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Inhibitors of Monoamine Oxidase III: 9-Substituted-β-Carbolines

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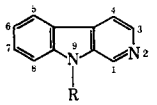
Abstract □ A series of 9-alkyl aromatic β-carbolines was synthesized and evaluated as inhibitors of mitrochondrial monoamine oxidase. The possible existence of a hydrophobic or hydrophilic region on the enzyme was explored. Substitution of an electron-withdrawing group such as acetyl on N-9 position reduced the inhibitory activity. This suggested that the increase in the inhibitory activity of 9-methyl-β-carboline was at least partially due to the increase of its electron density by the methyl group, thus making the β-carboline bind better to the enzyme.

Keyphrases □ Monoamine oxidase (MAO) inhibitors—synthesis □ β-Carbolines, 9-substituted—synthesis □ Pharmacological screening—β-carbolines, MAO inhibitors □ IR spectrophotometry—structure □ UV spectrophotometry—structure

From previous work (1), a 35-fold increase in inhibitory activity of tetrahydro-β-carboline was observed when a methyl group was introduced on the indolic nitrogen. This was attributed to either the increase in the binding of the indole nucleus as a result of the electron-donating property of the methyl group or the binding of the methyl group itself hydrophobically to the enzyme, or both. Although the methylation of the indole nitrogen of the fully aromatic β-carboline (I) did not give such a large increase in the inhibitory activity, the resulting 9-methyl compound (II) was three times more active than the parent compound. It would seem worthwhile to explore the effect of other 9-alkyl groups on the inhibitory activities of aromatic β-carbolines. The hydrophobic region of an enzyme has been demonstrated to be advantageous to the design of inhibitors, because it would contribute to a great extent to the binding of inhibitors to the enzyme (2).

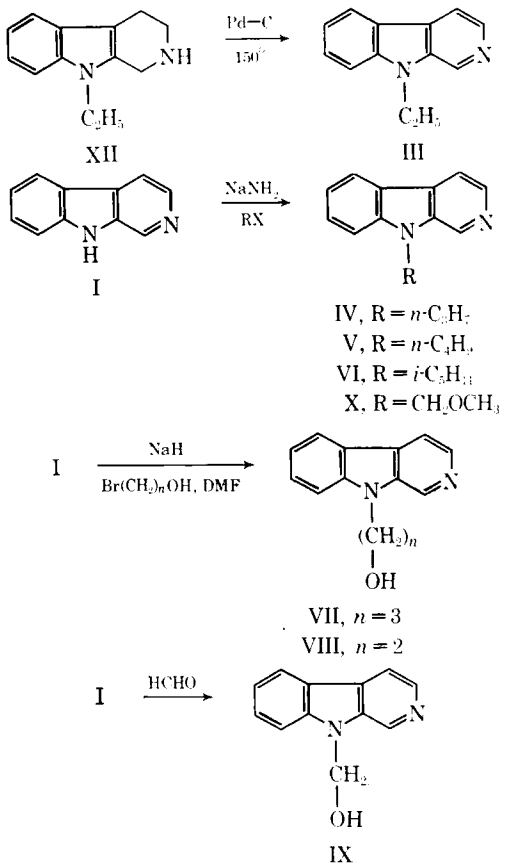
Table I showed that the inhibitory activities of 9-

Table I—Inhibition of MAO by



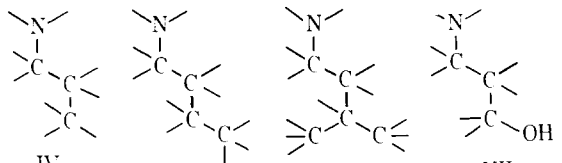
Compd	R	I_{50}, mM[a]
I	H	0.029[b]
II	CH_3	0.010[b]
III[c]	C_2H_5	0.048
IV[c]	n-C_3H_7	0.15
V[c]	n-C_4H_9	0.14
VI	i-C_5H_{11}	1.8
VII	$CH_2CH_2CH_2OH$	0.48
VIII	CH_2CH_2OH	0.11
IX[c]	CH_2OH	0.034
X[c]	CH_2OCH_3	0.40
XI	$COCH_3$	0.15

[a] Concentration of an inhibitor giving 50% inhibition of the enzyme. [b] Data from *Reference 1*. [c] Hydrochloride salt.



substituted-β-carbolines decreased as the length of the alkyl chain increased up to the propyl. A fivefold decrease in activity resulted from 9-methyl (II) to 9-ethyl (III) and a threefold decrease from 9-ethyl (III) to 9-propyl (IV). The 9-butyl (V) and 9-propyl (IV) compounds were equally as active. The decrease in activity from 9-methyl to 9-ethyl was, most likely, due to the increase in bulk of the ethyl group rather than to the projection of the terminal hydrophobic CH_3 of the ethyl into a hydrophilic region of the enzyme. This conclusion was based on finding that 9-hydroxymethyl (IX) and 9-methoxymethyl (X), each bearing an oxygen-containing group capable of forming hydrogen bonding with the enzyme, were not better inhibitors of MAO than III. A similar argument can also be held to account for the threefold decrease in activity from 9-ethyl (III) to 9-propyl (IV) by a comparison of inhibitory activity of IV with that of the 9-hydroxyethyl compound (VIII).

Branching of the 9-butyl to 9-isoamyl (VI) caused a decrease in activity of more than tenfold. This branched chain could be sterically unfavorable to the binding of VI on the enzyme. Another rationalization for the cause



of this decrease in activity of VI would be the projection of one of its two terminal methyl groups into a hydrophilic region of the enzyme. The latter was ruled out because the 9-hydroxypropyl compound (VII) was found to be a poorer inhibitor than V. The terminal OH of VII would otherwise contribute to the binding through a formation of hydrogen bonding with the enzyme.

Introduction of a 9-acetyl group to I gave an inhibitor XI which was 15 times less active than the 9-methyl compound (II). This finding not only showed the decrease in the activity of β-carboline by an electron-withdrawing group, such as CH_3CO, but once more suggested that the increase in the inhibitory activity of β-carboline was at least partially due to the increase in the electron density of the β-carboline nucleus by the CH_3, thus making the β-carboline bind better. The influence of 9-substituent on the electron density of aromatic β-carbolines was obvious when their ultraviolet spectra were compared. Unsubstituted β-carboline (I) showed maximum absorption at 337 and 350 mμ. A bathochromic shift of these two peaks to 346 and 357 mμ was observed in the 9-methyl compound (II), whereas a hypsochromic shift to 313 and 324 mμ was found in the 9-acetyl compound (XI).

The vast difference in the effect of 9-alkyl groups on the inhibitory activities between aromatic β-carbolines (Table I) and tetrahydro congeners (3) indicated that the two series of compounds were probably bound to the enzyme in different conformations.

CHEMISTRY

9-Ethyl-β-carboline (III) was prepared by the palladium-on-charcoal catalyzed dehydrogenation of the corresponding tetrahydro compound (XII). The reaction was performed in a Parr bomb as previously described (4).

Alkylation of β-carboline (I) was carried out by refluxing a solution of I in toluene with sodium amide and the appropriate alkyl halides. An attempt was made to prepare VI by the treatment of I with sodium amide and 1-bromo-3-methylbutane in liquid ammonia. From this reaction only the starting material was isolated. It would seem that alkylation of I with higher alkyl halides ($R > CH_3$) might require higher temperature. Chloromethyl methyl ether reacted with the sodio β-carbolines (I) in tetrahydrofuran at room temperature to yield 9-methoxymethyl-β-carboline (X). The same reaction did not take place in liquid ammonia.

9-Hydroxypropyl-β-carboline (VII) and 9-hydroxyethyl-β-carboline (VIII) were obtained by the reaction of I with sodium hydride and appropriate ω-bromoalkanols in dimethylformamide. When toluene was substituted for dimethylformamide as the solvent in the preparation of VII, a mixture of the unreacted I and the product was isolated. An attempt to prepare VII by the same method as that described for IV–VI was unsuccessful; treatment of I with either 3-chloropropanol or 3-bromopropanol in toluene in the presence of sodium amide did not afford VII. 9-Hydroxymethyl-β-carboline (IX) was the product from a reaction of I with formaldehyde at room temperature.

EXPERIMENTAL[1]

Alkylation of β-Carboline—A mixture of 0.01 mole of β-carboline, an equal molar amount of sodium amide, and 25–50 ml. of toluene was refluxed with stirring for 4 to 5 hr. After cooling, 0.01 mole of the appropriate alkyl halides was added. (Iodopropane was dissolved in 10 ml. of toluene, 1-bromo-3-methylbutane in 10 ml. of ether, whereas iodobutane was added without solvent.) Refluxing was continued for an additional 1 hr. (6 hr. in the case of VI). Water (10–25 ml.) was added and the organic layer was separated, dried, then evaporated *in vacuo*.

9-*n*-Propyl-β-carboline (IV)—The oily residue from the reaction was converted into its hydrochloride salt and twice recrystallized from ethanol; m.p. 229–231°, yield 20%. $\lambda_{max.}$ (KBr): 4.0 (broad NH^+), 6.13, 6.22, 6.53, 6.66 (C=N, C=C), and 13.4 μ (indole CH).

Anal.—Calcd. for $C_{14}H_{15}ClN_2$: C, 68.2; H, 6.13; N, 11.4. Found: C, 68.0; H, 6.13; N, 11.4.

9-*n*-Butyl-β-carboline (V)—The oily residue was converted into its hydrochloride salt and twice recrystallized from absolute ethanol; m.p. 223–225°, yield 17%.

Anal.—Calcd. for $C_{15}H_{17}ClN_2$: C, 69.1; H, 6.57; N, 10.7. Found: C, 69.4; H, 6.60; N, 10.8.

9-Isoamyl-β-carboline (VI)—The solid residue from the reaction was recrystallized three times from benzene; m.p. 212–213°, yield 15%.

Anal.—Calcd. for $C_{16}H_{18}N_2$: C, 80.6; H, 7.61; N, 11.8. Found: C, 80.4; H, 7.67; N, 11.8.

9-Ethyl-β-carboline (III)—A mixture of 570 mg. (3 mmoles) of 9-ethyl-1,2,3,4-tetrahydro-β-carboline, obtained by neutralization of its hydrochloride salt (I), in 15 ml. of benzene and 200 mg. of 5% palladium-on-charcoal catalyst was heated in a Paar bomb at 150° for 5 hr. After cooling, the mixture was filtered and the filtrate evaporated *in vacuo* leaving an oil. A solution of the product in ether was mixed with an excess of ethereal HCl. The yellow hydrochloride salt was collected on a filter; yield, 500 mg. (71%), m.p. 240–243°. Two recrystallizations from absolute ethanol gave 256 mg. (51%), m.p. 249–252°. $\lambda_{max.}$ (KBr): 3.8 (broad NH^+), 6.10, 6.21, 6.52, 6.65 (C=N, C=C), and 13.15 μ (indole CH); $\lambda_{max.}$ (95% ethanol): 388, 362, 305, 290, 284, 260, 252, 243, 237, 218, and 210 mμ.

The picrate salt of the product was also prepared. Its melting point (226–227°) agreed with the reported melting point (227–228°) for III-picrate, prepared from platinum-catalyzed dehydrogenation of corresponding tetrahydro compound at 160–170° in the absence of a solvent (5).

9-(3-Hydroxypropyl)-β-carboline (VII)—To a solution of 2.5 g. (15 mmoles) of β-carboline (I) in 25 ml. of dimethylformamide was

[1] Melting points are corrected and were taken on a Fisher-Johns apparatus. IR spectra were obtained with a Perkin-Elmer spectrophotometer model 237B. For qualitative UV spectra a Beckman spectrophotometer model DB-G was used.

added with cooling 0.7 g. (15 mmoles) of sodium hydride (50% suspension in mineral oil). After stirring for 1 hr. at ambient temperature, a solution of 2.1 g. (15 mmoles) of 3-bromopropanol in 10 ml. of dimethylformamide was added slowly with cooling, and the reaction mixture was stirred overnight. Water (200 ml.) was added and the solution was extracted with chloroform (3 × 50 ml.). The combined chloroform extracts were washed with water (4 × 50 ml.), then 10% hydrochloric acid (2 × 25 ml.). The acid extracts were combined and washed with 50 ml. of chloroform. After neutralization of the aqueous solution with 10% aqueous sodium hydroxide, the product was extracted into chloroform (3 × 50 ml.). The combined chloroform extracts, after being washed twice with water, were dried with anhydrous sodium sulfate, then evaporated *in vacuo* to yield a crude solid. Recrystallization from chloroform gave 2.1 g. (64%), m.p. 126–129°. Another recrystallization from the same solvent yielded 1.7 g. (50%), m.p. 131–132°. One more recrystallization did not raise the melting point. $\lambda_{max.}$ (KBr): 3.19 (OH), 6.17, 6.41 (C=C, C=N), 9.8 (C—O), and 13.4 μ (indole CH); $\lambda_{max.}$ (CH_3OH): 360 (s), 345, 286, 280, 257 (s), 248 (s), 232, and 210 mμ.

Anal.—Calcd. for $C_{14}H_{14}N_2O$: C, 74.3; H, 6.24; N, 12.4. Found: C, 74.5; H, 6.20; N, 12.4.

A portion of 9-(3-hydroxypropyl)-β-carboline was converted into its picrate salt, m.p. 201–202°.

Anal.—Calcd. for $C_{14}H_{14}N_2 \cdot C_6H_3N_3O_7$: C, 52.8; H, 3.76; N, 15.4. Found: C, 52.6; H, 3.55; N, 15.2.

9-(2-Hydroxyethyl)-β-carboline (VIII)—By a reaction similar to that described in the preparation of VII, 9-(2-hydroxyethyl)-β-carboline was obtained in 86% yield, m.p. 131–145°. Recrystallization of this crude product from chloroform gave 29%, m.p. 157–159°. Another recrystallization from chloroform–heptane yielded 24%, m.p. 159–160°. One more recrystallization from the same solvent did not change the melting point of the compound.

Anal.—Calcd. for $C_{13}H_{12}N_2O$: C, 73.6; H, 5.70; N, 13.2. Found: C, 73.8; H, 5.78; N, 13.2.

9-Hydroxymethyl-β-carboline (IX)—To a stirred suspension of 2 g. (12 mmoles) of β-carboline (I) was added dropwise concentrated hydrochloric acid until a solution was obtained. After being adjusted to pH between 5.5 and 6 with 10% aqueous sodium acetate, 10 ml. of 37% formaldehyde was added, and the mixture was allowed to stand at room temperature for 4 days. Stirring of the resulting solution caused precipitation of an off-white solid, which was collected on a filter and washed with 5 ml. of water; yield, 1.1 g. (39%) of hydrochloride salt, m.p. 218–219°. $\lambda_{max.}$ (KBr): 3.10 (OH), 6.11, 6.22, 6.35, 6.65 (C=N, C=C), and 13.1 μ (indole CH); $\lambda_{max.}$ (EtOH): 215, 235, 250 (s), 283 (s), 289, 304, 354 (s), and 375 mμ.

Anal.—Calcd. for $C_{12}H_{11}ClN_2O \cdot 0.5H_2O$: C, 59.1; H, 4.96; N, 11.5. Found: C, 59.2; H, 4.99; N, 11.5.

9-Methoxymethyl-β-carboline (X)—To a stirred suspension of 1.4 g. (30 mmoles) of sodium hydride (50% suspension in mineral oil) in 25 ml. of tetrahydrofuran was added over a period of 20 min. a solution of 3.3 g. (20 mmoles) of β-carboline (I) in 40 ml. of tetrahydrofuran. After a 2-hr. stirring at room temperature, 2.8 g. (35 mmoles) of chloromethyl methyl ether in 10 ml. of tetrahydrofuran was added over 10 min. Stirring was continued for 20 min., 10 ml. of water was added, and the mixture was allowed to stand overnight at room temperature. The solution was concentrated to about 20 ml. and the oily product, which was liberated upon the addition of 50 ml. of water, was extracted into ether (2 × 100 ml.). The combined ethereal extracts were dried with anhydrous sodium sulfate, treated with charcoal, and filtered. When the filtrate was mixed with ether–HCl, the hydrochloride salt separated as a dark oil, which was extracted into water (2 × 50 ml.). The combined aqueous extracts were made strongly basic with 10% aqueous potassium hydroxide and extracted with ether (3 × 50 ml.). After being dried with anhydrous sodium sulfate and treated with charcoal the ether was evaporated *in vacuo* leaving 1.4 g. (33%) of golden oil. An ethereal solution of this oily product when mixed with ether–HCl gave a solid hydrochloride salt, which was then recrystallized from 50% aqueous ethanol; yield, 1.0 g. (20%), m.p. 233°. $\lambda_{max.}$ (KBr): 6.10, 6.20, 6.22, 6.50, 6.65 (C=N, C=C), and 13.25 μ (indole CH); $\lambda_{max.}$ (EtOH): 208, 215, 236, 249 (s), 281 (s), 287, 303, 351 (s), and 373 mμ.

Anal.—Calcd. for $C_{13}H_{13}ClN_2O$: C, 62.8; H, 5.26; N, 11.3. Found: C, 62.6; H, 5.38; H, 11.3.

9-Acetyl-β-carboline (XI)—A mixture of 2 g. (12 mmoles) of β-carboline (I), 7 g. of anhydrous sodium acetate, and 28 ml. of acetic anhydride was refluxed with stirring for 4 hr. With external cooling a mixture of crushed ice and water was added and the solution was adjusted to about pH 11 with 10% aqueous KOH. The precipitate was collected on a filter and dried *in vacuo*; yield, 2.2 g. (88%) of light tan solid, m.p. 120–126°. This crude product was dissolved in 17 ml. of hot benzene, treated with charcoal, and filtered through diatomaceous earth. To the filtrate was added 45 ml. of heptane, and upon cooling the hot mixture 1.35 g. of needles, m.p. 123–125°, deposited. Another recrystallization from benzene–heptane gave 0.63 g. (25.2%) of product, m.p. 129–130°. $\lambda_{max.}$ (KBr): 5.90, 6.18, 6.38 (C=O, C=N, C=C); and 13.35 μ (indole CH); $\lambda_{max.}$ (EtOH): 208, 224 (s), 229, 265, 273 (s), 282, 313, and 324 mμ.

Anal.—Calcd. for $C_{13}H_{10}N_2O$: C, 74.3; H, 4.79; N, 13.3. Found: C, 74. 2;H, 4.83; N, 13.47.

To the authors' knowledge the preparation of XI has not yet been reported. Literature cited in the subject index of *Chemical Abstracts* does not refer to 9-acetyl-β-carboline (6).

Assay—Mitochondrial monoamine oxidase from beef liver was isolated and purified as previously described (1). All the stock solutions of the hydrochloride salts of inhibitors were prepared in water. Compounds VI, VII, and VIII were dissolved in 0.01 *N* HCl and Compound X in dimethyl sulfoxide. Incubation was carried out with tryptamine-2-^{14}C hydrochloride according to the previously described procedure (1).

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