performed in duplicate. The results generally differed from each other by less than 5%. Nonspecific binding was determined by incubating samples with 100 and 300 nM nonradioactive (−)-alprenolol at 40 and 37 °C, respectively, and was subtracted from the total amount of [^{3}H]dihydroalpronolol bound in order to obtain the amounts of [^{3}H]dihydroalprenolol specifically bound. Radioactivity associated with cells was separated from unbound [^{3}H]dihydroalprenolol by filtering and washing of samples over glass-fiber filters with ice-cold buffer. Radioactivity bound to the filters was counted in PCS scintillation fluid (Amersham Searle) in an ambient temperature scintillation counter at 50% efficiency.

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Registry No. (±)-**2**, 138-65-8; (±)-(*R**,*R**)-**6**, 84417-30-1; (±)-(*R**,*R**)-**6**, 84418-14-4; (±)-(*R**,*R**)-**6**·H_3PO_4, 84417-61-8; (±)-(*R**,*S**)-**6**·H_3PO_4, 84418-29-1; (±)-(*R**,*R**)-**7**, 84417-31-2; (±)-(*R**,*S**)-**7**, 84418-15-5; (±)-(*R**,*R**)-**7**·H_3PO_4, 84417-62-9; (±)-(*R**,*S**)-**7**·H_3PO_4, 84418-30-4; (±)-(*R**,*S**)-**8**, 84417-32-3; (±)-(*R**,*S**)-**8**, 84417-86-7; (±)-(*R**,*R**)-**8**·H_3PO_4, 84417-63-0; (±)-(*R**,*S**)-**8**·H_3PO_4, 84417-87-8; (±)-(*R**,*R**)-**9**, 84417-33-4; (±)-(*R**,*S**)-**9**, 84417-88-9; (±)-(*R**,*R**)-**9**·H_3PO_4, 84417-64-1; (±)-(*R**,*S**)-**9**·H_3PO_4, 84417-89-0; **10**, 84417-34-5; **11**, 84417-35-6; **12**, 83086-06-0; **13**, 84417-36-7; **14**, 84417-37-8; **15**, 84417-38-9; **16**, 84417-39-0; **17**, 84417-40-3; **18**, 84417-41-4; **19**, 84417-42-5; **20**, 84417-43-6; (±)-(*R**,*R**)-**21**·HCl, 84417-44-7; (±)-(*R**,*S**)-**21**·HCl, 84418-16-6; (±-)(*R**,*R**)-**21**·H_3PO_4, 84417-66-3; (±)-(*R**,*S**)-**21**·H_3PO_4, 84417-91-4; (±)-(*R**,*R**)-**22**·HCl, 84417-45-8; (±)-(*R**,*S**)-**22**·HCl, 84418-17-7; (±)-(*R**,*R**)-**22**·H_3PO_4, 84417-68-5; (±)-(*R**,*S**)-**22**·H_3PO_4, 84417-93-6; (±)-(*R**,*R**)-**23**·HCl, 84417-46-9; (±)-(*R**,*S**)-**23**·HCl, 84418-18-8; (±)-(*R**,*R**)-**23**·HOAc, 84417-57-2; (±)-(*R**,*R**)-**23**·H_3PO_4, 84417-69-6; (±)-(*R**,*S**)-**23**·H_3PO_4, 84417-95-8; (±)-(*R**,*S**)-**23**·HOAc, 84418-28-0; (±)-(*R**,*R**)-**24**·HCl, 84417-47-0; (±)-(*R**,*S**)-**24**·HCl, 84418-19-9; (±)-(*R**,*R**)-**24**·H_3PO_4, 84417-71-0; (±)-(*R**,*S**)-**24**·H_3PO_4, 84417-97-0; (±)-(*R**,*R**)-**25**·HCl, 84417-48-1; (±)-(*R**,*S**)-**25**·HCl, 84418-20-2; (±)-(*R**,*R**)-**25**·H_3PO_4, 84432-82-6; (±)-(*R**,*S**)-**25**·H_3PO_4, 84417-99-2; (±)-(*R**,*R**)-**26**·HCl, 84417-49-2; (±)-(*R**,*S**)-**26**·HCl, 84418-21-3; (±)-(*R**,*R**)-**26**·H_3PO_4, 84417-73-2; (±)-(*R**,*S**)-**26**·H_3PO_4, 84418-01-9; (±)-(*R**,*R**)-**27**·HCl, 84417-50-5; (±)-(*R**,*S**)-**27**·HCl, 84418-22-4; (±)-(*R**,*R**)-**27**·H_3PO_4, 84417-75-4; (±)-(*R**,*S**)-**27**·H_3PO_4, 84418-03-1; (±)-(*R**,*R**)-**28**·HCl, 84417-51-6; (±)-(*R**,*S**)-**28**·HCl, 84418-23-5; (±)-(*R**,*R**)-**28**·H_3PO_4, 84417-77-6; (±)-(*R**,*S**)-**28**·H_3PO_4, 84418-05-3; (±)-(*R**,*R**)-**29**·HCl, 84417-52-7; (±)-(*R**,*S**)-**29**·HCl, 84418-24-6; (±)-(*R**,*R**)-**29**·H_3PO_4, 84417-79-8; (±)-(*R**,*S**)-**29**·H_3PO_4, 84418-07-5; (±)-(*R**,*R**)-**30**·HCl, 84417-53-8; (±)-(*R**,*S**)-**30**·HCl, 84418-25-7; (±)-(*R**,*R**)-**30**·H_3PO_4, 84417-81-2; (±)-(*R**,*S**)-**30**·H_3PO_4, 84418-09-7; (±)-(R*,R*)-**31**·HCl, 84417-54-9; (±)-(*R**,*S**)-**31**·HCl, 84418-26-8; (±)-(*R**,*R**)-**31**·H_3PO_4, 84417-83-4; (±)-(*R**,*S**)-**31**·H_3PO_4, 84418-11-1; (±)-(*R**,*R**)-**32**·HCl, 84417-55-0; (±)-(*R**,*S**)-**32**·HCl, 84418-27-9; (±)-(*R**,*R**)-**32**·H_3PO_4, 84417-85-6; (±)-(*R**,*S**)-**32**·H_3PO_4, 84418-13-3; (±)-**33**, 84417-58-3; **34**, 84417-59-4; **35**, 84417-60-7; levulinic acid, 123-76-2; 4-acetylbutyric acid, 3128-06-1; 6-oxoheptanoic acid, 3128-07-2; 2-methylcyclohexanol, 583-59-5; 7-oxooctanoic acid, 14112-98-2; 2-acetylcyclohexanone, 874-23-7; *p*-toluidine, 106-49-0; *p*-butylaniline, 104-13-2; *N*-methyl-*p*-toluidine, 623-08-5; *o*-trifluoromethylaniline, 88-17-5; *m*-trifluoromethylaniline, 98-16-8; *p*-trifluoromethylaniline, 455-14-1; *p*-methoxyaniline, 104-94-9; butylamine, 109-73-9; cyclohexylamine, 108-91-8; dopamine, 51-61-6.

β-Carbolines as Benzodiazepine Receptor Ligands. 1. Synthesis and Benzodiazepine Receptor Interaction of Esters of β-Carboline-3-carboxylic Acid

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Several esters of β-carboline-3-carboxylic acid were synthesized and tested in respect to their affinity for the benzodiazepine receptor in bovine cortex membranes. Out of these derivatives, the methyl, ethyl, and *n*-propyl ester were clearly the most potent, while the *n*-butyl, benzyl, and 3-pyridylmethyl ester were considerably less active. Moreover, several β-carboline-3-carboxylates with ethanol derivatives as ester alcohol components were all less active than the ethyl or *n*-propyl ester themselves. It is concluded that the affinity of β-carboline-3-carboxylates to the benzodiazepine receptor is profoundly dependent on molecular size, as well as hydrophobic and electronic parameters of the ester alcohol component.

Today, a variety of evidence suggest that the pharmacological effects of the benzodiazepines are mediated through CNS specific binding sites.[1-4] These "benzodiazepine receptors" exhibit a very high specificity for pharmacologically and clinically active benzodiazepines, and only a small number of compounds chemically different from the benzodiazepines bind to these receptors with high affinity.[1-4] A case in point is the group of β-carbolines, where some derivatives have been related to the endogenous ligand of the benzodiazepine receptor.[5-9] Furthermore, quite remarkable benzodiazepine receptor affinities have been reported for some β-carboline-3-carboxylates (low nanomolar range) that are about three orders of magnitude higher than that of the parent compound β-carboline-3-carboxylic acid,[9] making these β-

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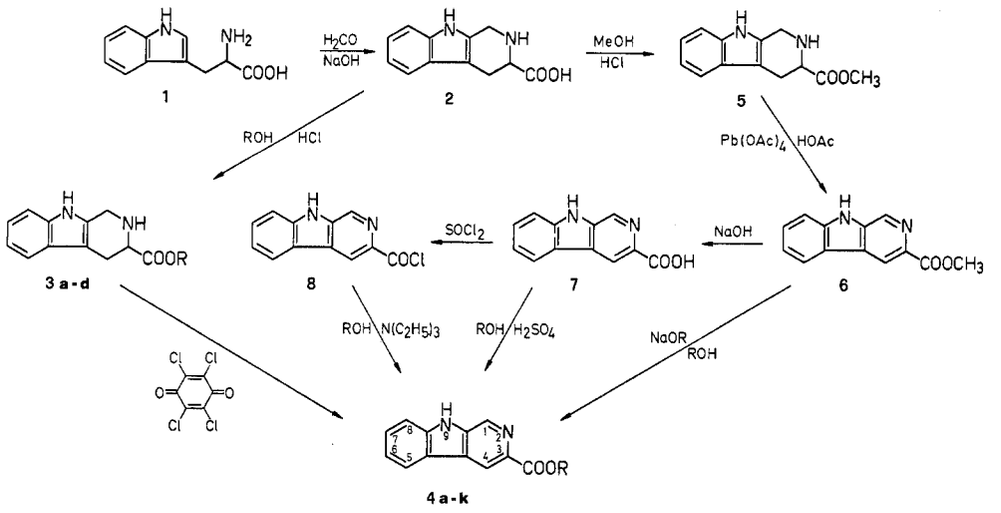
Scheme I



carboline derivatives important tools to study benzodiazepine receptor function.[10-13] Moreover, the β-carboline structure might become an important basis for the design of new benzodiazepine-related drugs.[14]

This article describes a relatively simple approach to synthesize β-carboline-3-carboxylates with usually high yield, combining known with new steps for the final synthetic sequence. Furthermore, the new derivatives were tested in respect for their benzodiazepine receptor affinity in order to acquire more information regarding the structural requirements of β-carboline-3-carboxylates as ligands of the benzodiazepine receptor.

Chemistry. The preparation of the esters of β-carboline-3-carboxylic acid, compounds **4a–k**, was carried out as outlined in Scheme I. To begin with, the cyclization of tryptophan (1) in alkaline solution[15] was so optimized that 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (2) was obtainable in 97% yield. The cyclization in acid solution[16,17] gives significantly lower yields. The key step in the synthetic sequence was the dehydrogenation of the esters of 2, compounds **5** and **3a–d**. The dehydrogenation with sulfur in refluxing xylene or with chloranil in refluxing *s*-tetrachloroethane[14,17] gave poor yields. By far the best method proved to be the dehydrogenation with lead tetraacetate in glacial acetic acid. According to this method, methyl β-carboline-3-carboxylate (**6**) was obtained in good yield, especially since **6** can be easily separated as the hydrogen oxalate from the reaction mixture. The esters **4** can be obtained either directly from **6** by base-catalyzed transesterification or indirectly through esterification of β-carboline-3-carboxylic acid (**7**) or its acid chloride (**8**).

Binding to the Benzodiazepine Receptor. The data reported confirm and considerably extend previous observations,[8,9] indicating that the ester formation of β-carboline-3-carboxylic acid (**7**) with methanol, ethanol, or 1-propanol leads to derivatives whose affinities for the benzodiazepine receptor are about three orders of magnitude higher (**6**, **4b**, and **4g** in Table II) than that of the parent compound **7** (Table II). Since there is little difference between the affinities of β-carboline (norharmane, IC_{50} = 3 μM[11]) and β-carboline-3-carboxylic acid (**7**) (Table II), the incorporation of suitable substituents hand in hand with the loss of the ionizable group might be the crucial step for the dramatic increase in receptor affinity. The data give some evidence that the size of these substituents is quite limited, since beginning with the methyl derivative **6**, maximal affinity is obtained in the case of the ethyl derivative **4b** and the affinity decreases again with increasing size of the ester group, e.g., **4g** (*n*-propyl), **4i** (*n*-butyl), **4j** (benzyl), and **4k** (3-pyridylmethyl) (Table II). The reason why the substituted ethyl esters **4c–f,h** have lower affinities than the comparable *n*-propyl derivative **4g** is not obvious in all details (Table II). The different affinity cannot be solely due to lipophilicity. For the esters possessing very high receptor affinity (**6** and **4b,g**), lipophilic parameters with R_{m_0} values (Table II) between 1.97 and 2.84 were obtained. The very lipophilic **4i,j** excepted, all other esters investigated lay in this lipophilic range. Furthermore, substituted ethyl esters with electron-withdrawing substituents (**4c,d,h**) have significantly higher affinities than those with electron-releasing substituents (**4e,f**). This indicates that size, lipophilicity, and electronic effects are responsible for high receptor affinity. Compared to the β-carboline-3-carboxylates, there is a much smaller affinity-increasing effect of the ester formation in the case of several tetrahydro-β-carboline-3-carboxylates, (**5** and **3a–d**), whose affinities are only about one order of magnitude higher than that of the parent free acid **2**, except the methyl derivative **5**, whose affinity is somewhat higher (Table I).

All β-carboline-3-carboxylates investigated so far also inhibit benzodiazepine receptor binding in vivo after iv administration to mice (Table II). There seems to be no general correlation between in vitro affinity (IC_{50} in Table II) and in vivo potency, since compounds **4c,i** and especially compound **4d** seem to be more potent in vivo relative to the in vitro affinity than the already known derivatives **6**,**4b**, and **4g**. The tetrahydro-β-carboline derivative **5** is only a very weak inhibitor of benzodiazepine receptor binding in vivo (Table I).

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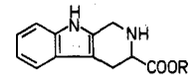
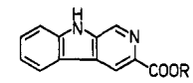
Table I. Esters of 1,2,3,4-Tetrahydro-β-carboline-3-carboxylic Acid



no.	R	mp, °C	yield, %	recrystn solvent	formula	anal.	R_{m_0}[c]	[³H]flunitrazepam binding: IC_{50},[d] nM	n_H[e]	% inhibn in vivo[f]
2	H	306[a]	97		$C_{12}H_{12}N_2O_2$	C, H, N		22 000 ± 4000	0.65	
5	Me	193-194[b]	90	MeOH	$C_{13}H_{14}N_2O_2$	C, H, N	1.54	250 ± 20	0.55	6.1 ± 2.3
3a	Et	149-150	80	EtOH	$C_{14}H_{16}N_2O_2$	C, H, N	1.93	6 000 ± 1300	0.87	
3b	*n*-Pr	142-143	70	*n*-PrOH	$C_{15}H_{18}N_2O_2$	C, H, N	2.55	4 400 ± 1400	0.61	
3c	*n*-Bu	136-137	70	*n*-BuOH	$C_{16}H_{20}N_2O_2$	C, H, N	3.09	4 800 ± 1100	0.59	
3d	Bzl	226-227	50	BzlOH	$C_{19}H_{19}ClN_2O_2$	C, H, N, Cl	3.30	2 700 ± 700	0.91	

[a] Literature[15] mp 306 °C; literature[16] mp 310 °C. [b] Literature[24] mp 186 °C. [c] Partition data obtained via reversed-phase TLC; $R_m = \log(1/R_f - 1)$; $R_{m_0} = R_m$ at 0% acetone as noted under Experimental Section. [d] Concentrations necessary for 50% inhibition (IC_{50}) are Means plus or minus SEM of four determinations. [e] Hill coefficients for the inhibition of specific [³H]flunitrazepam binding. [f] Inhibition of specific [³H]flunitrazepam binding in the mouse brain in vivo 10 min after iv administration of 15 μmol/kg according to ref 21. Data are given as percent inhibition of specific [³H]flunitrazepam binding and are means plus or minus SEM of four determinations.

Table II. Esters of β-Carboline-3-carboxylic Acid



no.	R	mp, °C	yield, %	recrystn solvent	formula	anal.	R_{m_0}[g]	[³H]flunitrazepam binding: IC_{50},[h] nM	n_H[i]	% inhibn in vivo[j]
7	H	310[a]	96		$C_{12}H_8N_2O_2$	C, H, N		7200 ± 900	0.52	
6	CH_3	261-262[b]	57	MeCN	$C_{13}H_{10}N_2O_2$	C, H, N	1.97	2.5 ± 0.5	0.56	51.2 ± 6.3
4a	CD_3	260-261	80	MeCN	$C_{13}H_7D_3N_2O_2$	C, H, N	1.95	5.3 ± 1.3	0.72	
4b	C_2H_5	231-232[c]	33	EtOH	$C_{14}H_{12}N_2O_2$	C, H, N	2.36	1.4 ± 0.1	0.75	61.7 ± 2.4
4c	CH_2CH_2F	220-221	60	MeCN	$C_{14}H_{11}FN_2O_2$	C, H, N	2.15	27 ± 8.0	0.79	55.3 ± 2.7
4d	CH_2CH_2Cl	179-180	70	MeCN	$C_{14}H_{11}ClN_2O_2$	C, H, N, Cl	2.80	22 ± 5.0	0.75	89.0 ± 4.2
4e	$CH_2CH_2OCH_3$	189-190	65	MeCN	$C_{15}H_{14}N_2O_3$	C, H, N	2.10	145 ± 30	0.79	34.6 ± 6.4
4f	$CH_2CH_2N(CH_3)_2$	164-165[d]	55	MeCN	$C_{16}H_{17}N_3O_2$	C, H, N	1.97	330 ± 70	0.83	13.4 ± 2.0
4g	*n*-C_3H_7	187-188[e]	35	MeCN	$C_{15}H_{14}N_2O_2$	C, H, N	2.84	4.6 ± 0.9	0.50	62.7 ± 6.7
4h	CH_2CH_2CN	194-195	17	MeCN	$C_{15}H_{11}N_3O_2$	C, H, N	2.01	27 ± 4.0	0.79	44.4 ± 4.5
4i	*n*-C_4H_9	210-211	30	MeCN	$C_{16}H_{16}N_2O_2$	C, H, N	3.35	29 ± 4.0	0.58	54.9 ± 2.0
4j	$CH_2C_6H_5$	233-234[f]	52	MeCN	$C_{19}H_{14}N_2O_2$	C, H, N	3.58	21 ± 3.0	0.73	38.4 ± 3.3
4k	CH_2-3-pyr	196-197	50	MeCN	$C_{18}H_{13}N_3O_2$	C, H, N	2.45	34 ± 3.0	0.90	49.4 ± 5.7

[a] Literature[20] mp 309-310 °C. [b] Literature[20] mp 262 °C. [c] Literature[14] mp 230-233 °C. [d] Literature[14] mp 165-168 °C. [e] Literature[14] mp 195-197 °C. [f] Literature[14] mp 234-238 °C. [g-j] See footnotes *c-f* in Table I.

During these experiments on the benzodiazepine receptor binding in vivo, none of the β-carbolines investigated showed either pronounced sedative actions, which are observed after comparable doses of benzodiazepines (relative to receptor occupation in vivo), or pronounced convulsant actions, which have been reported for the excitatory β-carboline derivative methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate at comparble iv doses (relative to receptor occupation in vivo).[18] This might give a first indication that all the derivatives belong more or less to the group of "neutral"[18] benzodiazepine receptor ligands. However, a more detailed analysis of the pharmacological properties of the derivatives that will reveal even small differences of the intrinsic activities is currently under progress.

Besides the assumption that the possible endogenous ligand of the benzodiazepine receptor might be a β-carboline derivative,[5-9] β-carbolines have received increasing attention, since some of these compounds seem to have a certain degree of specificity for one subclass of the benzodiazepine receptor, the cerebellar BZ_1 type.[10-13,19] We have previously shown that *n*-propyl β-carboline-3-carboxylate (compound **4g**) has different affinities for two benzodiazepine receptor subclasses, which account in the bovine cortex for about 60 (BZ_1 receptor) or 40% (BZ_2 receptor) of the total receptor population, and that the apparent Hill coefficient for inhibiting specific [³H]flunitrazepam binding in bovine cortex, which is about 0.5 in the case of *n*-propyl β-carboline-3-carboxylate, might serve as an indication for the relative subclass specificity of these compounds.[11] Benzodiazepines, which do not have any subclass specificity, have Hill coefficients of about unity in this system (see Experimental Section, receptor binding assay). Although it might be premature to use the Hill coefficients as final proof for the subclass specificity, the Hill coefficients found for the derivatives synthesized in this study might suggest that a pronounced subclass specificity (Hill coefficients below 0.6) is only present for two other esters, the methyl and the *n*-butyl derivatives (**6** and **4i** in Table II), and possibly also for the methyl tetrahydro-β-carboline-3-carboxylate (**5** in Table I). Thus, the data strongly suggest that the structural parameters leading to high affinity or high subclass specificity may differ fundamentally. In conclusion, our data show that

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the affinity-increasing effect of the ester formation with β-carboline-3-carboxylic acid is profoundly dependent on molecular size, as well as hydrophobic and electronic parameters of the ester alcohol component.

Experimental Section

Melting points were determined in open glass capillary tubes, with a Büchi-Tottoli melting point apparatus, and are uncorrected. Each analytical sample was homogeneous by TLC. The TLC experiments were carried out on Merck 60 F_{254} silica gel plates. For the R_{m_0} value[22,23] determination, these plates were impregnated with liquid paraffin as the stationary phase, and acetone/water mixtures served as the mobile phase. Elemental analyses (C, H, N, and Cl) were performed by the Mikroanalytisches Laboratorium, Universität Mainz, and were within ±0.4% of the theoretical values. The structures of all compounds were confirmed by infrared spectra (Beckman IR 4220 spektrophotometer; KBr pellets), ^{1}H NMR spectra (Varian EM-360A, 60 MHz), with Me_4Si as internal standard, and mass spectroscopy (Varian-MAT CH7A).

Tritiated flunitrazepam was obtained from New England Nuclear (Dreieich, FRG) and had a specific activity of 88 Ci/mmol and a radiochemical purity >99%. Diazepam, clonazepam, and chlordiazepoxid were gifts of Hoffmann-La Roche (Basel, Switzerland). All other chemicals were of reagent grade and obtained from commerical suppliers. 1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid (**2**),[15,16] methyl 1,2,3,4-tetrahydro-β-carboline-3-carboxylate (**5**),[24] β-carboline-3-carboxylic acid (**7**),[20] methyl β-carboline-3-carboxylic acid (**6**),[20] ethyl β-carboline-3-carboxylate (**4b**),[14] 2-(dimethylamino)ethyl β-carboline-3-carboxylate (**4f**),[14] *n*-propyl β-carboline-3-carboxylate (**4g**),[14] and benzyl β-carboline-3-carboxylate (**4j**)[14] are known compounds.

1,2,3,4-Tetrahydro-β-carboline-3-carboxylic Acid (2). A mixture of (±)-tryptophan (102 g, 0.5 mol), NaOH (20 g, 0.5 mol), and H_2O (200 mL) was stirred until clear, and then formalin (30%; 50 g, 0.5 mol) was added. The mixture was stirred at room temperature for 2 h, refluxed for 3 h, and then neutralized (pH 5) with HCl (6 N, 83 mL) and cooled. The precipitate was collected, washed well with H_2O, MeOH, and CH_2Cl_2, and dried in vacuo (20 mmHg, 100 °C).

Preparation of 1,2,3,4-Tetrahydro-β-carboline-3-carboxylates (3a–d and 5). Dry hydrogen chloride gas was bubbled into a suspension of **2** (10.8 g, 50 mmol) in 150 mL of alcohol (**5**, CH_3OH; **3a**, C_2H_5OH; **3b**, *n*-C_3H_7OH; **3c**, *n*-C_4H_9OH; **3d**, $C_6H_5CH_2OH$). The temperature was kept under 40 °C. The mixture cleared slowly, and then a white precipitate formed readily. The reaction was monitored by TLC (*n*-BuOH/AcOH/H_2O, 4:1:1). The crystalline product was collected, washed sparingly with the corresponding alcohol, stirred into H_2O (300 mL), neutralized ($NaHCO_3$), and extracted with CH_2Cl_2 (1 × 300 mL, 3 × 100 mL). The combined CH_2Cl_2 extracts were dried (Na_2SO_4) and evaporated. In the case of **3d**, the clear reaction mixture was evaporated to one-third its volume and left to stand overnight. The precipitate was collected, stirred into CH_2Cl_2 (50 mL), and collected again.

Methyl β-Carboline-3-carboxylate (6). To a cooled, rapidly stirring solution of **5** (11.5 g, 50 mmol) in glacial AcOH (200 mL) was added $Pb(OAc)_4$ (44.5 g, 100 mmol), and the resulting mixture was stirred for 15 min. Oxalic acid (50 g) was added, and stirring was continued for 60 min. The pale yellow precipitate was collected, washed sparingly with MeOH, suspended in a mixture of H_2O (500 mL) and CH_2Cl_2 (1000 mL), and neutralized with $NaHCO_3$. The emulsion was filtered, the precipitate was washed well with CH_2Cl_2, and the aqueous layer was further extracted with CH_2Cl_2 (3 × 150 mL). The combined CH_2Cl_2 extracts were dried (Na_2SO_4) and evaporated.

β-Carboline-3-carboxylic Acid (7). A mixture of **6** (11.3 g, 50 mmol), NaOH (8.0 g, 200 mmol), EtOH (100 mL), and H_2O (200 mL) was refluxed for 30 min, and the EtOH was removed on the rotary evaporator. The mixture was neutralized (pH 5) with HCl (6 N, 33 mL) and cooled. The precipitate was collected, washed with H_2O and MeOH, and dried in vacuo (20 mmHg, 100 °C).

Preparation of β-Carboline-3-carboxylates (4). Method A (4b,g,i). A solution of the corresponding tetrahydro compound **3a–c** (50 mmol) and chloranil (100 mmol) in *s*-tetrachloroethane (200 mL) was refluxed for 90 min. The resulting black solution was poured into cold HCl (0.2 N, 1000 mL). The aqueous layer was washed with CH_2Cl_2 (3 × 250 mL), neutralized ($NaHCO_3$), and extracted with CH_2Cl_2 (1 × 1000 mL, 3 × 200 mL). The combined CH_2Cl_2 extracts were dried (Na_2SO_4) and evaporated.

Method B (4a,c–e). A solution of **7** (2.12 g, 10 mmol), H_2SO_4 (9.8 g, 100 mmol), and the corresponding alcohol (250 mmol; **4a**, CD_3OH; **4c**, FCH_2CH_2OH; **4d**, $ClCH_2CH_2OH$; **4e**, $H_3COCH_2CH_2OH$) was stirred at 100 °C (internal), and the reaction was monitored by TLC (*n*-BuOH/AcOH/H_2O, 4:1:1). The reaction mixture was poured over ice, neutralized ($NaHCO_3$), and extracted with CH_2Cl_2, and the extract was dried (Na_2SO_4) and evaporated.

Method C (4f,h,k). A mixture of $SOCl_2$ (50 mL) and **7** (2.12 g, 10 mmol) was refluxed for 1 h and evaporated to dryness. The residue (crude **8**) was slurried with dry toluene (30 mL) and evaporated to dryness, and the appropriate alcohol [250 mmol; **4f**, $(H_3C)_2NCH_2CH_2OH$; **4h**, $NCCH_2CH_2OH$; **4k**, pyr-3-CH_2OH] containing triethylamine (5 g, 50 mmol) was added. This mixture was stirred at room temperature for 2 h, and then poured into ice–water (400 g) containing HCl (20 mL) and washed with CH_2Cl_2 (3 × 200 mL). The aqueous layer was neutralized ($NaHCO_3$) and extracted with CH_2Cl_2 (1 × 400 mL, 3 × 100 mL). The combined CH_2Cl_2 extracts were dried (Na_2SO_4) and evaporated.

Benzyl β-Carboline-3-carboxylate (4j). A suspension of Na (115 mg, 5 mmol) in dry benzyl alcohol (27 g, 250 mmol) was stirred until clear, **6** (2.26 g, 10 mmol) was added, the temperature was raised to 75–80 °C (internal), and the reaction was monitored by TLC (2-butanone). The solution was poured over ice (200 g) and extracted with CH_2Cl_2 (3 × 200 mL). The combined CH_2Cl_2 extracts were dried (Na_2SO_4), and the CH_2Cl_2 was evaporated. The oily remainder was left at room temperature overnight. The white crystalline precipitate that formed was collected, washed with benzyl alcohol, and dried in vacuo (2 mmHg, 140 °C).

Receptor Binding Assay. Bovine brains were obtained from a local slaughterhouse and stored at −20 °C after dissection. For the receptor binding assay about 1.5 g of frozen bovine cortex was homogenized in 40 mL of 50 mM Tris-HCl buffer, pH 7.4. After centrifugation at 48000*g* for 10 min, the supernatant was discarded, and the pellet was resuspended in 200 mL of the same buffer; 900-μL aliquots of this homogenate were incubated for 45 min together with 50 μL of buffer containing [^{3}H]flunitrazepam at a final concentration of 0.2 nM and 50 μL of buffer containing the blank (diazepam) or different concentrations of the displacers. The incubation was terminated by rapid filtration through Whatman GF-B filters. The filters were washed three times with 3 mL of ice-cold incubation buffer, placed in minivials, and dried for 30 min at 60 °C. Radioactivity on the filters was determined by liquid scintillation spectrophotometry in 4 mL of Quickszint 402 (Zinsser, Frankfurt, FRG). Nonspecific binding was determined by parallel experiments containing diazepam (10 μM) and accounted for less than 10% of total binding. The concentrations of the β-carboline derivatives that inhibit specific [^{3}H]flunitrazepam binding by 50% (IC_{50}) were determined by log probit analysis with four to six concentrations of the displacers, each performed in triplicate. Under these experimental conditions, the two benzodiazepine derivatives clonazepam and chlordiazepoxid had IC_{50} values of 4 and 1000 nM, respectively, with apparent Hill coefficients (n_H) not different from unity. The data are means of four to six individual determinations.

Inhibition of specific [^{3}H]flunitrazepam binding in vivo after iv administration of the β-carbolines to mice was determined as previously described.[21]

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K.-P.L. and W.W. The excellent technical assistance of A. Stillbauer is gratefully acknowledged.

Note Added in Proof: After this paper had been submitted, the recent work of Cain et al.[25] also describing the synthesis and benzodiazepine receptor interaction of several β-carboline derivatives came to our attention.

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Registry No. DL-1, 54-12-6; (±)-2, 41509-88-0; (±)-3a, 84518-77-4; (±)-3b, 84454-27-3; (±)-3c, 84454-28-4; (±)-3d·HCl, 84454-29-5; 4a, 84454-30-8; 4b, 74214-62-3; 4c, 84454-31-9; 4d, 84454-32-0; 4e, 84454-33-1; 4f, 78538-73-5; 4g, 76808-18-9; 4h, 84454-34-2; 4i, 84454-35-3; 4j, 78538-68-8; 4k, 84454-36-4; (±)-5, 42021-11-4; 6, 69954-48-9; 7, 74214-63-4; 8, 84454-37-5; CD_3OH, 1849-29-2; FCH_2CH_2OH, 371-62-0; $ClCH_2CH_2OH$, 107-07-3; $H_3COCH_2CH_2OH$, 109-86-4; $(H_3C)_2NCH_2CH_2OH$, 108-01-0; $NCCH_2CH_2OH$, 109-78-4; pyr-3-CH_2OH, 100-55-0; $C_6H_5CH_2OH$, 100-51-6.

Alkylation of Opioid Receptor Subtypes by α-Chlornaltrexamine Produces Concurrent Irreversible Agonistic and Irreversible Antagonistic Activities[1]

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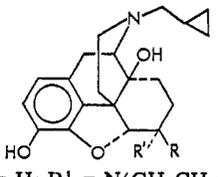
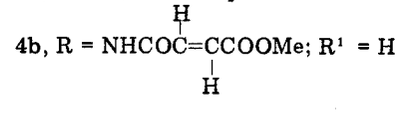
α-Chlornaltrexamine (**1a**, α-CNA), the C-6 epimer of the opioid receptor affinity label β-CNA (**1b**), has been synthesized and tested in vitro and in vivo. In vitro, α-CNA appears to alkylate opioid receptor subtypes (μ, κ, and δ) and is similar to β-CNA in its ability to produce irreversible antagonism at all three subtypes. However, **1a** differs from **1b** in that it exhibits additionally an irreversible agonist activity in the guinea pig ileum preparation but not in the mouse vas deferens preparation. This latter activity is discussed in terms of an irreversible mixed agonism–antagonism at κ receptors, or, alternatively, it may reflect differences between μ receptors in the two in vitro preparations.

The affinity label β-chlornaltrexamine (**1b**, β-CNA) has



1a, R = H; R^1 = $N(CH_2CH_2Cl)_2$
1b, R = $N(CH_2CH_2Cl)_2$; R^1 = H
2a, R = H; R^1 = $N(CH_2CH_2OH)_2$
3a, R = H; R^1 = NH_2



found considerable use in the pharmacological characterization of opioid receptors.[2-9] This ligand irreversibly inhibits the stereospecific binding of [^{3}H]naloxone in the opiate receptor binding assay[3] and behaves as an irreversible narcotic antagonist in the guniea pig ileum (GPI)[4,5] and mouse vas deferens (MVD)[6] preparations. Moreover, β-CNA produces ultralong-lasting antagonism (≥3 days) of morphine in vivo.[7-9] At least three opioid receptor subtypes (μ, κ, and δ) are apparently covalently bound by interaction with β-CNA.

In view of the properties of β-CNA (**1b**), it was therefore of interest to investigate the pharmacological profile of its C-6 epimer, α-CNA (**1a**), because its nitrogen mustard electrophile is in a different orientation and, hence, might alkylate different receptor nucleophiles. In this report we describe the synthesis and remarkable biological properties of α-CNA. A key feature distinguishing α-CNA from β-CNA is its behavior as an irreversible agonist in addition to its irreversible antagonist activity.

Table I. Agonist Effect of α-CNA on the Untreated and β-FNA-Treated GPI

GPI preparation	IC_{50} ± SE,[a] nM	
	EKC	α-CNA
untreated	0.72 ± 0.21	5.5 ± 2.0
β-FNA treated[b]	0.69 ± 0.22	2.4 ± 0.8

[a] All IC_{50} values represent means plus or minus standard errors determined from four GPI preparations. [b] GPI was incubated with 2×10^{-7} M β-FNA for 60 min and washed thoroughly 20 times. Such preparations have been shown to be μ-less, since all available μ sites are irreversibly bound by β-FNA and become essentially preparations with κ sites. The IC_{50} values of the κ agonist, EKC (ethylketazocine), indicate that the κ sites are intact in the β-FNA-treated GPI.

Chemistry. α-CNA was prepared in two steps from α-naltrexamine (**3a**)[10] by exhaustive reductive alkylation of the latter with glycoaldehyde ($NaBH_3CN$, MeOH) and

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