 71 nM; β-CEA, 280 nM.

[3] It should be noted that, *in vivo*, β-CMC also demonstrates a higher affinity than the β-carboline esters for the cerebral benzodiazepine receptors of mice (36), probably indicating more favorable pharmacokinetic factors in the case of β-CMC.

rings (37, 38). This situation is quite distinct from that in β-CCM and β-CEA, in which the carbonyl group tends to drain electrons away from the adjacent aromatic ring. The result in the case of β-CMC should be an increase in the electron density of the pyridine nitrogen atom with respect to that observed in the other two β-carbolines, with a concomitantly higher potential for this atom [N(2)] to enter into bonding interactions with cationic centers on the receptor. This energy gain in β-CMC may compensate some loss of affinity as a result of the displacement of the carbonyl group away from the aromatic ring.

Alternatively, N(14)—H(14) may interact directly with a binding site on the receptor, in particular, with an anionic residue. This would be fundamentally different from the situation in which a carbonyl is present at this position (i.e., β-CCM and β-CEA), this group being expected to interact with a cationic site on the receptor (28, 39).[4] The importance of N(14)—H(14) in receptor binding is also suggested by the packing characteristics of β-CMC (see below) in which these atoms are observed to participate in a strong hydrogen bond with N(2).

The importance of N(14) in binding interactions of β-CMC with the receptor (either by resonance interaction with the aromatic ring or by direct interaction with an anionic site on the receptor) is further demonstrated by the fact that, in compound *9*, in which this atom is replaced by a carbon atom incapable of such donation, a much lower affinity is observed for the benzodiazepine receptor (IC_{50} = 1430 nM) (40). Thus, N(14) does not serve merely as a spacer, moving the carbonyl group away from the heterocycle, but must actively participate in receptor binding.

A notable difference between the three-dimensional crystal structures of β-CCM and β-CMC lies in the relative positions of their carbonyl group oxygens with respect to N(2), these being *cis* in the first case (27, 28) and *trans* in the second. The carbonyl group oxygen of β-CEA, however, is *trans* to N(2), as in β-CMC (26).

The FTIR spectra of β-CMC in dilute solution also yield some information regarding the conformation of the side-chain. For secondary amide-type carbamates such as β-CMC, four different planar conformations are possible: two in which the substituents are *trans* or *cis* about the oxygen-carbonyl bond (Fig. 3), each of which can be *cis* or *trans* about the nitrogen-carbonyl bond. For steric reasons, and as has been shown spectroscopically (35), only the conformers that are *trans* with respect to the nitrogen-carbonyl bond (as depicted in Fig. 3) are observed in solution. This is also the geometry exhibited by β-CMC in the crystalline state, as the X-ray structure indicates. With regard to the geometry about the bond joining O(17) and the carbonyl group, FTIR spectroscopy of β-CMC indicates (as explained above) a favored *cis* position in dilute solution (Fig. 3b) in contrast to the exclusively *trans* relationship found in the crystalline state.

We cannot, of course, be certain whether the conformational preferences of the β-carboline side-chains expressed in their crystalline state or in solution are the same conformations recognized by the receptor. Loew *et al.* (39) have shown that, in β-CCM, for instance, the energy differences between the various conformational possibilities of the methoxycarbonyl moiety were very small. Nevertheless, the most energetically favorable conformer was, in fact, subsequently that shown to exist in the crystalline state [i.e., with the carbonyl oxygen and N(2) coplanar and *cis*]. This was also the most favorable geometry of the side-chain of β-CCE found by Crippen's distance geometry analysis of various benzodiazepine receptor ligands (41). Although the benzodiazepine receptor no doubt recognizes a single conformer of the β-carboline side-chains, no conclusions can as yet be drawn regarding eventual relationships to the pharmacological activity of these compounds until conformationally restrained analogues are synthesized and tested.

Since in none of the β-carbolines studied by X-ray crystallography is there any evidence of intramolecular hydrogen bonding, it can be reasonably assumed that the intermolecular hydrogen-bonding patterns observed in the crystalline state, as shown by the packing characteristics, are a good reflection of the potential interactions between the molecule and its receptor (42, 43). In this respect, however, β-CMC can be significantly distinguished from β-CCM and β-CEA both in the number and the quality of these potential hydrogen bonds with the receptor.

All three β-carbolines possess a strong hydrogen bond between the indolic hydrogen [H(9)] and the oxygen atom of the carbonyl group of a neighboring molecule, the bond lengths and angles being remarkably similar for the three compounds despite their quite different packing arrangements [H(9) . . . O: β-CCM, 2.06 Å; β-CEA, 1.96 Å; β-CMC, 1.99 Å; N(9)—H(9) . . . O angle: β-CCM, 140°; β-CEA, 154°; β-CMC, 151°). This bond is in fact weakest in β-CCM, wherein the same H(9) has been shown (27, 28) to simultaneously take part in a hydrogen bond with the pyridine atom N(2). No such three-centered interactions are observed in β-CMC; the pyridine nitrogen atom N(2) and H(14) of the exocyclic amino group of one molecule form two distinct hydrogen bonds with, respectively, the corresponding H(14) and N(2) of a second molecule. The two molecules of the "dimer" so formed are thus tightly bound by the formation of this pseudo-six-membered ring (Fig. 2). The strength of this interaction is also indicated in the FTIR spectrum of solid β-CMC, in which the absorption of its side-chain NH at 3196 cm^{-1} is 100 cm^{-1} lower than that generally observed in other hydrogen-bonded carbamates (30, 31).

In contrast to β-CCM and β-CMC, the packing characteristics of β-CEA show no involvement of N(2) in any hydrogen bond (26). In view of the acknowledged importance of this atom in assuring a high affinity of the β-carboline compounds for the benzodiazepine receptor [analogous carbazole derivatives have practically no affinity for the receptor (44)], this implies a certain limitation in extrapolating crystal data to potential ligand-receptor interactions.

If, as others have suggested (29), the short O(17)—H(1) distances in crystalline β-CMC correspond to weak hydrogen bonding between these atoms, these interactions may play a role in stabilizing the geometry of the dimers. These attractive interactions could thus be responsible for forcing the side-chain of β-CMC into its less stable *trans* conformation about the oxygen-carbonyl bond (Fig. 3a). In terms of receptor-ligand interactions, this could indicate the binding of C(1) to an anionic site on the receptor.

The crystal packing characteristics of β-CMC also differ from those of β-CCM and β-CEA in lacking both intramolecular and intermolecular π-π interactions. The carbonyl group of β-CMC,

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[4] P. Clapier, R. H. Dodd, C. Minot, C. Ouannès, P. Potier, and E. Tran Huu Dau, submitted for publication.

being displaced from the aromatic ring system by the interposition of an amine, is less constrained to planarity with this ring system and, in fact, the carbonyl group lies at 24.3° to the plane of the ring. In both β-CCM and β-CEA, the proximity of the carbonyl group to the aromatic ring makes coplanarity of these two moieties energetically advantageous. Intermolecular π-π interactions are particularly important in β-CEA, wherein the C(3) side-chain containing the carbonyl group is stacked above the A-ring of a second molecule (26). No analogous interactions were observed in β-CCM, although the packing data presented for this compound (28) implied that the molecules tend to lie in planes such that stacking interactions of the π-π type between the "layers" are possible. However, in β-CMC, neither of these possible stacking interactions is evident in the crystalline state; molecules surrounding each dimeric species lie across the plane defined by the dimer rather than *in* this plane, the geometry being stabilized by the O(16) . . . H(9) hydrogen bond. Although this does not imply, of course, that π-π interactions between the planar aromatic rings of β-CMC and aromatic or carbonyl groups of the benzodiazepine receptor are absent, it does suggest that these interactions may be different from those of β-CCM and β-CEA. In particular, if it is assumed that the carbonyl groups of all three β-carbolines interact with the same site on the benzodiazepine receptor, then it is likely that the planar aromatic rings of β-CMC, being farther removed from this carbonyl group compared to β-CCM and β-CEA, could interact with different π-donating residues of the receptor.

This would be in line with Codding and Muir's recent model (44) of agonist-antagonist binding to the benzodiazepine receptor. In this model, the carbonyl groups of β-CCM, Ro 15-1788 (*4*) (exocyclic carbonyl), and CGS-8216 (a receptor antagonist) were considered as a common structural feature of these compounds. The distance of this moiety from their respective aromatic A-rings being in all three cases similar, the shared, antagonist actions of these compounds were correlated to this distance. Shorter distances, as in the benzodiazepines, would give rise to agonist activity by virtue of the different conformation imposed on the receptor. According to this model, then, β-CMC, whose important carbonyl group is even farther removed from its phenyl ring, should evidently provoke still different conformational changes in the receptor. This difference between β-CMC and the other antagonists is apparently translated into a selectivity of antagonist action rather than a simple decrease in affinity.

However, this model, although plausible, does not distinguish between antagonists and inverse agonists and fails in the case of the agonist β-carboline-3-carboxylates ZK 91296 (*5*) and ZK 93423 (*6*). The assumption that benzodiazepines and β-carbolines occupy precisely the same sites on the receptor may not be valid. Other experimental evidence also points to this conclusion (45, 46).

Although the crystalline structures of β-CMC and other β-carbolines may help to explain their relative affinities for the benzodiazepine receptor *in vitro*, extension of these data to prediction of pharmacological activity of a molecule *in vivo* seems at present hazardous.[5] X-ray crystal structures of other pharmacologically well described benzodiazepine receptor ligands will perhaps permit more precise correlations between structure and activity.

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[5] Whereas β-CCM is regarded as a pure inverse agonist, the pharmacological activity of β-CEA, other than *in vitro* binding data, has not been reported. However, the methyl amide analogue of β-CEA (known as FG 7142), whose packing characteristics in the crystalline state could be expected to be unchanged, is both proconvulsant (47) and anxiogenic (48) with an *in vitro* IC_{50} of 657 nM (6).

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