Keyphrases

Thalictrum alkaloids
Alkaloid isolation–*Thalictrum fendleri*
Chromatography, partition and adsorption–separation
TLC and paper chromatography–separation
NMR spectrometry
IR spectrophotometry–structure
UV spectrophotometry–structure

Inhibitors of Monoamine Oxidase

Influence of Methyl Substitution on the Inhibitory Activity of β-Carbolines

By BENG T. HO, WILLIAM M. MCISAAC, KENNETH E. WALKER, and VICENTE ESTEVEZ

A number of tetrahydro and aromatic β-carbolines, mostly with a methyl substituent at various positions, were synthesized and their *in vitro* inhibitory activities on monoamine oxidase evaluated. Substitution of a methyl group at the N-9 nitrogen of tetrahydro-β-carboline gave a potent competitive inhibitor of the enzyme. Methyl groups at C-1 of both tetrahydro and aromatic β-carbolines generally reduced the potency, whereas introduction of a methyl group at the N-2 nitrogen of tetrahydro-β-carboline gave a compound of equal activity.

FOR MANY YEARS, since the discovery of iproniazid, inhibitors of the enzyme monoamine oxidase have been used as chemotherapeutic agents for the treatment of depression. However, the many potent antidepressants of the hydrazine class are hepatotoxic, and for this reason have fallen into disuse. Attempts, therefore, have been made to search for other antidepressants with more rapid onset and lower toxicity.

A number of β-carbolines have been found to be potent inhibitors of monoamine oxidase (1–3), yet little is known about the way these compounds bind to the enzyme. In this phase of these studies, a number of tetrahydro and aromatic β-carbolines, mostly with methyl substituents at various positions, were synthesized and their *in vitro* inhibitory activities evaluated. Information on the mode of binding of these compounds to the enzyme will be useful in the design and synthesis of more potent inhibitors which may become useful in the treatment of depression.

DISCUSSION

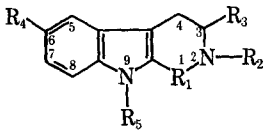
A fivefold decrease in inhibitory activity was observed when a methyl group was placed on C-1 of the tetrahydro-β-carbolines I and IV (Table I) and the aromatic β-carboline (XX)(Table II) to give II, V, and XXI, respectively. Ethyl substitution on C-1 of I and XX resulted in an even greater decrease in activity; III was seven times less active as an inhibitor than I, and XIII was 16 times less than XX. This loss of inhibitory activity could be attributed to steric hindrance by the bulk of the alkyl group. When a larger group such as COOH was substituted on C-1 of tetrahydro-β-carbolines, an even greater loss of activity occurred; the activity of XV was about 18 times less than I and four times less than II. However, the possibility of a repulsion between COO^- of XV and an anion site on the enzyme could not be entirely ruled out. Likewise, a methyl substituent on C-3 caused a threefold decrease in activity when I was compared with XVII. A COOH group on C-3 gave an inactive compound (XVIII).

Introduction of a methoxy group to C-6 of the ring decreased the inhibitory activity; a fourfold loss in activity was observed in both cases when I was converted into IV, or II into V. An eightfold decrease in activity was observed when 1,9-dimethyl-β-carboline (XII) was compared to its 6-methoxy derivative (XIII). If the indole nucleus binds as an electron donor to an electron-poor locus of the enzyme in a charge-transfer complex (4), the electron-donating methoxy group should make IV and V bind better. Thus, the decreased activity of 6-methoxytetrahydro-β-carbolines can be attributed either to the bulk of the methoxy group or to its polar nature, which is unfavorable for the binding. Studies to obtain greater binding of the indole nucleus by other substituents are in progress.

Neither methyl nor propyl substitution on N-2 of II affected the inhibitory activity; VI and VII were equally as active as II, indicating that a long alkyl

Received June 12, 1967, from the Biological Research Division, Texas Research Institute of Mental Sciences, and the Departments of Biochemistry and Psychiatry, Baylor University College of Medicine, Houston, TX 77025
Accepted for publication September 12, 1967.
This work was supported by grant MH-11168 from the U.S. Public Health Service, Bethesda, Md., and by the Britton Fund.
The authors thank Miss Patricia Kralik for her technical assistance in providing the enzyme assay data.

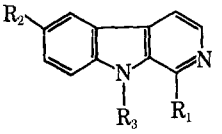
TABLE I—INHIBITION OF MONOAMINE OXIDASE BY TETRAHYDRO-β-CARBOLINES



Compd.	R_1	R_2	R_3	R_4	R_5	% Inhibition	mM Concn. Inhibitor
	Iproniazid					50	0.025
I[a]	CH_2	H	H	H	H	50	0.34
II[b]	$CHCH_3$	H	H	H	H	50	1.5
III[a]	$CHCH_2CH_3$	H	H	H	H	50	2.5
IV	CH_2	H	H	CH_3O	H	50	1.3
V[b]	$CHCH_3$	H	H	CH_3O	H	50	6.3
VI[a,b]	$CHCH_3$	CH_3	H	H	H	50	1.5
VII[a,b]	$CHCH_3$	n-C_3H_7	H	H	H	50	1.3
VIII	$CHCH_3$	CH_3CO	H	H	H	24	1.0[c]
IX	$CHCH_3$	CH_3CO	H	CH_3O	H	2	1.0
X[a]	$CHCH_3$	CH_3	H	H	CH_3	50	0.90
XI[a]	CH_2	H	H	H	CH_3	50	0.0098
XII[d]	$CHCH_3$	H	H	H	CH_3	50	0.085
XIII[a,b]	$CHCH_3$	H	H	CH_3O	CH_3	50	0.98
XIV	CHCOOH	H	H	H	CH_3	50	0.79
XV	CHCOOH	H	H	H	H	50	6.0
XVI	CHCOOH	H	H	CH_3O	H	6	1.0[e]
XVII	CH_2	H	CH_3	H	H	50	0.92
XVIII[b]	CH_2	H	COOH	H	H	50	16
XIX[b]	CO	H	H	H	H	50	2.5

[a] Hydrochloride salt. [b] Commercial sample. [c] Assay was run in the presence of 40 μl. of dimethylsulfoxide; this amount of dimethylsulfoxide alone gives 40% inhibition of the enzyme. [d] Sulfate salt. [e] Highest tested concentration.

TABLE II—INHIBITION OF MONOAMINE OXIDASE BY β-CARBOLINES



Compd.	R_1	R_2	R_3	% Inhibition	mM Concn. Inhibitor
XX[a]	H	H	H	50	0.029
XXI[a]	CH_3	H	H	50	0.14
XXII	CH_2CH_3	H	H	50	0.47
XXIII	H	H	CH_3	50	0.010

[a] Commercial sample.

chain like propyl can be tolerated by the enzyme provided the basicity of the N-2 nitrogen is not appreciably decreased. That a basic N-2 nitrogen is required for the binding of the β-carbolines to the enzyme is indicated by the fact that the acetyl derivatives (VIII and IX) of II and V show a marked decrease in activity. This was further observed in XIX where the basicity of the N-2 nitrogen was abolished by formation of a lactam linkage. Other evidence is seen from the work of McIsaac and Estevez (3), who found that replacement of the N-2 nitrogen of β-carbolines by CH completely abolished monoamine oxidase inhibitory activity.

An increase of 35-fold in inhibitory activity was observed in XI by the introduction of a methyl group at N-9 nitrogen of I. Only an eighteenfold increase was observed when a methyl group was placed at N-9 nitrogen of II; this difference is probably due to the steric interaction between the two adjacent methyl groups in XII. The effect of substitution on N-9, however, is not as great in the aromatic series. For example, the introduction of an N-9 methyl group to XX (Table II) resulted in XXIII which is only three times as active as XX.

In summary, tetrahydro-β-carbolines in general are not as good inhibitors of monoamine oxidase as aromatic β-carbolines *in vitro;* an approximately tenfold difference in inhibitory activity between these two series can be seen when I is compared to XX, and II to XXI. However, by substituting a methyl group at the N-9 nitrogen of the tetrahydro-β-carboline (I) a 35-fold increase in activity occurs, and the resulting XI becomes as potent as the aromatic β-carboline (XXIII). From the assay, XI appears to be three times more active than iproniazid (Table I). A Lineweaver-Burk plot showed that XI was a competitive inhibitor of the monoamine oxidase $Km = 8.1 \times 10^{-6}$ M and $Ki = 7.4 \times 10^{-6}$ M (Fig. 1). To our knowledge XI is so far the only

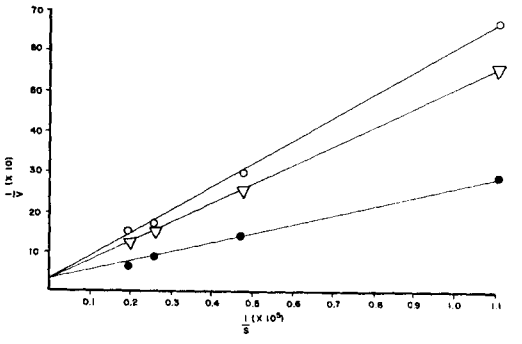


Fig. 1—Lineweaver-Burk plot of inhibition of MAO by XI. Key: ●, *no inhibitor,* ○, 1×10^{-6} M *XI,* ▽, 8×10^{-6} M *XI.*

TABLE III—PHYSICAL CONSTANTS OF β-CARBOLINES

Compd.	Method[a]	% Yield	M.p., °C.	Anal.,% C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
I	*A*	90	280[b,c]	...	...	...	...	...	...
III	*B*	45	261–262[d]	65.9[d]	66.0	7.23	7.30	11.8	12.0
XVI	*A*	50	224–226[b]	63.4	63.3	5.72	5.75	11.4	11.4
IV	*A*	87	268–269 dec.[d]	60.2[d]	60.0	6.31	6.45	11.7	11.9
VIII	*C*	30	205.5–206[e]						
IX	*C*	31	202–202.5[e]						
X	*D*	50	224–225 dec.[f,g]	52.4[f]	54.0	4.77	4.96	15.8	16.0
XI	*D*	50	257–258[d,f]						
XII	*D*	63	233 dec.[f,g]	53.1[g]	53.1	4.45	4.55	16.3	16.2
XVII	*A*	66	240–241[b]	77.4	77.2	7.57	7.65	15.0	15.2
XXII	*E*	7.2	218–219[d]	67.1[d]	66.9	5.62	5.70	12.0	11.8
XXIII	*D*	52	108–109[b]	79.1	79.2	5.53	5.57	15.4	15.5

[a] Method *A* is the condensation of the appropriate indolealkylamine with glyoxylic acid, followed by decarboxylation of the 1-carboxy derivative (see example under *Synthesis*); method *B* is the condensation of tryptamine with aldehyde; method *C* is the acetylation by acetic anhydride; method *D* is the methylation using sodium in liquid ammonia; method *E* is the cyclization and decarboxylation of *N*-acetyltryptophan with $POCl_3$ and polyphosphoric acid. [b] Free base. [c] Lit. m.p. 282–284° (7). [d] Hydrochloride salt. [e] Prepared from acetic anhydride and sodium acetate; lit. m.p. 205–207° (VIII) and 203–204.5° (IX) prepared from acetic anhydride alone (11). [f] Free base was an oil. [g] Monopicrate salt.

tetrahydro-β-carboline whose inhibitory activity is comparable to the most potent of the aromatic β-carbolines. There is no obvious explanation for the greater inhibitory activity of XI other than that the methyl group on N-9 makes the indole nucleus bind strongly to the enzyme. This methyl group may itself bind to the monoamine oxidase by a combination of van der Waals forces and hydrophobic bonding. However, the maximum increase in binding expected for one methyl group from both hydrophobic bonding and van der Waals forces would be only tenfold (5). Studies of the effect of other substituents of N-9 and N-2 of β-carbolines on inhibitory activity are in progress.

CHEMISTRY

Three tetrahydro-β-carbolines (IV, XI, and XVII, Table III) were synthesized from the condensation of the appropriate indolealkylamine with glyoxylic acid, followed by decarboxylation of the intermediates (XVI, XIV, and XXIV). Compound XVII prepared according to this method had a melting point of 240–241°, whereas 201–203° was recorded for this compound prepared by reacting α-methyltryptamine with formaldehyde (6). The synthesis of I by this route has been reported (7). The starting material for XIV, 1-methyltryptamine, was prepared according to the method of Potts and Saxton (8). In our preparation this oily amine was converted to its hydrochloride salt, m.p. 174–175°, in 42% yield. The melting point (239.5–240.5°) of the picrate of XI agreed with that in the literature (9) prepared by reduction of the 1-oxo derivative. Although the preparation of IV (10) and XI have been reported, our procedure seems to be more convenient than the reported method *via* the Na–BuOH reduction of the corresponding 1-oxo derivatives. At pH 4, 1-carboxytetrahydro-β-carboline precipitated, while glyoxylic acid and the salt of the unreacted tryptamine remained in the aqueous solution. In the next decarboxylation step, the product was obtained by precipitation from the basic medium in which unreacted amphoteric starting material, if any, was soluble in basic medium. Thus, in both steps the product can be obtained in a reasonably pure state and subsequent recrystallization should not be necessary. The decarboxylation was performed at 65–70° for 45 min. However, in a later run comparable yields were obtained when the decarboxylation was carried out at reflux temperature for only 30 min.

The condensation of tryptamine with propionaldehyde in aqueous acid medium was carried out at reflux temperature. 1-Ethyltetrahydro-β-carboline (III) was isolated as its hydrochloride salt.

The preparation of 1-ethyl-β-carboline (XXII) started from the acylation of tryptophan with propionyl chloride. Cyclization and subsequent decarboxylation of the resulting *N*-propionyltryptophan with $POCl_3$ and polyphosphoric acid gave XXII in a low yield of 7.2%. No attempt was made to improve the yield.

Alkylation of the sodium salts of 1-methyl-1,2,3,4-tetrahydro-β-carboline and its 2-methyl derivative with methyl iodide gave XII and X, respectively. 9-Methyl-β-carboline (XXIII) was prepared in the similar manner. An attempt was made to prepare X from XII. Treatment of XII with formaldehyde and formic acid did not give the desired product. Since the hydrochloride salts of both X and XII as well as the sulfate salt of X were hygroscopic, these two compounds were characterized as their picrate salts (Schemes I and II).

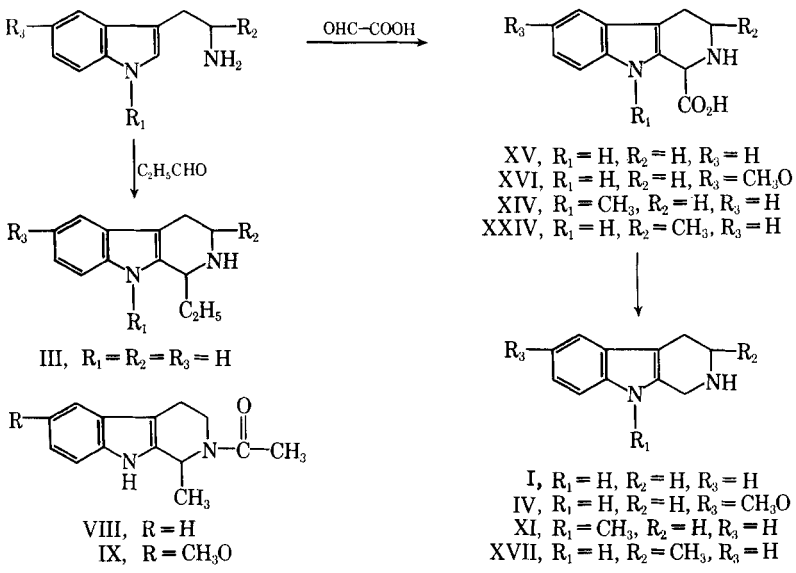
EXPERIMENTAL

Melting points were taken on a Fisher-Johns apparatus and are corrected.

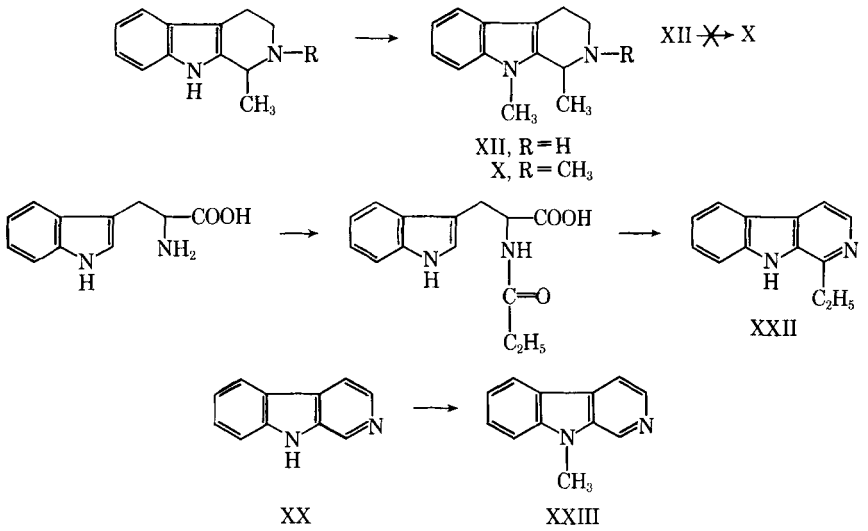
Synthesis

1-Ethyl-1,2,3,4-tetrahydro-β-carboline(III)—To a stirred suspension of 2.0 Gm. (12.5 mmoles) of tryptamine in 50 ml. of water was added 10% H_2SO_4 until the pH of the solution reached between 2–3. A stream of helium was flushed over the surface while the solution was heated to reflux. Propionaldehyde (3 ml., 40 mmoles) was added and the solution was allowed to stand for 30 min. without further heating. The condenser was removed and the solution was heated to boiling for an additional 30 min. It was made strongly basic with 10% KOH and extracted with ether (3 × 50 ml.). The ethereal extracts were combined and dried with anhydrous Na_2SO_4. Anhydrous hydrogen chloride was passed into the ethereal solution to precipitate the hydrochloride; yield, 2.2 Gm. (76%).

Thin-layer chromatography on silica using *n*-



Scheme I



Scheme II

propanol–concentrated NH_4OH (95:5) showed contamination of the product by tryptamine. One recrystallization from absolute ethanol–ether gave 1.3 Gm. (45%) of solid, m.p. 261–262°, which was free of starting material. $\nu_{max.}$ (KBr) 3240 (NH); 2950, 2900, 2760, 2670, 2620, 2575, 2480, 2380, 2290 (CH, NH^+); 1620, 1590 (C=C, NH^+); 745 cm.$^{-1}$ (indole CH). (See Table III for analytical data.)

6-Methoxy-1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid (XVI)—A solution of 400 mg. (4 mmoles) of glyoxylic acid monohydrate in 22 ml. of water was added with magnetic stirring to a solution of 1 Gm. (4 mmoles) of 5-methoxytryptamine hydrochloride in 8 ml. of water. The mixture was adjusted to pH 4 with 10% KOH and the product precipitated after a short time. After being stirred for 5 hr. at room temperature, the solid was collected on a filter and washed several times with small amounts of water; yield, 450 mg. (50%), m.p. 224–226°, with evolution of CO_2. $\nu_{max.}$ (KBr) 3300 (NH) 2900–2875, 2790, 2530 (CH, NH^+); 1645, 1590, 1570 cm.$^{-1}$ (COO^-, NH^+, C=C); $\lambda_{max.}$ (1% HCl) 220, 273, 293 (s), 367 mμ. (See Table III for analytical data.)

6-Methoxy-1,2,3,4-tetrahydro-β-carboline (IV)—A mixture of 400 mg. (1.7 mmoles) of XVI and 3 ml. of concentrated hydrochloric acid in 10 ml. of water was heated at 65–70° for 45 min., during which time a white solid deposited in the solution. Just enough water was added to dissolve the solid and the product was precipitated by adding excess of 10% KOH; yield, 276 mg. (87%), m.p. 216–218°. $\nu_{max.}$ (KBr) 3270 (NH); 1625, 1590, 1560 cm.$^{-1}$ (C=C); $\lambda_{max.}$ (EtOH) 220, 273, 293 (s), 362 mμ.

A melting point of 220–223° has been recorded for this compound prepared by reduction of 6-methoxy-1-oxo-1,2,3,4-tetrahydro-β-carboline (10).

For analysis this product was converted to the

hydrochloride salt, m.p. 268–269° dec.; $\nu_{max.}$ (KBr) 3190 (NH); 2900–2880, 2780–2740, 2640, 2570, 2560, 2500, 2440 (CH, NH^+); 1620, 1590, 1580, 1565 $cm.^{-1}$ (NH^+, C=C). (See Table III for analytical data.)

1-Ethyl-β-carboline (XXII)—To a solution of 5 Gm. (24.5 mmoles) of tryptophan in 200 ml. of ice cold 10% KOH was added a solution of 20 ml. of propionyl chloride in 200 ml. of benzene and the mixture was stirred vigorously. An additional small amount of solid KOH plus crushed ice was added to make the aqueous solution strongly basic; this was followed by addition of 5 ml. of propionyl chloride. This procedure was repeated once more. The aqueous layer, after being made strongly basic with solid KOH, was separated. It was then acidified with concentrated HCl to about pH 1 and extracted with chloroform (3 × 200 ml.). The combined chloroform extracts were dried with Na_2SO_4 and the product was precipitated by adding 1 L. of petroleum ether (b.p. 60–110°); yield, 4.3 Gm. (67%), m.p. 161–161.5°. $\nu_{max.}$ (KBr) 1715 (acid C=O); 1615, 1540 $cm.^{-1}$ (amide C=O and NH).

A vigorously stirred mixture of 4.2 Gm. (16 mmoles) of the above *N*-propionyltryptophan, 42 Gm. of polyphosphoric acid, and 9 ml. of $POCl_3$ was heated at 125° for 45 min., then poured onto 100 Gm. of crushed ice. After removal of the resinous material by filtration, the solution was made strongly basic with 10% KOH and extracted with ether (3 × 15 ml.). The ethereal extracts were combined and dried with anhydrous Na_2SO_4. Anhydrous hydrogen chloride was passed into the ethereal solution to precipitate the hydrochloride; yield, 1.2 Gm. (32%), m.p. 135–145°. This crude product was recrystallized several times from absolute ethanol–ether then from ethanol–heptane giving 270 mg. (7.2%) of beige solid, m.p. 218–219°, which was chromatographically pure [solvent, *n*-propanol–concentrated NH_4OH (95:5)]. $\nu_{max.}$ (KBr) 3340 (NH); 3125, 3040, 2950, 2900, 2850, 2800, 2750 (CH, NH^+); 1630, 1620, 1570, 1525, 1505 (C=C, NH^+); 820 (pyridine CH); 755 $cm.^{-1}$ (indole CH); $\lambda_{max.}$ (H_2O) 212, 233, 250 (s), 280 (s), 287, 335, 347. (See Table III for analytical data.)

1,9-Dimethyl-1,2,3,4-tetrahydro-β-carboline (XII)—1-Methyl-1,2,3,4-tetrahydro-β-carboline (1.86 Gm., 0.01 mole) was added slowly with stirring to a sodamide solution prepared by reacting 0.25 Gm. (0.011 mole) of Na with 60–70 ml. of liquid ammonia in the presence of a catalytic amount of ferric nitrate. After all the amine had dissolved (about 15 min.), 1.56 Gm. (0.011 mole) of methyl iodide in 20 ml. of ether was added slowly over a period of 30 min. Stirring was continued for an additional 20 min. The volatile solvents were removed by a stream of helium while the flask was immersed in hot water. The residue was extracted with benzene (3 × 5 ml.) and the combined extracts dried with anhydrous Na_2SO_4. An excess amount of 5% methanolic H_2SO_4 was added to the benzene solution and the sulfate salt solidified upon addition of ether. It was collected on a filter, washed with ether, and dried *in vacuo;* yield, 1.7 Gm. (63%), m.p. 239–242° dec.. $\nu_{max.}$ (KBr), 3350, 3275, 3225, 3200, 3000, 2930, 2890, 2850, 2800 (NH, CH, NH^+); 1600, 1555 (C=C); 745 $cm.^{-1}$ (indole CH); $\lambda_{max.}$ (1% HCl) 222, 274, 281, 288 mμ. For analysis the sulfate was converted to the monopicrate which was purified by recrystallization from absolute ethanol. (See Table III for analytical data.)

9-Methyl-β-carboline (XXIII)—β-Carboline (800 mg., 4.6 mmoles) was treated with a mixture of 114 mg. (5 mmoles) of sodium in liquid ammonia and 468 mg. (6.6 mmoles) of methyl iodide as in the preparation of 1,9-dimethyl-1,2,3,4-tetrahydro-β-carboline. The reaction residue was extracted with benzene, evaporated, and the residue dissolved in the minimum amount of petroleum ether (b.p. 30–60°). After removal of the insoluble starting material by filtration, the petroleum ether was evaporated *in vacuo* leaving a white solid. Recrystallization from heptane gave 434 mg. (52%) of product, m.p. 108–109°; $\nu_{max.}$ (KBr) 1620, 1595, 1555, 1495 (C=N, C=C); $\lambda_{max.}$ (EtOH) 216, 236, 241 (s), 251 (s), 264 (s), 284, 290, 346, 357 mμ. (See Table III for analytical data.)

A m.p. of 107° (12) and 109° (13) has been recorded for this compound prepared by the treatment of 9-methyl-1-oxo-β-carboline with $POCl_3$.

Assay

Beef liver was homogenized in 10 vol. of 0.25 *M* sucrose and the cell debris was removed by centrifugation. The supernatant was centrifuged at 13,000×g for 90 min. to sediment the mitochondrial pellet which was then suspended in 0.01 *M* phosphate buffer pH 7.6 and subjected to sonication at 20 kc./sec. for 20 min. The sonicated preparation was then centrifuged at 100,000×g for 60 min., and the supernatant containing monoamine oxidase (340 mμmole/hr./mg. protein) was used as the enzyme source. This procedure gives approximately sevenfold purification.

All the stock solutions of the hydrochloride salts of inhibitors were prepared in water and the solutions of free amines in 0.01 *N* HCl. Compound VIII was dissolved in dimethylsulfoxide, IX and XIX in 50% aqueous dimethylsulfoxide, XIV in 0.01 *N* NaOH, and XVI and XVIII in 0.05 *N* NaOH. Incubation was carried out at 37° for 30 min. in a solution containing 60 mμmoles of the substrate, tryptamine-2-^{14}C hydrochloride, varying amounts of inhibitor, 20 μl. of enzyme, phosphate buffer pH 7.4, and water to make a final volume of 1 ml. The product, a mixture of indoleacetaldehyde and indoleacetic acid, was extracted with ether in strong acidic medium. After the removal of solvent, the product was assayed for ^{14}C in a liquid scintillation spectrometer and the concentration of inhibitor at which enzyme activity was 50% inhibited was determined.

REFERENCES

(1) Udenfriend, S., Witkop, B., Redfield, B. G., and Weissbach, H., *Biochem. Pharmacol.*, **1**, 160(1958).
(2) Pletscher, A., Besendorf, H., Bachtold, H. P., and Gey, K. F., *Helv. Physiol. Pharmacol. Acta*, **17**, 202(1959).
(3) McIsaac, W. M., and Estevez, V., *Biochem. Pharmacol.*, **15**, 1625(1966).
(4) Cilento, G., and Giusti, P., *J. Am. Chem. Soc.*, **81**, 3801(1959).
(5) Baker, B. R., Ho, B. T., and Santi, D. V., *J. Pharm. Sci.*, **54**, 1415(1965).
(6) Terzyan, A. G., Akapyan, Zh. G., and Tatevosyan, G. T., *Izv. Akad. Nauk. Arm. SSR, Khim. Nauki*, **16** (No. 1) 87(1963); through *Chem. Abstr.*, **59**, 7501(1963).
(7) Vejdělek, Z. J., Trška, V., and Protiva, M., *J. Med. Pharm. Chem.*, **3**, 427(1961).
(8) Potts, K. T., and Saxton, J. E., *J. Chem. Soc.*, **1954**, 2641.
(9) Abramovich, R. A., *ibid.*, **1956**, 4593.
(10) Pachter, J. J., Mohibacher, R. J., and Zacharias, D. E., *J. Am. Chem. Soc.*, **83**, 635(1961).

(11) Taborsky, R. G., and McIsaac, W. M., *J Med. Chem.*, **7**, 135(1964).
(12) Moza, B. K., and Trojaneh, J., *Coll. Czech. Chem. Commun.*, **28**, 1419(1963); through *Chem. Abstr.*, **59**, 7584 (1963).
(13) Henecka, H., Timmler, H., and Lorenz, R., *Med. Chem. Abhandl. Med-Chem. Forschungsstaetten Farbenfabriker Bayer A: G.*, **7**, 277(1963); through *Chem. Abstr.*, **60**, 5472 (1964).

Keyphrases

Monoamine oxidase inhibitors
β-carbolines–synthesis
Methyl and methoxy substitution–activity effect
Ethyl substitution–activity effect
Propyl substitution–activity effect
IR spectrophotometry–structure
UV spectrophotometry–structure

Drug Release from Wax Matrices I

Analysis of Data with First-Order Kinetics and with the Diffusion-Controlled Model

By JOSEPH B. SCHWARTZ, ANTHONY P. SIMONELLI, and WILLIAM I. HIGUCHI

The release of drug into aqueous media from wax matrices has been investigated using benzoic acid and salicylic acid as the active ingredients. The data have been examined from the viewpoint of the first-order kinetic theory and that of a diffusion-controlled process. It was found that the experimental data are best analyzed using a diffusion-controlled model and square root of time release profiles. Parameters controlling the release are presented.

SEVERAL WORKERS (1–4) have recently reported the results of investigations regarding the factors influencing drug release from inert, insoluble matrices. Matrices composed of plastic polymers have been shown to exhibit release profiles which are best described by the linear square root of time dependence indicating that a diffusion-controlled mechanism is operative (2). Waxes, on the other hand, present a much more complicated system, both experimentally and theoretically, due to various physical-chemical factors and properties not present in the plastic systems.

As a result, the mechanism of release from wax matrices has not been as firmly established. In view of the large number of patents issued in this area, it would be highly desirable that the release rates from these systems also be quantitated by use of the diffusion model and the determination of the necessary controlling parameters made.

Theoretical treatment has shown that drug release from an insoluble, inert matrix is described by the T. Higuchi equation (5) if the rate-determining process is diffusion; and this is given by:

$$Q = \sqrt{\frac{D\epsilon}{\tau}(2A - \epsilon C_s)C_s t} \qquad \text{(Eq. 1)}$$

where Q is the amount of drug released per unit area of the disk exposed to the solvent; D is the diffusion coefficient of the drug in the solvent; ϵ is the porosity of the matrix; τ is the tortuosity of the matrix; A is the concentration of solid drug in the matrix; C_s is the solubility of the drug in the solvent; and t is time. Each of the above variables lends itself to experimental determination. Any units may be used for the above variables and constants, provided they are mutually consistent.

It was the purpose of this study to establish the applicability of the diffusion model to wax systems and to determine the controlling variables.

EXPERIMENTAL

Tablets composed of a drug homogeneously distributed throughout a wax matrix were prepared by the following method. The drug was suspended or dissolved in the melted waxes contained in an evaporating dish. Following the removal of heat, continual stirring until complete solidification had

Received April 14, 1967, from the College of Pharmacy, University of Michigan, Ann Arbor, MI 48104
Accepted for publication September 12, 1967.
Presented to the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Las Vegas meeting, April 1967.