Inhibitors of Monoamine Oxidase IV: 6(or 8)-Substituted Tetrahydro-β-carbolines and Their 9-Methyl Analogs

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Abstract □ Several analogs of tetrahydro-β-carboline (I) and 9-methyltetrahydro-β-carboline (VII) with substituents on C-6 or C-8 were synthesized. Methylation of the N-9 position of a tetrahydro β-carboline is generally achieved in the presence of a base. However, the preparations of 8,9-dimethyltetrahydro-β-carboline and 8-chloro-9-methyltetrahydro-β-carboline from 8-methyltetrahydro-β-carboline and 8-chlorotetrahydro-β-carboline, respectively, required the blockage of the N-2 position, otherwise methylation would favor this position over the N-9 due to the steric interference caused by the C-8 substituent. Although C-6 substitution in general resulted in a decrease in inhibitory activity, introduction of a methyl group to C-8 of I and VII did not affect the activities of I and VII. When the 8-methyl groups of the two series were replaced by chlorine atoms, a fourfold increase in activity was observed. The inhibitory activity of the resulting 8-chloro-9-methyltetrahydro-β-carboline was even greater than VII, the best inhibitor previously reported.

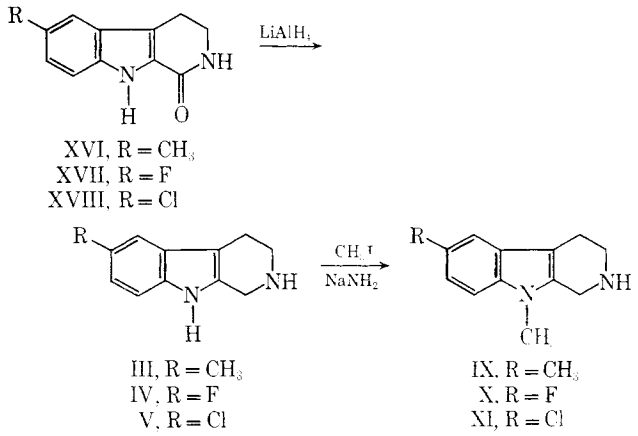
Keyphrases □ Monoamine oxidase inhibitors—synthesis □ Tetrahydro-β-carbolines, 6 (or 8)-substituted and 9-methyl analogs—synthesis □ Structure-activity relationship—monoamine oxidase inhibition □ IR spectrophotometry—analysis

Substitution of hydrogen on C-6 position of tetrahydro-β-carboline (I), in general, resulted in a slight decrease in inhibitory activity (see Table I—Compounds II–VI). An even greater loss of activity was observed when the same position of 9-methyltetrahydro-β-carboline (VII) was substituted (see Table I, Compounds VIII–XI). It is most likely that both the steric interaction of these 6-substituents with the enzyme and their electronic nature could account for the decrease in activity of I or VII. Between atoms or groups of similar size, the methoxy group of II being an electron-donating group caused a fourfold decrease in inhibitory activity of I, compared to the electron-withdrawing bromine atom (in VI) which decreased the inhibition by only 1.4-fold. Also, the 6-methyl compound (III) was 1.6-fold less active than the 6-chloro compound (V). In the 9-methyl series, a 367-fold decrease resulted from the substitution of the C-6 position of VII by an electron-donating methoxy group (see VIII).

A methyl group on C-8 of XII and XIII did not seem to affect the inhibition of the enzyme. Compounds XII and I were inhibitors of the same activity, and Compound XIII was only slightly less active than Compound VII. Chlorine substitution on C-8 of the two series, on the other hand, gave better inhibitors: XIV was 3.4 times more active than I, and XV was 2.6 times as active as VII. The possibility that a formation of hydrophobic bonding between the chlorine atom and the enzyme was ruled out in view of the finding that the 8-methyl compounds (XII and XIII) were not better inhibitors than the corresponding 8-hydrogen compounds (I and VII). It remains to be determined if the chlorine atom affected the electron density of the indole ring and thus made XIV and XV complex better with the enzyme. This study is currently being pursued in these laboratories.

CHEMISTRY

All 6- and 8-substituted tetrahydro-β-carbolines except the 6-bromotetrahydro-β-carboline (VI) were obtained by the reduction of the corresponding 1-oxo compound with $LiAlH_4$ (Schemes I and II).

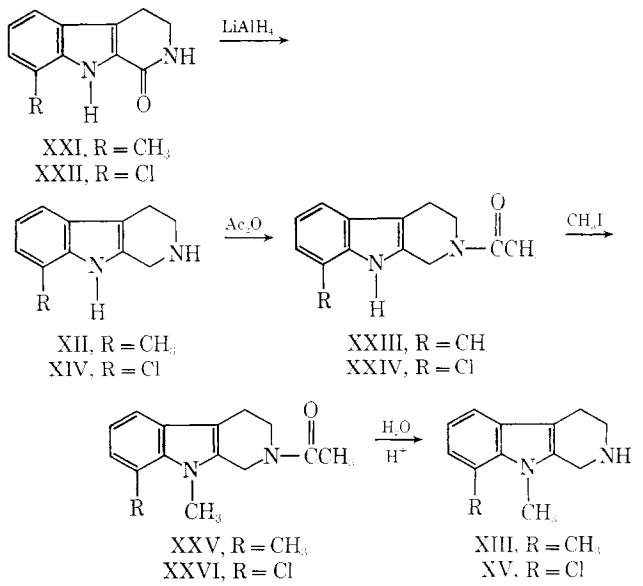


XVI, R = CH_3
XVII, R = F
XVIII, R = Cl

III, R = CH_3
IV, R = F
V, R = Cl

IX, R = CH_3
X, R = F
XI, R = Cl

Scheme I



XXI, R = CH_3
XXII, R = Cl

XII, R = CH_3
XIV, R = Cl

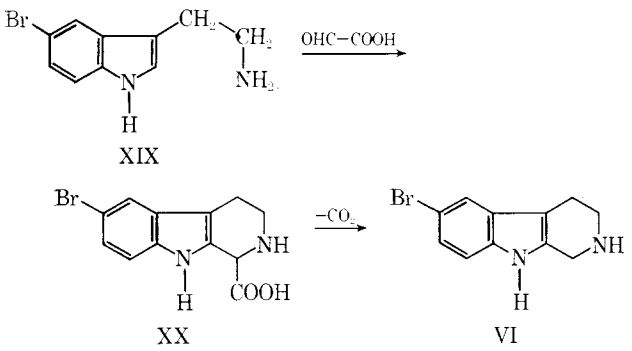
XXIII, R = CH
XXIV, R = Cl

XXV, R = CH_3
XXVI, R = Cl

XIII, R = CH_3
XV, R = Cl

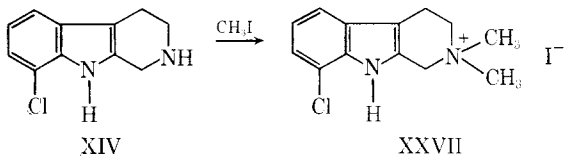
Scheme II

For the preparation of VI, 5-bromotryptamine (XIX) was first condensed with glyoxylic acid to give 6-bromotetrahydro-β-carboline-1-carboxylic acid (XX), which, upon refluxing in aqueous hydrochloric acid, decarboxylated and formed VI (Scheme III).



Scheme III

Methylation of III, IV, and V in liquid ammonia in the presence of sodium amide afforded the corresponding 9-methyltetrahydro-β-carbolines (IX–XI, Scheme I). However, when a similar reaction was carried out on 8-chlorotetrahydro-β-carboline (XIV), the only product isolated was the quaternary compound XXVII (Scheme IV). The crowded situation on the indolic nitrogen (N-9) caused by



Scheme IV

the adjacent 8-Cl group seemed to favor methylation on the piperidino nitrogen (N-2). This difficulty was finally overcome by blocking N-2 with an acetyl group before the introduction of the methyl group on N-9, and the subsequent hydrolysis of the amide linkage on N-2 yielded the 8-chloro-9-methyltetrahydro-β-carboline (XV, Scheme III). Methylation of 2-acetyl-8-chlorotetrahydro-β-carboline (XXIV) in the presence of sodium hydride afforded a better yield (76%) of the 9-methyl compound (XXVI) than the procedure with sodium amide (52%). For the preparation of 8,9-dimethyltetrahydro-β-carboline (XIII), the same method used for XV was followed (Scheme II).

EXPERIMENTAL[1]

6-Chloro-1,2,3,4-tetrahydro-β-carboline (V)—A solution of 3.3 g. (15 mmoles) of 6-chloro-1-oxo-1,2,3,4-tetrahydro-β-carboline in 25 ml. of tetrahydrofuran was added over a period of 45 min. to a stirred suspension of 2.3 g. (60 mmoles) of lithium aluminum hydride in 25 ml. of tetrahydrofuran. After being refluxed for 1 hr., the mixture was cooled in ice and 5 ml. of water was added. A total of 10 ml. of tetrahydrofuran was added to facilitate the stirring. The precipitate was filtered and washed with 50 ml. of tetrahydrofuran. The combined filtrate and washings were evaporated *in vacuo* leaving 2.6 g. (84%) of solid, m.p. 230–232°. Recrystallization of this product from toluene yielded 2.4 g. with the melting point unchanged; $\lambda_{max.}$ (KBr): 2.95, 3.05 (NH); 6.15, 6.34, 6.68 μ (C=C, CH_2).

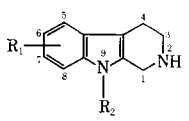
Anal.—Calcd. for $C_{11}H_{11}ClN_2$: C, 63.9; H, 5.36; N, 13.6. Found: C, 63.9; H, 5.27; N, 13.4.

6-Fluoro-1,2,3,4-tetrahydro-β-carboline (IV)—In a similar manner as in the preparation of V, 5.0 g. (25 mmoles) of 6-fluoro-1-oxo-1,2,3,4-tetrahydro-β-carboline in 50 ml. of tetrahydrofuran was reduced with 3.8 g. (100 mmoles) of lithium aluminum hydride in 50 ml. of tetrahydrofuran to yield 4.1 g. (86%) of product, m.p. 207–208°. One recrystallization from toluene gave 3.7 g. (79%), m.p. 212–313°.

A m.p. of 226–228° has been recorded for this compound prepared from 5-fluorotryptamine, formaldehyde, and formic acid (2).

[1] Melting points are corrected and were taken on a Fisher-Johns or Mel-Temp apparatus. IR spectra were taken with a Perkin-Elmer spectrophotometer model 237B.

Table I—Inhibition of Monoamine Oxidase by



Compd.	R_1	R_2	I_{50}[a], m*M*
I	H	H	0.34[b]
II	6-OCH_3	H	1.30
III[c]	6-CH_3	H	0.68
IV[c]	6-F	H	0.52
V	6-Cl	H	0.42
VI	6-Br	H	0.46
VII	H	CH_3	0.010[b]
VIII[d]	6-OCH_3	CH_3	3.60
IX[c]	6-CH_3	CH_3	0.10
X[c]	6-F	CH_3	0.12
XI[c]	6-Cl	CH_3	0.18
XII	8-CH_3	H	0.38
XIII[c]	8-CH_3	CH_3	0.016
XIV	8-Cl	H	0.10
XV[c]	8-Cl	CH_3	0.0038

[a] Concentration of an inhibitor giving 50% inhibition of the enzyme. [b] Data from *Reference 1*. [c] Hydrochloride salt. [d] B. T. Ho, *et al.*, to be published.

For analysis the product was converted to its hydrochloride salt, m.p. 310–311° (ethanol-ether); $\lambda_{max.}$ (KBr): 3.18, 3.46, 3.60, 3.70, 3.74, 3.80, 3.84, 3.92, 3.96, 4.02, 4.15 (NH, CH, NH^+); 6.17, 6.30, 6.38 μ (NH^+, C=C).

Anal.—Calcd. for $C_{11}H_{11}FN_2 \cdot HCl$: C, 58.3; H, 5.34; N, 12.4. Found: C, 58.5; H, 5.57; N, 12.2.

6-Methyl-1,2,3,4-tetrahydro-β-carboline (III)— In a similar manner as in the preparation of V, 1.0 g. (5 mmoles) of 6-methyl-1-oxo-1,2,3,4-tetrahydro-β-carboline in 100 ml. of hot benzene was reduced with 1.1 g. (30 mmoles) of lithium aluminum hydride in 50 ml. of ether. The product from the reaction was isolated as the hydrochloride salt; yield, 0.75 g. (68%), m.p. 291–293° (ethanol); $\lambda_{max.}$ (KBr): 2.90, 3.19, 3.40, 3.45, 3.60, 3.85, 3.95, 4.05, 4.10 (NH, CH, NH^+); 6.20, 6.29, 6.35, 6.40, 6.48, 6.80 μ (NH^+, C=C, CH_2).

Anal.—Calcd. for $C_{12}H_{14}N_2 \cdot HCl$: C, 64.7; H, 6.79; N, 12.6. Found: C, 64.5; H, 7.08; N, 12.7.

In a later preparation, the reduction was carried out in tetrahydrofuran and the product was isolated as the free base, m.p. 189–190°; yield, 93% [lit. (3), m.p. 189–190°].

6 - Bromo - 1,2,3,4 - tetrahydro - β - carboline - 1 - carboxylic Acid (XX)—To a solution of 1.0 g. (3.6 mmoles) of 5-bromotryptamine hydrochloride (4) in 15 ml. of water was added with stirring a solution of 335 mg. (3.6 mmoles) of glyoxylic acid monohydrate in 1 ml. of water. The product precipitated while the pH of the solution was adjusted to about 4.5 with 10% aqueous sodium hydroxide. After stirring at room temperature for 4 hr., the precipitate was collected on a filter and washed with 10 ml. of ice water; yield, 500 mg.(43%), m.p. 221° dec., with evolution of CO_2; $\lambda_{max.}$ (KBr): 3.00 (NH, OH); 3.34, 3.36, 3.44, 3.50, 3.56, 3.74, 3.82, (CH, NH^+); 6.05, 6.10, 6.16, 6.28, 6.36, 6.42 μ (COO^-, NH^+, C=C).

Anal.—Calcd. for $C_{12}H_{10}BrN_2O_2$: C, 48.8; H, 3.74; N, 9.49. Found: C, 49.0; H, 3.79; N, 9.44.

6-Bromo-1,2,3,4-tetrahydro-β-carboline (VI)—A mixture of 1.6 g. (6.4 mmoles) of 6-bromo-1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid (XX), 6 ml. of concentrated hydrochloric acid, and 20 ml. of water was refluxed with stirring for 90 min. during which time light brown crystals deposited. This hydrochloride salt was collected on a filter, redissolved in water, and converted into the free base by the addition of 10% sodium hydroxide to about pH 11. The precipitate, 950 mg., was recrystallized from toluene to yield 700 mg. (51%), m.p. 232–234° dec. $\lambda_{max.}$ (KBr): 2.95, 3.04 (NH); 6.17, 6.35, 6.69, 6.89, 6.92, 6.97 μ (C=C, CH_2).

Anal.—Calcd. for $C_{11}H_{11}BrN_2$: C, 52.6; H, 4.42; N, 11.2. Found: C, 52.8; H, 4.46; N, 11.0.

6,9-Dimethyl-1,2,3,4-tetrahydro-β-carboline (IX)—6-Methyl-1,2,3,4-tetrahydro-β-carboline (III) (1.86 g., 0.01 mole) was added portionwise with stirring to a sodium amide solution, prepared by allowing 0.25 g. (0.011 g. atom) of sodium to react with 100 ml. of liquid ammonia in the presence of a catalytic amount of ferric ni-

trate. After 15 min., a solution of 1.56 g. (0.011 mole) of methyl iodide in 2 ml. of ether was slowly added and the mixture was stirred at room temperature for 30 min. The ammonia was evaporated on a warm water bath and the residue was partitioned between 50 ml. of water and 50 ml. of ether. The aqueous phase was separated and extracted with 50 ml. of chloroform. The combined ether and chloroform extracts were evaporated under reduced pressure leaving an oil which solidified upon drying in a desiccator *in vacuo;* yield, 2.0 g., m.p. 105–110°. Recrystallization from cyclohexane gave 850 mg. (42%), m.p. 114–115°. One more recrystallization from the same solvent gave 600 mg., m.p. 115–116°.

When the combined mother liquors were treated with ether–HCl 800 mg. of hydrochloride salt, m.p. 267–277°, was obtained. Recrystallization from ethanol gave 400 mg., m.p. 288–290°. One more recrystallization from ethanol gave 300 mg. (13%), m.p. 292–293°. Thus, the combination of the free base and hydrochloride afforded a total yield of 55%, $\lambda_{max.}$ (KBr): 2.95, 3.32, 3.40, 3.44, 3.46, 3.52, 3.60, 3.70, 3.76, 3.84, 3.94, 4.06, 4.14, 4.32, 4.52, 4.79 (NH, CH, NH^+); 6.29, 6.32, 6.72, 6.82, 6.86, 6.95 μ (C=C, CH_2).

Anal.—Calcd. for $C_{13}H_{16}N_2 \cdot HCl$: C, 66.0; H, 7.24; N, 11.8. Found: C, 66.1; H, 7.31; N, 11.7.

6-Fluoro-9-methyl-1,2,3,4-tetrahydro-β-carboline (X)—In a similar manner as in the preparation of IX reaction of 0.01 mole of 6-fluoro-1,2,3,4-tetrahydro-β-carboline (IV) with 0.011 mole of sodium amide and 0.011 mole of methyl iodide gave 2.0 g. of oil. When a benzene solution of the product was mixed with ether–HCl, the hydrochloride salt, m.p. 289–292°, was obtained in a 96% yield. Recrystallization from ethanol gave 51%, m.p. 293–294°. A second recrystallization from ethanol–ether yielded 36%, m.p. 301–302°; $\lambda_{max.}$ (KBr): 2.95, 3.31, 3.40, 3.45, 3.61, 3.68, 3.76, 3.84, 3.94, 4.04, 4.12, 4.32, 4.54 (NH, CH, NH^+); 6.16, 6.29, 6.32, 6.72, 6.85, 6.94, 6.96 μ (C=C, CH_2).

Anal.—Calcd. for $C_{12}H_{13}FN_2 \cdot HCl$: C, 59.9; H, 5.86; N, 11.6. Found: C, 60.0; H, 5.95; N, 11.6.

6-Chloro-9-methyl-1,2,3,4-tetrahydro-β-carboline (XI)—Methylation of the 6-chloro-1,2,3,4-tetrahydro-β-carboline (V), as in the preparation of X, gave 85% of the product isolated as hydrochloride salt, m.p. 262–267°. Recrystallization from ethanol gave 39%, m.p. 302–303°; $\lambda_{max.}$ (KBr): 2.94, 3.40, 3.42, 3.46, 3.02, 3.60, 3.69, 3.76, 3.84, 3.87, 4.08, 4.38 (NH, CH, NH^+); 6.29, 6.35, 6.79, 6.88, 6.96μ (C=C, CH_2).

Anal.—Calcd. for $C_{12}H_{13}ClN_2 \cdot HCl$: C, 56.0; H, 5.49; N, 10.9. Found: C, 56.1; H, 5.56; N, 10.9.

8-Methyl-1,2,3,4-tetrahydro-β-carboline (XII)—The reduction of 8-methyl-1-oxo-1,2,3,4-tetrahydro-β-carboline was carried out in a similar manner as described in the preparation of V except the refluxing time was 4 hr. A crude product, m.p. 204–207° dec., was obtained in 79% yield. Recrystallization from benzene gave 59% of needles, m.p. 213–215°. The melting point remained unchanged after a second recrystallization. $\lambda_{max.}$ (KBr): 2.95, 3.05 (NH): 3.20, 3.29, 3.38, 3.41, 3.44, 3.48, 3.52, 3.65 (CH); 6.18, 6.30, 6.68, 6.75, 6.90, 6.95 μ (C=C, CH_2).

Anal.—Calcd. for $C_{11}H_{11}ClN_2$: C, 77.4; H, 7.58; N, 15.0. Found: C, 77.3; H, 7.62; N, 15.2.

2-Acetyl-8-methyl-1,2,3,4-tetrahydro-β-carboline (XXIII)—8-Methyltetrahydro-β-carboline (XII) 2.4 g. (13 mmoles) was mixed with 10 ml. of acetic anhydride. The solid dissolved but precipitation occurred almost instantly. The mixture was poured into 150 ml. of water. The solid was collected on a filter and washed successively with 25 ml. of 10% HCl, 25 ml. of 2 *N* NaOH, and 25 ml. of water; yield, 2.6 g. (90%), m.p. 208–209°. Recrystallization from aqueous ethanol gave 2.0 g. (69%), m.p. 211–212°; $\lambda_{max.}$ (KBr): 3.06 (indole NH); 3.30, 3.37, 3.45, 3.52, 3.55 (CH); 6.10, 6.19, 6.31, 6.70, 6.81, 6.89, 6.92, 6.99, 7.05 μ (C=O, C=C, CH_2).

Anal.—Calcd. for $C_{14}H_{16}N_2O$: C, 73.7; H, 7.06; N, 12.3. Found: C, 73.8; H, 7.08; N, 12.2.

2-Acetyl-8,9-dimethyl-1,2,3,4-tetrahydro-β-carboline (XXV)—A mixture of 4.30 g. (20 mmoles) of 2-acetyl-8-methyltetrahydro-β-carboline (XXIII), 1.56 g. (40 mmoles) of sodium amide, and 50 ml. of toluene was refluxed with stirring for 6 hr. After cooling, 5.68 g. (40 mmoles) of methyl iodide in 10 ml. of toluene was added slowly over a period of 15 min., and the mixture was refluxed for 2 hr. Water (25 ml.) was added, and the aqueous phase, after being separated from the toluene phase, was washed with 25 ml. of chloroform. The combined chloroform and toluene extracts were washed with 10% HCl (3 × 25 ml.), dried, and evaporated *in vacuo* to yield 4.3 g. (90%) of product, m.p. 144–146°. Recrystallization from ethanol, with decolorizing charcoal added, gave 2.2 g., m.p. 149–150°. One more recrystallization from ethanol gave 2.0 g. (42%), m.p. 152–153°; $\lambda_{max.}$ (KBr): 3.29, 3.32, 3.39, 3.41, 3.44, 3.52 (CH); 6.14, 6.22, 6.30, 6.70, 6.83, 6.93 μ (C=O, C=C, CH_2).

Anal.—Calcd. for $C_{15}H_{18}N_2O$: C, 74.4; H, 7.49; N, 11.6. Found: C, 74.6; H, 7.55; N, 11.5.

8,9-Dimethyl-1,2,3,4-tetrahydro-β-carboline (XIII)—A mixture of 1.5 g. (6 mmoles) of 2-acetyl-8,9-dimethyltetrahydro-β-carboline (XXV) and 50 ml. of 20% aqueous sulfuric acid was refluxed for 1 hr. The resulting solution was extracted with chloroform (3 × 25 ml.). After separation the aqueous phase was made basic with 2 *N* NaOH, and the product extracted with chloroform (3 × 25 ml.). The combined chloroform extracts were washed with 25 ml. of water, dried with anhydrous sodium sulfate, and then evaporated *in vacuo*. When a solution of the residue in 25 ml. of benzene was mixed with ether–HCl, the hydrochloride salt precipitated; yield, 1.2 g. (82%), m.p. 250–253°. Recrystallization from ethanol gave 800 mg. (55%), m.p. 251–253°. Another recrystallization from the same solvent did not raise the melting point. $\lambda_{max.}$ (KBr): 2.95, 3.39, 3.43, 3.55, 3.60, 3.69, 3.78, 3.83, 3.93, 4.02, 4.09, 4.12 (NH, CH, NH^+); 6.20, 6.35, 6.72, 6.85, 6.92 μ (NH^+, C=C, CH_2).

Anal.—Calcd. for $C_{13}H_{16}N_2 \cdot HCl \cdot {}^1/_2H_2O$: C, 63.5; H, 7.38; N, 11.4. Found: C, 63.9; H, 7.20; N, 11.4.

8-Chloro-1,2,3,4-tetrahydro-β-carboline (XIV)—In a similar manner as in the preparation of XII, reduction of 8-chloro-1-oxo-1,2,3,4-tetrahydro-β-carboline gave 92% of crude product, m.p. 170–180°. The product was dissolved in tetrahydrofuran, then mixed with ether–HCl to yield the hydrochloride salt. Two recrystallizations from aqueous ethanol gave 41%, m.p. 324° dec. $\lambda_{max.}$ (KBr): 3.10 (NH), 3.30, 3.42, 3.54, 3.57, 3.65, 3.71, 3.75, 3.80, 3.99, 4.08 (CH, NH^+); 6.16, 6.25, 6.31, 6.41, 6.70, 6.78, 6.86, 6.98 μ (C=C, CH_2).

Anal.—Calcd. for $C_{11}H_{11}ClN_2 \cdot HCl$: C, 54.3; H, 4.97; N, 11.5. Found: C, 54.4; H, 4.91; N, 11.5.

A portion of the hydrochloride salt in hot water was treated with NaOH to yield 39% of the free amine, m.p. 213–214°.

2-Acetyl-8-chloro-1,2,3,4-tetrahydro-β-carboline (XXIV)—Acetylation of 8-chloro-tetrahydro-β-carboline, as in the preparation of XXIII, gave 97% of the product, m.p. 176.5–177.5°. Recrystallization from ethanol did not raise the melting point. $\lambda_{max.}$ (KBr); 2.95, 3.16, 3.21, 3.25, 3.33, 3.42, 3.48, 3.52 (NH, CH); 6.20, 6.30, 6.75, 6.89, 6.95, 7.05 μ (C=O, C=C, CH_2).

Anal.—Calcd. for $C_{13}H_{13}ClN_2O$: C, 62.8; H, 5.27; N, 11.3. Found: C, 63.0; H, 5.31; N, 11.4.

2-Acetyl-8-chloro-9-methyl-1,2,3,4-tetrahydro-β-carboline (XXVI)—To a solution of 3.5 g. (14 mmoles) of 2-acetyl-8-chloro-tetrahydro-β-carboline (XXIV) in 25 ml. of dimethylformamide was added with cooling 0.68 g. (14 mmoles) of sodium hydride (50% suspension in mineral oil). An initial precipitation gradually dissolved during a 2-hr. stirring at ambient temperature. Upon cooling, 2.8 g. (20 mmoles) of methyl iodide was added and the reaction mixture was stirred overnight. Water (200 ml.) was added and the product was extracted into chloroform (3 × 50 ml.). The combined chloroform extracts were washed with water (5 × 100 ml.) to remove the dimethylformamide, dried with anhydrous sodium sulfate, and evaporated *in vacuo* leaving 4.3 g. of product, m.p. 98–100°. Recrystallization from heptane gave 2.8 g. (76%), m.p. 107–108°. The melting point remained unchanged after another recrystallization. $\lambda_{max.}$ (KBr): 6.12 (C=O); 6.20, 6.31, 6.48, 6.80, 7.00 μ (C=C, CH).

Anal.—Calcd. for $C_{14}H_{15}ClN_2O$: C, 64.0; H, 5.75; N, 10.7. Found: C, 63.8; H, 5.77; N, 10.5.

8-Chloro-9-methyl-1,2,3,4-tetrahydro-β-carboline (XV)—A mixture of 2.5 g. (9.5 mmoles) of 2-acetyl-8-chloro-9-methyltetrahydro-β-carboline (XXVI) and 20 ml. of 20% aqueous H_2SO_4 was refluxed for 6 hr. After cooling, the solid was collected on a filter and washed with a small amount of water. The crude sulfate salt was dissolved in 500 ml. of hot water, made strongly basic with 30% NaOH, and the free amine was extracted into chloroform (4 × 100 ml). The combined chloroform extracts were dried with anhydrous sodium sulfate, and evaporated *in vacuo* leaving a solid m.p. 67–69°. This amine in 25 ml. of benzene was mixed with ether–HCl to yield 1.4 g. (58%) of hydrochloride salt, m.p. 293–295°. Recrystallization from aqueous ethanol gave 1.0 g. (42%), m.p. 295–296°. $\lambda_{max.}$ (KBr): 3.43, 3.64–3.68 (broad), 3.77, 3.85, 4.08 (CH, NH^+); 6.20, 6.32 (broad), 6.48, 6.75, 6.88, 6.95 μ (NH^+, C=C).

Anal.—Calcd. for $C_{12}H_{13}ClN_2 \cdot HCl$: C, 56.0; H, 5.49; N, 10.9. Found: C, 56.0; H, 5.47; N, 10.7.

2,2-Dimethyl-8-chloro-1,2,3,4-tetrahydro-β-carbolinium Iodide (XXVII)—A mixture of 0.8 g. (3.9 mmoles) of 8-chlorotetrahydro-β-carboline (XIV), 0.16 g. (4 mmoles) of sodium amide, and 50 ml. of toluene was refluxed with stirring for 4 hr. After cooling 0.57 g. (4 mmoles) of methyl iodide was added, and the mixture was first stirred at room temperature for 1 hr. then refluxed for 1 hr. The solvent was evaporated *in vacuo* and the residue washed with water; yield, 1.0 g., m.p. 188–208°. Another washing with benzene gave 700 mg. (48%), m.p. 240–242°. Recrystallization from ethanol gave 250 mg., m.p. 238–239°. The product gave a positive test for iodine (5). $\lambda_{max.}$ (KBr): 2.93, 3.15, 3.32, 3.42, 3.51 (NH, CH); 6.18, 6.28, 6.45, 6.79, 6.85, 6.95 μ (C=C, CH_2).

Anal.—Calcd. for $C_{13}H_{16}ClIN_2$: C, 43.1; H, 4.45, N, 7.73. Found: C, 43.3; H, 4.42; N, 7.62.

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 except XI·HCl were prepared in water. Compounds VI, VIII, and XII were dissolved in 0.01 *N* HCl, Compounds V and XI in dimethyl sulfoxide, and Compound XIV in 25% aqueous dimethyl sulfoxide. Incubation was carried out with tryptamine-2-^{14}C hydrochloride according to the previously described procedure (1).

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Comparative Pharmacokinetics of Coumarin Anticoagulants VI: Effect of Plasma Protein Binding on the Distribution and Elimination of Bishydroxycoumarin by Rats

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Abstract □ The partitioning of bishydroxycoumarin (BHC) from rat plasma to an organic solvent phase decreases with increasing drug concentration to a minimum value and then increases as the concentration is further increased. The same type of profile is observed in the partitioning of BHC from rat plasma to the liver, both *in vitro* and *in vivo*. The elimination of large doses (60 mg./kg.) of BHC in the rat is much more rapid than the elimination of smaller (2-20 mg./kg.) doses. A plot of the elimination rate constant of BHC as a function of dose yields a curve which is similar to the partitioning profiles of BHC from plasma to liver and from plasma to organic solvent. The minimum concentration ratio, liver:plasma, in a perfused liver system and in intact animals, and the minimum *in vivo* elimination rate constant, occur at the same plasma concentration of BHC. These results reflect the unusual concentration dependence of the plasma protein binding of this drug. They demonstrate the pronounced effect of protein binding on the distribution of BHC, and the effect of distribution on the elimination of this drug.

Keyphrases □ Coumarin anticoagulants—pharmacokinetics □ Bishydroxycoumarin (BHC)—distribution, elimination □ Elimination rates—dose relationship □ Plasma protein binding—BHC distribution, elimination

Bishydroxycoumarin (BHC) is a widely used anticoagulant that is extensively bound to plasma albumin and is eliminated from the body almost exclusively by biotransformation in the liver (1). Its unusual pharmacokinetic characteristics in man (2), which are also evident in the rhesus monkey (3), have stimulated numerous investigations (reviewed in *Reference* 4). Because BHC also shows an unusual concentration dependence in its binding to plasma protein (4), this drug is particularly suitable as a model to determine the effect of plasma protein binding on the distribution of a drug in the body, and to assess the effect of changes in distribution on the kinetics of its elimination. Accordingly, the plasma protein binding, distribution, and kinetics of elimination of BHC have been studied over a wide concentration range and will be described here.

EXPERIMENTAL

The extractability of BHC from rat plasma (4), the isolated rat liver perfusion system (1), and the method of determination of BHC in rat plasma (4) have been described in previous papers in this series. About 60 mg./kg. body weight of BHC was administered intravenously or intraperitoneally to male Sprague-Dawley rats weighing 240-600 g. A 5-mg./kg. dose was administered 1 week later. All other experimental conditions were as described previously (3), except that 6 mg./kg. vitamin K_1 (Aqua Mephyton, Merck Sharp and Dohme, West Point, Pa.) was administered intraperitoneally in all experiments immediately before injection of BHC. The apparent volume of distribution (V_d), the biologic half-life