SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF C(1)- AND N(2)-SUBSTITUTED β-CARBOLINE DERIVATIVES§

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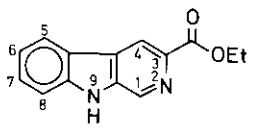
Abstract- A series of C(1) as well as N(2)-hetero-substituted β-carbolines has been synthesized from indoles *via* N-hydroxy-tryptophan derivatives (Schemes I and II). Preliminary pharmacodynamical data are provided (Tabel I). Of the β-carbolines **4,5,14-16** prepared, the compounds **4,5** and **14** show appreciable *in vitro* affinity towards the benzodiazepine receptor.

INTRODUCTION

Compounds containing the β-carboline structure have aroused considerable interest in neuropharmacology. It was found that β-carboline-3-carboxylic acid derivatives **1** are potent inhibitors of the specific binding of ^{3}H-diazepam to its brain receptors[1a,b]. It has been proposed that such β-carbolines, which have been isolated from human urine and brain tissue compose part of the structure of the endogenous ligand of the benzodiazepine receptors. Subsequently, the formation of ethyl β-carboline-3-carboxylate (**1**) was considered to be an artefact due to



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the isolation procedure[2]. However, recent experimental evidence points again to the presence of an endogeneous ligand with β-carboline-like properties[3].
Anyhow, β-carbolines remain useful tools of investigation due to their selective action on the central type benzodiazepine receptors. These observations, the occurrence of tetrahydro-β-carbolines in human urine, and the potential implication of β-carbolines in mechanisms which operate in alcoholism and mental illness deserve more studies in this area[4].

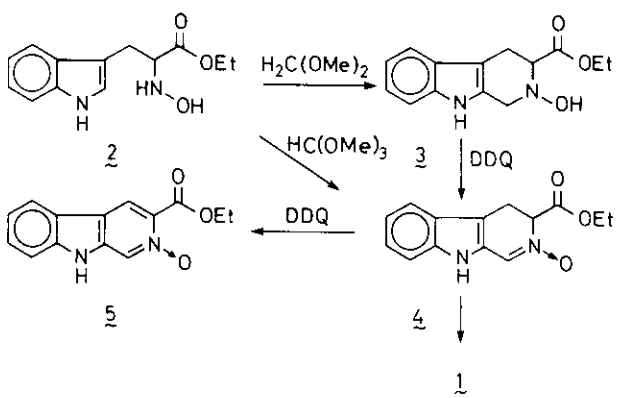
So the β-carboline structure might become an important basis for the design of new benzodiazepine-related drugs[5]. For that reason several 1-alkyl- and 4-alkyl β-carboline-carboxylates as well as amide congeners have been prepared[5] *via* different methods. Studies on structure-activity relationships (SAR)[5e,6] have demonstrated that 3,4-dihydro-, or 1,2,3,4-tetrahydro-β-carbolines show decreased activity as compared to the corresponding fully aromatic compounds. Introduction of substituents at positions C(1) and/or N(9) of the ring-system results in loss of activity, while substitutions at positions C(4),C(5),C(6) or C(7) do not affect the binding affinity considerably. The presence of an alkyloxycarbonyl group at position C(3) appeared to be essential to ensure high receptor binding affinity.

In order to study the effect of the presence of functional groups at C(1) or N(2) on the activity of β-carbolines we prepared the β-carbolines **4**,**5**,**14-16** and investigated their affinity towards the benzodiazepine receptor.

SYNTHESIS

As part of our synthetic studies on N-hydroxytryptophan derivatives we reported[7] recently the conversion of **2** into the N-hydroxytetrahydro-β-carboline derivative **3** (Scheme I). In addition we described[7] that the nitrone **4** could be prepared either by DDQ oxidation of **3** or - more efficiently - in a single step from **2** by

Scheme I



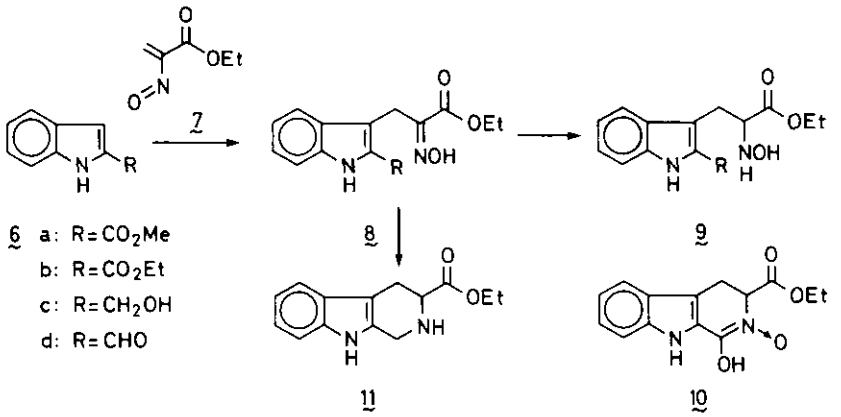
acid-catalyzed condensation with triethyl orthoformate. Here we report that the N-oxo-β-carboline carboxylate **5** can be prepared quantitatively from **4** by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) oxidation.

Another reaction of **4** is worthwhile being mentioned in this context. When a solution of **4** in CH_2Cl_2/MeOH (3/1,v/v) is kept for three weeks, the β-carboline **1** is isolated quantitatively. Thus the reaction sequence **2** →**4**→**1** provides a novel and convenient route to **1**.

Prior to the development of this scheme we had studied another approach to nitrone **4**. Although this approach was unsuccessful, it is elaborated here as it gave an entry to the novel, C(1)-substituted β-carbolines **14-16**. We anticipated that **4** might be accessible by intramolecular condensation of **9d** (Scheme II). As a

precursor for 9d we had selected 8d, the synthesis of which is briefly outlined here. Cycloaddition[8] of indole aldehyde 6d - prepared by literature procedure from 6b *via* 6c[9] - with the nitrosoolefin 7 - derived *in situ* from ethyl β-(hydroxyimino)-α-bromopropanoate - gave the tryptophan derivative 8d in 69% yield[10].

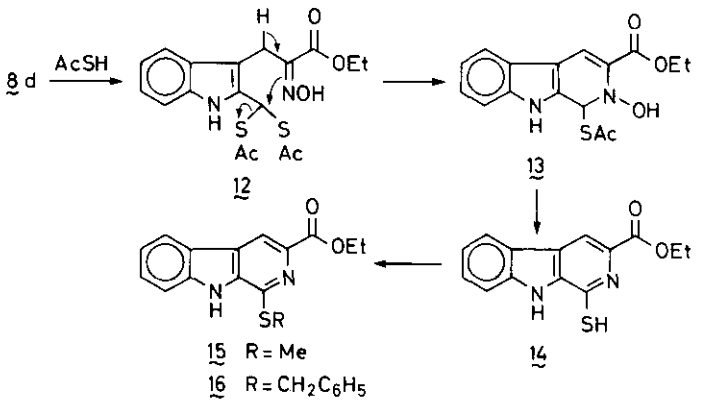
Scheme II



Attempts to reduce selectively the oxime function of 8d failed. Treatment with $Me_3N{\cdot}BH_3$ - a reagent we have employed successfully for the reduction of α-oximino carboxylic esters[11] - led to complete decomposition of 8d. When $NaBH_4$ was used as the reducing agent the alcohol 8c was obtained in 87% yield[12] (Procedure II). Treatment of 8d with Zn and NH_4Cl in aqueous tetrahydrofuran gave the tetrahydro-β-carboline 11 in 80% yield.

Based on these experiences we decided to protect the aldehyde function before reducing the oxime group. For this purpose, we chose the thioacetylation[13]. Treatment of 8d with a mixture of CH_3COSH and CF_3CO_2H at 0 °C gave a compound to which we assigned structure 14 on the basis of spectroscopic evidence (see Experimental Section). This assignment could be substantiated by the following experiments. Desulphurisation of 14 was achieved by treatment with activated zinc in acetic acid to give 1 in 82% yield. Alkylation with methyl iodide or benzyl bromide gave 15 (87%) and 16 (88%), respectively. The unprecedented and surprising formation

Scheme III



of **14** from **8d** can be rationalized as depicted in Scheme III. The initially formed dithioacetal **12** is apparently unstable and yields **13** under elimination of AcSH. Spontaneous aromatization of **13** by loss of water yields a β-carboline derivative, which is deacylated to yield **14**.

Finally, we attempted to prepare the C(1)-functionalized dihydro-β-carboline oxide **10** by ring closure of **9b**. However, treatment of **9b** with Me_3COK failed to give **10**; instead only slow decomposition of the starting material was observed.

Compound **9b** was prepared efficiently by reaction of **6b** with the transient nitroso-olefin **7** to yield (70%) **8b**[10] (*cf.* the preparation of **8c** and **8d**, *vide supra*). Subsequent reduction with $Me_3N{\bullet}BH_3$[11] gave **9b** in 94% yield. Problems encountered in the related conversion **8d**→**9d** were not encountered in this reduction step.

RECEPTOR BINDING ASSAYS

Receptor binding assays were performed as reported earlier[14]. Table I shows the IC_{50} values of the compounds tested for inhibition of ^{3}H-diazepam binding to rat brain synaptic membranes.

The activity of compound **5** is lower than that of **1**, but comparable to that of diazepam. The analogue **4** exerts an activity which is about one order of magnitude lower than that of the aromatic analogue **5**. The activities of **4** and **5** are considerably higher than that of the agonist chlorodiazepoxide, though all three compounds feature a ni- trone function in conjugation with an aromatic ring system. Of the C(1) substituted derivatives **14-16** only 1-thio-β-carboline **14** shows an appreciable activity, whereas the S-alkylated congeners **15** and **16** are devoid of activity.

TABLE I Inhibition of specific ^{3}H-diazepam binding to rat brain synaptic membranes. The drug dose causing 50% inhibition is calculated and expressed as IC_{50}.

COMPOUND	IC_{50} [nM]
Diazepam	21.4
Chlorodiazepoxide	1820
1	0.63
4	182
5	21.4
14	4200
15	no inhib. at 1000
16	no inhib. at 1000

Our present findings support the data obtained previously from structure-activity relationship investigations[5]. The fully aromatic β-carbolines are more potent inhibitors of diazepam than the partially saturated congeners. As observed with C(1)-alkyl-substituted β-carbolines the benzodiazepine receptor has a low or no affinity to C(1)-functionalized β-carbolines. Moreover, N-oxide formation causes a drop in activity, though the fully aromatic analogue still preserves an appreciable affinity towards the receptor.

Finally, it is worthwile to mention that we observed a shift of IC_{50} values when the binding studies were performed in the presence of 10^{-5} M aminobutyric acid. This shift is indicative of the pharmacological profile of the substance tested. By this criterion compounds **4** and **14** both appear to behave as intermediates between agonists and antagonists, a behaviour not observed with **1** and related convulsants.
More detailed studies of the mode of action are currently under investigation.

EXPERIMENTAL

Melting points were taken on a Koefler hot stage (Leitz-Wetzlar) and are uncorrected. Ultraviolet spectra were measured with a Perkin-Elmer spectrometer, Model 555.
Proton magnetic resonance spectra were measured on a Varian Associa tes Model T-60 or a Bruker WH-90 spectrometer. Chemical shifts are reported as δ-values (parts per million) relative to tetramethyl silane as an internal standard. Mass spectra were obtained with a double-focussing VG 7070E spectrometer. Thin layer chromatography (TLC) was carried out by using Merck precoated silica gel F-254 plates (thickness 0.25 mm). Spots were visualized with a UV hand lamp, iodine vapor, Cl_2-TDM[15], cinnamaldehyde/HCl for indole detection[16] and $AgNO_3/Na_2CrO_7$ for the detection of sulfides[17]. A miniprep LC (Jobin Yvon) has used for preparative HPLC; as stationary phase Merck silicagel H (type 60) was used. Merck silicagel (Type 60) was used for flash chromatography.

3-(Ethoxycarbonyl)-β-carboline (1)

Compound **14** (*vide infra*) (0.1 mmol, 27 mg) was treated for two days at 40 °C with activated zinc in acetic acid (5 ml). The reaction mixture was then filtered, the filtrate concentrated to dryness and the residue subjected to flash column chromatography ($MeOH/CH_2Cl_2$, 5/95, v/v) to give 82% (18 mg) of crystalline **1**, mp 225-227 °C (lit.[18] mp 224-229 °C). Spectroscopical data are identical with those reported in literature[1a].

2-(Hydroxymethyl)indole (6c) was prepared by $LiAlH_4$ reduction[19] of **2-(methoxycarbonyl)indole** (6b)[20]; **2-(formyl)indole** (6d) was prepared by MnO_2 oxidation of 6c[9] according to literature procedures.

2-Oxo-3-(ethoxycarbonyl)-β-carboline (5)

To a stirred solution of **4**[7] (0.45 mmol, 117 mg) in dry CH_2Cl_2 (12 ml) was added dropwise DDQ (0.45 mmol, 103 mg) in CH_2Cl_2 (10 ml). The reaction mixture was monitored by tlc. After stirring for 1 h the reaction mixture was concentrated to dryness, the residue was dissolved in EtOAc and washed with 0.1N $NaHCO_3$. The $NaHCO_3$ solution was washed 10 times with EtOAc (total 500 ml). Evaporation of the

organic layer gave quantatively crystalline 5 (115 mg), which was recrystallized from ethanol, mp 250-252 °C. UV (MeOH) λ_{max} 366 (sh), 349 (sh), 328, 278, 259 (sh), 209 nm; λ_{min}=306, 230 nm. EIMS (70 eV) m/z 256 ([M]$^+$, 26%), 240 ([$C_{14}H_{12}N_2O_3$]$^+$, 12%), 195 ([$C_{12}H_7N_2O_3$]$^+$, 49%), 168 ([$C_{11}H_8N_2$]$^+$, 83%), 167 ([$C_{11}H_7N_2$]$^+$, 100%), exact mass calcd. for $C_{14}H_{12}N_2O_3$ 256.0848, found: 256.0857; ^{1}H-NMR (90 MHz, d^6-DMSO) δ 8.64 and 8.51 (2s, 2H, C(1)H and C(4)H), 8.21-7.19 (m, 4H, C(5)-C(8)H), 4.33 (q, 2H, OCH_2CH_3), 1.33 (t, 3H, OCH_2CH_3). Anal. calcd. for $C_{14}H_{12}N_2O_3$ (M 256.262) C 65.62, H 4.72, N, 10.93, found C 65.58, H 4.72, N 10.83.

Ethyl α-(Hydroxyimino)-β-(2-ethoxycarbonylindol-3-yl)propanate (8b)

A solution of ethyl α-(hydroxyimino)-β-bromopropanoate (4 mmol, 840 mg) in CH_2Cl_2 (25 ml) was added dropwise to a stirred solution of 6b[19] (4 mmol, 700 mg) and a suspension of Na_2CO_3 (8 mmol, 825 mg) in dry CH_2Cl_2 (25 ml) at room temperature under argon. Stirring in an argon atmosphere was continued at room temperature for 16 h. Then the mixture was filtered through a thin layer of silica gel 60 and concentrated to dryness. The residue was subjected to column chromatography (silicagel 60, MeOH/CH_2Cl_2, 4/96, v/v) to yield crystalline 8b (70%) mp 174-176 °C which was recrystallized from ethyl acetate. UV (MeOH) λ_{max} 294, 223 nm; λ_{min} 254 nm. CIMS (100 eV) m/z 319 ([M+1]$^+$, 100%), 303 (40%), 302 (61%), 301 (78%), 257 (16%), 229 ([$C_{13}H_{13}N_2O_2$]$^+$, 34%), 202 ([$C_{12}H_{12}NO_2$]$^+$, 34%), exact mass calcd. for $C_{16}H_{18}N_2O_5$ 319.1293, found: 319.1288; ^{1}H-NMR (90 MHz, $CDCl_3$) δ 8.90 (s(br), 1H, indole NH), 7.74 (d, J=8.1 Hz, 1H, indole C(4)H), 7.37-7.01 (m, 3H, indole C(5)-C(7)H), 4.25 (s, 2H, indole C(3)CH_2), 4.43 (q, 2H, indole C(2)-COOCH_2CH_3), 4.17 (q, 2H, OCH_2CH_3), 1.42 (t, 3H, indole C(2)COOCH_2CH_3), 1.16 (t, 3H, OCH_2CH_3). Anal. calcd. for $C_{16}H_{18}N_2O_5$ (M 318.329) C 60.37, H 5.70, N 8.80, found C 60.39, H 5.73, N 8.69.

Ethyl α-(Hydroxyimino)-β-(2-hydroxymethylindol-3-yl)propanoate (8c)

Procedure I: Reaction of 2-hydroxymethylindole (6c)[19] (2 mmol, 294 mg) with ethyl α-(hydroxyimino)-β-bromopropanoate (2 mmol, 420 mg) in the presence of Na_2CO_3 (4 mmol, 412 mg) was performed in CH_2Cl_2 (40 ml) as described for the preparation of 8b to give 8c in 77% (425 mg) yield after recrystallization from CH_2Cl_2/CCl_4, m p 117-122 °C. UV (MeOH) λ_{max} 290 (sh), 280, 220 nm, λ_{min} 246 nm. EIMS (70 eV) m/z 276 ([M]$^+$, 89%), 259 ([M-OH]$^+$, 22%), 195 (30%), 186 (19%), 185 (66%), 169 (29%), 168 (57%), 167 (42%), 160 ([$C_{10}H_{10}NO$]$^+$, 47%), 159 (26%), 158 (32%), 146 (71%), 143 ([$C_{10}H_9N$]$^+$, 100%), 159 (26%), 158 (32%), 146 (71%), 143 exact mass calcd. for $C_{14}H_{16}N_2O_4$ 276.1121, found 276.1110. ^{1}H-NMR (90 MHz, CD_2Cl_3) δ 9.0 (s(br), 1H, indole NH), 7.73-7.00 (m, 4H, indole C(4)-C(7)H), 4.85 (s, 2H, indole C(2)CH_2O), 4.20 (q, 2H, OCH_2CH_3), 4.08 (s, 2H, indole C(3)-CH_2), 1.22 (t, 3H, OCH_2CH_3).

Procedure II: A stirred solution of 8d (*vide infra*) (0.5 mmol, 137 mg) in ethanol (dry, 50 ml) was treated at 0 °C with $NaBH_4$ (0.55 mmol, 20 mg) under argon. After 1 h the reaction mixture was treated with 1N HCl (2 ml) and subsequently

concentrated to dryness. The residue was partioned between CH_2Cl_2 and water. The organic layer was washed with brine, dried (Na_2SO_4), filtered and the solvent evaporated. Recrystallization gave **8c** in 87% (120 mg) yield.

Ethyl α-(Hydroxyimino)-β-(2-formylindol-3-yl)-propanoate (8d)

Reaction of 2-formylindole **6d** (8.5 mmol, 1.23 g) with ethyl α-(hydroxyimino)-β-bromopropanoate (9 mmol, 1.79 g) in the presence of Na_2CO_3 (16 mmol, 1.65 g) was performed in CH_2Cl_2 (100 ml) as described for the preparation of **8b** to give **8d** in 69% (1.60 g) yield after recrystallization from CH_2Cl_2/n-hexane, m p 185-187 °C. UV (MeOH) λ_{max} 312, 230 nm; λ_{min} 262 nm. EIMS (70 eV) m/z 274 ($[M]^+$, 100%), 257 ($[M-OH]^+$, 27%), 243 (34%), 184 ($[C_{11}H_8N_2O]^+$, 90%), 158 ($[C_{10}H_8NO]^+$, 94%), 130 ($[C_9H_8N]^+$, 85%); exact mass calcd. for $C_{14}H_{14}N_2O_4$ 274.0954, found: 274.0950. ^{1}H-NMR (90 MHz, CD_3OD) δ 10.21 (s, 1H, indole C(2)CHO), 7.50 (d, J=7.9 Hz, 1H, indole C(4)H), 7.36-7.04 (m, 3H, indole C(5)-C(7)H), 4.42 (s, 2H, indole C(3)-CH_2), 4.16 (q, 2H, OCH_2CH_3), 1.18 (t, 3H, OCH_2CH_3). Anal. calcd. for $C_{14}H_{14}N_2O_4$ (M 274.276); C 61.31, H 5.15, N 10.21, found: C 60.77, H 5.14, N 10.17.

Ethyl α-(Hydroxyamino)-β-(2-ethoxycarbonylindol-3-yl)propanate (9b)

A solution of HCl in ethanol (5 ml of a 7 N solution) was added dropwise to a solution of **8b** (0.5 mmol, 160 mg) and triethylamine-borohydride (0.6 mmol, 45 mg) in ethanol (5 ml). The mixture was stirred at room temperature for 6 h and then concentrated to dryness *in vacuo*. The residue was dissolved in CH_2Cl_2, washed with brine and dried over Na_2SO_4. Concentration *in vacuo* afforded crystalline material which was recrystallized from CH_2Cl_2/n-hexane, m p 145-148 °C to give **9b** in 94% yield (150 mg). UV (MeOH) λ_{max} 293, 226 nm; λ_{min} 254 nm. CIMS (100 eV) m/z 321 ($[M+1]^+$, 100%), 303 ($[M-OH]^+$, 18%), 203 (40%), 202 (26%), exact mass calcd. for $C_{16}H_{20}N_2O_5$ 321.1450; found 321.1451. ^{1}H-NMR (90 MHz, CD_2Cl_2) δ=9.0 (s(br), 1H, indole NH), 7.68 (d, J=7.6 Hz, 1H, indole C(4)H), 7.39-7.04 (m, 3H, indole C(5)-C(7)H), 4.36 (q, 2H, indole C(2)$COOCH_2CH_3$), 4.14 (q, 2H, OCH_2CH_3), 4.30-4.10 (X part of ABX spectrum, 1H, indole C(3)-CH_2CH), 3.50 and 3.42 (AB part of ABX spectrum, 2H, indole C(3)CH_2), 1.40 (t, 3H, indole C(2)$COOCH_2CH_3$), 1.18 (t, 3H, OCH_2CH_3). Anal. calcd. for $C_{16}H_{20}N_2O_5$ (M 320.345) C 59.99, H 6.29, N 8.74, found C 59.68, H 6.19, N 8.69.

3-(Ethoxycarbonyl)-1,2,3,4-tetrahydro-β-carboline (11)

Small portions of activated zinc were added to a solution of **8d** (0.5 mmol, 137 mg) and NH_4Cl (100 mg) in THF/H_2O (15 ml, 2/1, v/v) at room temperature under argon. After stirring the reaction mixture for two days all starting material had been converted; the reaction was monitored by tlc; (R_f 0.2; MeOH/CH_2Cl_2, 4/96, v/v), the reaction mixture was filtered and concentrated to dryness *in vacuo*. The residue was dissolved in CH_2Cl_2 and the resulting solution was washed with water, dried with Na_2SO_4 and concentrated to dryness. Recrystallization of the residue from EtOAc/n-hexane gave **11** in 80% (98 mg) yield, m p 148-150 °C. UV (MeOH)

λ_{max} 290 (sh), 278, 240 nm. EIMS (70 eV) m/z 244 ($[M]^+$, 47%), 171 ($[C_{11}H_{11}N_2]^+$, 69%), 143 ($[C_{10}H_9N]^+$, 100%), exact mass calcd. for $C_{14}H_{16}N_2O_2$ 244.1212, found: 244.1204; ^{1}H-NMR (90 MHz, $CDCl_3$) δ 7.8 (s(br), 1H, N(9)H), 7.56-7.01 (m, 4H, C(5)-C(8)H), 4.26 (q, 2H, OCH_2CH_3), 4.14 (s(br), 2H, C(1)H_2), 3.79 (X part of ABX spectrum, $^3J_{AX}$=5.0 Hz, $^3J_{BX}$=9.7 Hz, 1H, C(3)H), 3.10 and 2.89 (AB part of ABX spectrum, $^3J_{AX}$=5.0 Hz, $^3J_{BX}$=9.7 Hz, $^2J_{AB}$=15.3 Hz, 2H, C(4)H_2), 1.92 (s(br), 1H, N(2)H), 1.32 (t, 3H, OCH_2CH_3).

1-Mercapto-3-ethoxycarbonyl-β-carboline (14)

Compound **8d** (1 mmol, 274 mg) was dissolved in thioacetic acid (5 ml) after which trifluoroacetic acid (1 ml) was added to the cooled (0 °C) solution. The reaction mixture was stirred under cooling (4 °C) for 18 h. Then CH_2Cl_2 was added and the solution was washed with water, 5% $NaHCO_3$ and brine, and dried (Na_2SO_4). Evaporation of the solvent and recrystallization of the residue from MeOH gave **14** in 81% (220 mg) yield, m p 236-239 °C. UV (MeOH) λ_{max} 380, 316, 280 (sh), 255, 222 nm; λ_{min} 348, 292, 250 nm. EIMS (70 eV) m/z 272 ($[M]^+$, 64%), 240 ($[M-S]^+$, 10%), 198 ($[C_{11}H_6N_2S]^+$, 100%), exact mass calcd. for $C_{14}H_{12}N_2O_2S$ 272.0619; found 272.0620. ^{1}H-NMR (90 MHz, CD_2Cl_2) δ 10.9 (s(br), 1H, SH), 9.5 (s(br), 1H, N(9)H), 8.20 (s, 1H, C(4)H, 8.00 (d, J=8.1 Hz, C(5)H), 7.60-7.20 (m, 3H, C(6)-C(8)H), 4.40 (q, 2H, OCH_2CH_3), 1.46 (t, 3H, OCH_2CH_3). Anal. calcd. for $C_{14}H_{12}N_2O_2S$ (M 272.326) C 61.75, H 4.44, N 10.29; found C 61.75, H 4.44, N 10.29.

1-Methylthio-3-(ethoxycarbonyl)-β-carboline (15)

Methyl iodide (1.25 mmol, 185 mg) was added dropwise to a stirred solution of **14** (1 mmol, 272 mg) and a suspension of K_2CO_3 (2.5 mmol, 350 mg) in CH_2Cl_2 (5 ml) at room temperature. Stirring was continued for 18 h at room temperature. The precipitate was then removed by filtration and the filtrate was concentrated to dryness *in vacuo*. The residue was partioned between CH_2Cl_2 and water, and the organic layer was washed with 1N HCl, with brine and then dried over Na_2SO_4. Flash chromatography (CH_2Cl_2) and recrystallization from CH_2Cl_2/n-hexane gave 250 mg of **15** (87%), m p 179-181 °C. UV (MeOH) λ_{max} 350, 334, 315, 282, 262, 233 nm; λ_{min} 342, 328, 302, 268, 248, 219 nm. EIMS (70 eV) m/z 286 ($[M]^+$, 62%), 212 (94%), 198 ($[C_{11}H_6N_2S]^+$, 22%). exact mass calcd. for $C_{15}H_{14}N_2O_2S$ 286.0776; found 286.0772. ^{1}H-NMR (90 MHz, CD_2Cl_2) δ 8.66 (s(br), 1H, N(9)H), 8.63 (s, 1H, C(4)H), 8.17 (d, J=7.0 Hz, 1H, C(5)H), 7.63-7.26 (m, 3H, C(6)-C(8)H), 4.46 (q, 2H, OCH_2CH_3), 2.83 (s, 3H, SCH_3), 1.46 (t, 3H, OCH_2CH_3). Anal. calcd. for $C_{15}H_{14}N_2O_2S$ (M 286.353) C 62.92, H 4.93, N 9.78; found C 62.59, H 4.88, N 9.69.

1-Benzylthio-3-(ethoxycarbonyl)-β-carboline (16)

Benzyl bromide (1.1 mmol, 188 mg) was added dropwise to a stirred solution of **14** (1 mmol, 272 mg) and a suspension of K_2CO_3 (2 mmol, 280 mg) in CH_2Cl_2 (5 ml). Stirring was continued for two days. Work-up as described for **15**, and recrystallization from CH_2Cl_2/n-hexane gave 320 mg of **16** (88%), m p 164-166 °C. UV

(MeOH) λ_{max} 350, 336, 312, 282, 264, 233 nm; λ_{min} 344, 328, 305, 269, 248, 219 nm. EIMS (70 eV) m/z 362 ([M]$^+$, 100%), 288 (63%), 255 (25%), 198 ([$C_{11}H_6N_2S$]$^+$, 24%), 91 ([C_7H_7], 90%), exact mass calcd. for $C_{21}H_{18}N_2O_2S$ 362.1089; found 362.1083. ^{1}H-NMR (90 MHz, CD_2Cl_2) δ 8.65 (s, 1H, C(4)H), 8.55 (s(br), 1H, N(9)H, 8.16 (d, J=7.6 Hz, 1H, C(5)H), 7.60-7.19 (m, 8H, C(5)-C(7)H and C_6H_5), 4.69 (s, 2H, S-$CH_2C_6H_5$), 4.59 (1, 2H, OCH_2CH_3), 1.49 (t, 3H, OCH_2CH_3). Anal. calcd. for $C_{21}H_{18}N_2O_2S$ (M 362.451) C 69.59, H 5.01, N 7.73; found C 69.58, H 5.01, N 7.72.

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Received, 20th June, 1986