Inhibitors of Monoamine Oxidase VI: Effects of Substitution on Inhibitory Activity of 6(or 8)-Substituted β-Carbolines

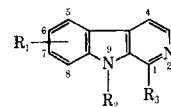BENG T. HO, KO-CHIN LI, K. E. WALKER, L. WAYNE TANSEY, PATRICIA M. KRALIK, and WILLIAM M. McISAAC

Abstract ☐ A number of 6(or 8)-substituted aromatic β-carbolines were synthesized, and their inhibitory activities toward monoamine oxidase were compared with their tetrahydro congeners. A considerable difference in the effects of 6(or 8)-substitution on the inhibitory activities existed between these aromatic and tetrahydro-β-carbolines. Influence of 9-methyl substitution on activities was greater with the tetrahydro than the aromatic series; as a result, 9-methyltetrahydro-β-carbolines were generally much better inhibitors of the enzyme than the corresponding 9-hydrogen-tetrahydro-β-carbolines. An amino group at C_1 of β-carboline caused a fivefold decrease in inhibitory activity. This decrease was likely due to the steric hindrance by the bulk of the amino group. Aromatic β-carbolines were prepared by the palladium-on-charcoal catalyzed dehydrogenation of the corresponding tetrahydro-β-carbolines. Methylation of the N_9 of aromatic β-carboline was carried out with methyl iodide in the presence of sodium hydride. Nitration of β-carboline gave a mixture of two isomeric products, 6- and 8-nitro-β-carbolines, which were separated with hot chloroform. Catalytic reduction converted the nitro compounds to 6- and 8-amino-β-carbolines, respectively. The positions of the amino group was confirmed by NMR spectrometry.

Keyphrases ☐ Monoamine oxidase inhibitors—synthesis ☐ β-Carbolines, 6(or 8)-substituted—synthesis ☐ Structure-activity relationship—monoamine oxidase inhibitors ☐ NMR spectroscopy—structure ☐ UV spectrophotometry—structure

Table I—Inhibition of Monoamine Oxidase

Compound	R_1	R_2	R_3	$I_{50},^a$ mM
II	H	H	H	0.029^b
III	H	CH_3	H	0.010^b
IV	H	H	CH_3	0.14^b
V	H	H	NH_2	0.15
VI	$6\text{-}OCH_3$	H	H	0.043
VII^c	$6\text{-}OCH_3$	CH_3	H	0.028
VIII	$6\text{-}CH_3$	H	H	0.12
IX	$6\text{-}CH_3$	CH_3	H	0.070
X	$6\text{-}Cl$	H	H	0.024
XI	$6\text{-}NH_2$	H	H	0.63
XII	$8\text{-}OCH_3$	H	H	0.12
XIII	$8\text{-}CH_3$	H	H	0.072
XIV^c	$8\text{-}CH_3$	CH_3	H	0.020
XV	$8\text{-}NH_2$	H	H	0.58

^{a} Concentration of an inhibitor giving 50% inhibition of the enzyme. ^{b} Data from *Reference 2*. ^{c} Hydrochloride salt.

In the previous papers (1, 2) the monoamine oxidase inhibitory activity of several 6(or 8)-substituted tetrahydro-β-carbolines and their 9-methyl analogs has been reported. It was found that, in general, replacement of hydrogen on the C_6 position of tetrahydro-β-carboline (Ia) caused a slight reduction in inhibitory activity. An even greater decrease of activity resulted when the same position of 9-methyltetrahydro-β-carboline (Ib) was substituted (1). Introduction of a methyl group on C_8 of Ia and Ib, however, did not affect the activity of these two compounds. In the present study, a number of 6(or 8)-substituted aromatic β-carbolines (V–XV) were synthesized, and their inhibitory activities were compared with those of the tetrahydro series.

A considerable difference in the effects of 6(or 8)-substitution on the inhibitory activities existed between these aromatic β-carbolines and their tetrahydro congeners. Replacement of the C_6-hydrogen of II by a methoxy group resulted in nearly a 1.5 time decrease in activity (Table I, Compound VI), whereas a greater loss (fourfold) of activity was observed by a similar substitution in the tetrahydro series (Table II Compounds Ia and Ic). When the 9-methyl compounds of each series were compared, 6-methoxy substitution on the 9-methyltetrahydro-β-carboline (Ib) gave Compound Id, which was 10 times less active than Ib as an inhibitor; however, only less than a threefold decrease in activity of 9-methyl-β-carboline (III) resulted from
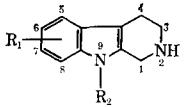
the conversion of III to 6-methoxy-9-methyl-β-carboline (VII). Substitution of chlorine on the C_6 position had less effect on both series, because X was equally as active as II, and Ie was only slightly less active than Ia. The greater (fourfold) loss of activity resulting from the methyl substitution of C_6 of II was unexpected in view of the similarity in sizes among CH_3, Cl, and OCH_3 and the weaker electron-donating nature of CH_3 than OCH_3.

It was reported earlier (1) that a methyl group on C_8 of Ia and Ib exerted little effect on the inhibitory activity (Table II, Compounds If and Ig). The same substitution on C_8 of the aromatic series, on the other hand, caused a reduction in the inhibition of the enzyme; XIII and XIV were, respectively, 2.5 and 2.0 times weaker inhibitors than II and III.

Effects of 9-methyl substitution were greater in the tetrahydro than in the aromatic series. For instance, a 34-fold increase in activity was achieved by the methylation of Ia to Ib, a 13-fold increase from Ic to Id,[1] and nearly a 24-fold increase from If to Ig (Table II). Introduction of a methyl group to N_9 of II gave only a threefold increase in inhibition; a 1.5 time increase was observed as a result of conversion of VI to VII, a 1.7 time increase from VIII to IX, and a 3.6 time increase from XIII to XIV (Table I). These data further indicated that if aromatic β-carbolines and their tetrahydro congeners were bound at the same site on monoamine

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[1] The previously reported value of 3.6 mM (1) should be the I_{50} for 2-methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline.

Table II—Inhibition of Monoamine Oxidase

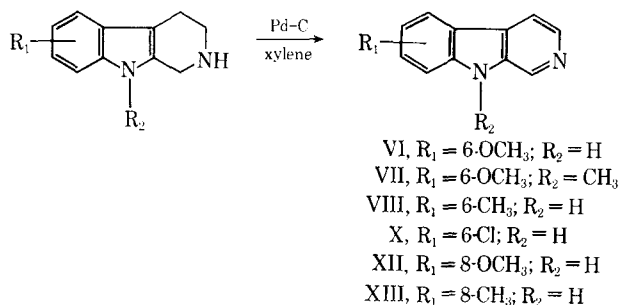| Compound	R_1	R_2	$I_{50},^a$ mM
Ia	H	H	0.34
Ib	H	CH$_3$	0.010
Ic	6-OCH$_3$	H	1.30
Id	6-OCH$_3$	CH$_3$	0.10
Ie	6-Cl	H	0.42
If	8-CH$_3$	H	0.38
Ig	8-CH$_3$	CH$_3$	0.016

^{a} All data from *References 1* and *2*.

oxidase, it would appear to have different binding conformations for each series (3).

Decreases of 22- and 20-fold, respectively, in activity were found when an amino group was placed on either C_6 or C_8 of II (Table I, Compounds XI and XV). Such magnitude of reduction in activity, however, was not observed with the 6-methoxy compound (VI). Since the amino group is smaller in size than the methoxy group, the greater loss of activity found with XI and XV was, therefore, not due to the steric hindrance caused by the substituent on C_6 or C_8.

Replacement of C_1-hydrogen of II with an amino group resulted in about a fivefold decrease in activity. In view of the finding that V was an inhibitor of equal activity as 1-methyl-β-carboline (IV), this decrease was most likely attributed to the steric hindrance by the bulk of the amino or alkyl group (2).

CHEMISTRY

Aromatic β-carbolines, such as 6-methoxy-(VI), 6-methyl-(VIII), 6-chloro-(X), 8-methoxy-(XII), and 8-methyl-(XIII), were prepared by the palladium-on-charcoal (Pd-C) catalyzed dehydrogenation of the corresponding 1,2,3,4-tetrahydro-β-carbolines (I) in boiling xylene (Scheme I). The reaction would appear to require a

VI, $R_1 = 6$-OCH$_3$; $R_2 = H$

VII, $R_1 = 6$-OCH$_3$; $R_2 = CH_3$

VIII, $R_1 = 6$-CH$_3$; $R_2 = H$

X, $R_1 = 6$-Cl; $R_2 = H$

XII, $R_1 = 8$-OCH$_3$; $R_2 = H$

XIII, $R_1 = 8$-CH$_3$; $R_2 = H$

Scheme I

fresh and active catalyst; in a few instances when an old catalyst was used in the preparation of methoxylated β-carbolines, only the starting material was recovered. Due to the availability of 8-methyl-1-oxotetrahydro-β-carboline commercially, the preparation of XIII by the present method was simpler and more economical than by the method of Cook et al. (4) who utilized the condensation of 7-methyltryptophan with formaldehyde, followed by $K_2Cr_2O_7$ oxidation. The synthesis of 8-methyltetrahydro-β-carboline (If) has previously been reported (1).

The very low yield of Compound X was apparently attributed to the dehalogenation of the 6-chloro atom by Pd-C during the dehydrogenation; unsubstituted β-carboline (XVI) was isolated as a by-product. Attempts to prepare X by other methods were unsuccessful. Substitution of Raney nickel for Pd-C as the

VIII, 6-CH$_3$
XIII, 8-CH$_3$

IX, 6-CH$_3$
XIV, 8-CH$_3$
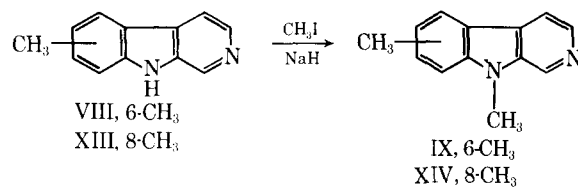
Scheme II

catalyst resulted in the isolation of an equal amount of the unchanged 6-chlorotetrahydro-β-carboline (Ie) and the dehalogenated product II. No reaction took place when 1-oxo-6-chlorotetrahydro-β-carboline was refluxed with phosphorus oxychloride. This reagent has been reported to give 9-alkyl-β-carbolines from 1-oxo-9-alkyl-1,2,3,4-tetrahydro-β-carbolines (5). Chloranil (tetrachloro-p-benzoquinone) has been shown to be an excellent dehydrogenating agent for the preparation of carbazoles from tetrahydrocarbazoles (6). However, the use of p-benzoquinone in the dehydrogenation of 6-chlorotetrahydro-β-carboline failed to give X.

Methylation of VIII and XIII with methyl iodide in the presence of sodium hydride gave the dimethyl-β-carbolines IX and XIV, respectively (Scheme II). 1-Amino-β-carboline (V) was obtained from the treatment of β-carboline (II) with sodium amide according to the procedure reported in the literature (9) (Scheme III).
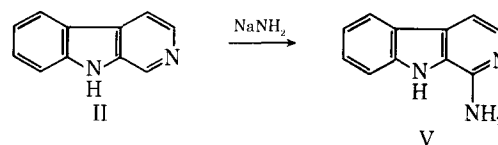
Scheme III

Nitration of β-carboline (II) and the reduction of the nitro-β-carboline to amino-β-carboline have been reported by Saxena (7). In his preparation, only one isomer was isolated. The position of the nitro group was not established other than to assume that it was the 6-nitro-β-carboline (XVI). No indication was given by Saxena on whether the 6-nitro-β-carboline prepared by him was free of contamination of other isomers. However, in carrying out the nitration of 1-methyl-β-carboline, Synder et al. (8) reported the isolation of two products. The higher-melting nitro compound (67% yield) was then reduced, diazotized, and converted to a bromo compound whose physical properties were identical with those of 6-bromo-1-methyl-β-carboline. The position of the nitro group in the lower-melting compound (20% yield) was assumed by them to be the isomer 8-nitro-1-methyl-β-carboline.

In the present work, two isomeric nitro-β-carbolines from the nitration of II (Scheme IV) were found to vary with their R_f values on TLC, retention times on gas chromatograms, as well as IR and UV spectra. Separation of these two isomers was achieved by the difference in their solubilities in hot chloroform. Reduction of the nitro group by Pd-C catalyst gave 6-amino-β-carboline (XI) and 8-amino-β-carboline (XV), respectively (Scheme IV). The authors chose to differentiate the positions of the amino group in the two products, XI and XV, by their NMR spectra; XI (60 Mc.p.s.): τ (D$_2$O-DCl),
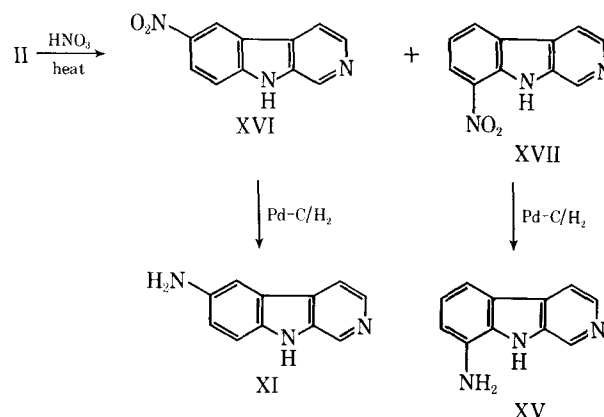
Scheme IV

1.31 (singlet, H-1), 1.80 (doublet, J = 6.0 c.p.s., H-3), 1.89 (doublet, J = 6.0 c.p.s., H-4), 1.94 (doublet, J = 2.0 c.p.s., H-5), 2.27 (doublet of doublets, J = 2.0 and 8.7 c.p.s., H-7), 2.57 (doublet, J = 8.7 c.p.s., H-8). Due to the limited solubility of XV in D_2O-DCl, its NMR spectrum was run with a 100 Mc.p.s. spectrometer: τ (D_2O-DCl), 0.82 (singlet, H-1), 1.44 (doublet, J = 6.3 c.p.s., H-3), 1.55 (doublet, J = 6.3 c.p.s., H-4), 1.67 (doublet, J = 7.9 c.p.s., H-5), 2.07 (doublet, J = 7.9 c.p.s., H-7), 2.48 (triplet, J = 7.9 c.p.s., H-6). An earlier attempt to convert 6-nitrotetrahydro-β-carboline into XVI, which could then be used for further characterization of the 6-nitro isomer, was unsuccessful.

EXPERIMENTAL[2]

Preparation of Substituted Aromatic β-Carbolines from Tetrahydro-β-carbolines—A mixture of 20 mmoles of the corresponding substituted 1,2,3,4-tetrahydro-β-carbolines (1, 2) in 150 ml. of xylene and 500 mg. of 5 or 10% Pd-C catalyst was refluxed for 6 hr. or overnight and then filtered hot. The filtrate was cooled in ice; the deposited product was collected on a filter and recrystallized from a suitable solvent. Accordingly, the following compounds were prepared: 6-methoxy-β-carboline (VI), 45%, m.p. 206–207° (toluene); λ_{max}. (EtOH) 217, 234, 249, 259 (s), 292, 299, 361, 371 mμ.

Anal.—Calcd. for $C_{12}H_{10}N_2O$: C, 72.71; H, 5.09; N, 14.14. Found: C, 73.01; H, 4.77; N, 14.09.

6-*Methyl-β-carboline* (*VIII*)—27%, m.p. 190–191° (benzene); λ_{max}. (EtOH) 220, 240, 255 (s), 265 (s), 285, 297, 357, 372 mμ.

Anal.—Calcd. for $C_{12}H_{10}N_2$: C, 79.09; H, 5.53; N, 15.37. Found: C, 79.19; H, 5.55; N, 15.11.

6-*Chloro-β-carboline* (*X*)—0.9%, m.p. 270–271° (chloroform); λ_{max}. (EtOH) 238, 249, 331, 345 mμ.

Anal.—Calcd. for $C_{11}H_7ClN_2$: C, 65.20; H, 3.48; N, 13.82. Found: C, 65.49; H, 3.38; N, 13.96.

8-*Methoxy-β-carboline* (*XII*)—62%, m.p. 204–205° (xylene); λ_{max}. (EtOH) 216, 243, 268, 278, 287, 343, 354 (s) mμ.

Anal.—Calcd. for $C_{12}H_{10}N_2O$: C, 72.71; H, 5.09; N, 14.14. Found: C, 72.68; H, 5.11; N, 14.04.

8-*Methyl-β-carboline* (*XIII*)—90%, m.p. 233–234.5° (benzene); λ_{max}. (EtOH) 217, 237, 338, 352 mμ. A 229–230° m.p. has been reported for this compound, which was prepared by the treatment of 7-methyltryptophan with formaldehyde followed by potassium dichromate oxidation (4).

6-*Methoxy-9-methyl-β-carboline* (*VII*)—Ether-HCl was added to the xylene filtrate and the hydrochloride salt of VII was collected on a filter; yield, 83%, m.p. 254–257°. Recrystallization from EtOH gave 50%, m.p. 258–259°; λ_{max}. (EtOH) 220, 235, 248, 270, 286 (s), 292, 299, 370, 380 mμ. A 262–263° m.p. has been recorded for the hydrochloride salt of VII prepared by the treatment of the 1-oxo-6-methoxy-9-methyltetrahydro-β-carboline with POCl$_3$ (5).

Alkylation of Substituted β-Carbolines–8,9-*Dimethyl-β-carboline* (*XIV*)—To a stirred suspension of 0.2 g. (12 mmoles) of 8-methyl-β-carboline (XIII) in 20 ml. of dimethylformamide was added slowly 0.6 g. (17 mmoles) of sodium hydride (50% suspension in mineral oil). After stirring at room temperature for 4 hr., the mixture was cooled in ice, and 1.8 g. (13 mmoles) of methyl iodide was added. Stirring was continued overnight, and 150 ml. of chloroform was then added. The chloroform layer was washed with water (ten 100-ml. portions), dried (anhydrous sodium sulfate), and evaporated *in vacuo*, leaving an oil. When a solution of this oil in 150 ml. of dry ether was mixed with an excess of ether-HCl, a hydrochloride salt of the product precipitated. Recrystallization from ethanol gave 1.8 g. (65%), m.p. 298–300°.

Anal.—Calcd. for $C_{13}H_{12}N_2$·HCl: C, 67.10; H, 5.63; N, 12.04. Found: C, 66.75; H, 5.86; N, 11.98.

A small protion of this hydrochloride salt was neutralized with 10% sodium hydroxide to yield 8,9-dimethyl-β-carboline (XIV), m.p. 69–71°; λ_{max}. (EtOH) 247, 292, 350, 364 mμ. A 68–70° m.p. has been recorded for the compound prepared from 1,7-dimethyltryptophan and formaldehyde followed by oxidation of the condensation product with potassium dichromate (4).

6,9-*Dimethyl-β-carboline* (*IX*)—In a similar manner as the preparation of XIV, a mixture of 1.6 mmoles of 6-methyl-β-carboline

(VIII) in 10 ml. of dimethylformamide and 2.8 mmoles of sodium hydride was stirred for 30 min. Methyl iodide (2.8 mmoles) was added, and the stirring was continued for 1 hr. The product, m.p. 121–122°, was obtained in a 38% yield. Recrystallization from benzene–hexane did not raise the melting point; λ_{max}. (EtOH) 220, 238, 255 (s), 265, 285, 297, 357, 367 mμ.

Anal.—Calcd. for $C_{13}H_{12}N_2$: C, 79.56; H, 6.16; N, 14.27. Found: C, 79.73; H, 6.24; N, 14.13.

1-Amino-β-carboline (V)—This was prepared from β-carboline (II) and NaNH$_2$ according to the procedure of Snyder *et al.* (9), m.p. 200–201° (lit. m.p. 198–200°); λ_{max}. (EtOH) 225, 243, 256 (s), 273 (s), 280, 290, 339, 353 mμ.

Nitration of β-Carboline—A mixture of 4.96 g. (29 mmoles) of -carboline (II) and 60 ml. of concentrated nitric acid was stirred at ice temperature for 2 hr. and then heated on a steam bath until the solid dissolved (approximately 15 min.). The hot solution was poured onto 30 g. of crushed ice, and the bright-yellow solid that precipitated was collected on a filter and washed several times with water. This nitrate salt was dissolved in a minimum amount of water (about 900 ml.). Upon neutralization with 10% sodium hydroxide, the free amine precipitated; yield 4.88 g. (78%). Both silica gel TLC [CHCl$_3$–MeOH (9:1)] and gas chromatography (3% SE 30 on Varaport 30) showed that it was a mixture of two products.

The mixture was treated with about 4 l. of hot chloroform and then immediately filtered. The insoluble solid, 2.64 g. (42%) and with R_f = 5.8, was recrystallized 3 times from acetone to give 850 mg., m.p. 340–342°; λ_{max}. (EtOH) 237, 267, 295, 350 mμ.

Anal.—Calcd. for $C_{11}H_7N_3O_2$: C, 61.97; H, 3.31; N, 19.71. Found: C, 61.83; H, 3.39; N, 19.54. The assignment of 6-nitro-β-carboline (XVI) to this compound was based on the NMR spectrum of its reduction product, 6-amino-β-carboline (XI).

When this chlorofom filtrate was evaporated *in vacuo*, 1.65 g. (26%) of bright-yellow solid was obtained. Two recrystallizations from a minimum amount of dimethylformamide gave 0.5 g., m.p. 320° dec.; R_f = 7.3. λ_{max}. (EtOH) 228, 263, 292, 302 mμ.

Anal.—Calcd. for $C_{11}H_7N_3O_2$: C, 61.97; H, 3.31; N, 19.71. Found: C, 62.01; H, 3.45; N, 19.68. On the basis of the NMR spectrum of its reduction product (XV), the compound was believed to be the 8-nitro-β-carboline (XVII).

6-Amino-β-carboline (XI)—A mixture of 0.85 g. (4 mmoles) of 6-nitro-β-carboline (XVI), 200 ml. of ethanol, and 0.5 g. of 5% Pd-C catalyst was shaken with hydrogen at an initial pressure of 3 atm. until the consumption of hydrogen ceased. After filtration, the solution was evaporated *in vacuo*, and the residue was twice recrystallized from ethanol yielding 0.33 g. (45%) of product, m.p. 302–303° [lit. (7) m.p. 268°]; λ_{max}. (EtOH) 242, 296, 304, 381 mμ. Silica gel TLC [butanol-acetic acid-water (4:1:1)]: R_f = 4.5.

Anal.—Calcd. for $C_{11}H_9N_3$: C, 72.11; H, 4.95; N, 22.94. Found: C, 71.81; H, 5.50; N, 22.69.

8-Amino-β-carboline (XV)—In a similar manner as described in the preparation of X, the 8-nitro-β-carboline (XVII) was catalytically reduced. The product, 8-amino-β-carboline was recrystallized from ethanol–ether to yield 62%, m.p. 242° dec.; λ_{max}. (EtOH) 232, 252, 292, 360 mμ. Silica gel TLC [*n*-butanol–acetic acid–water (4:1:1)]: R_f = 6.4.

Anal.—Calcd. for $C_{11}H_9N_3$: C, 72.11; H, 4.95; N, 22.94. Found: C, 71.90; H, 5.11; N, 22.69.

Assay—Mitochondrial monoamine oxidase from beef liver was isolated and purified as previously described (2). All the stock solutions of inhibitors, except VIII and IX, were prepared in 0.01 N HCl. Compounds VIII and IX were dissolved in 0.02 N HCl. Incubation was carried out with tryptamine-2-^{14}C hydrochloride according to the previously described procedure (2).

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Kinetics and Mechanisms of Action of Drugs on Microorganisms XI: Effect of Erythromycin and Its Supposed Antagonism with Lincomycin on the Microbial Growth of *Escherichia coli*

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Abstract ☐ The steady-state growth of *Escherichia coli* in broth cultures is inhibited by erythromycin with a new steady-state growth-rate constant ($k_{app.}$), which is linearly related to drug concentrations in the range 0–10.0 mcg. ml.$^{-1}$ as $k_{app.} = k_0 - k_E E$, where k_E is the inhibitory-rate constant for drug concentration E of drug-free rate constant k_0. The $k_{app.}$ at $E > 10.0$ mcg. ml.$^{-1}$ adheres to a kinetic model, which implies the saturation of a limited number of receptor sites in accordance with the equation $k_{app.} = k_0 - k_a E/(1 + k_b E)$, where k_a and k_b are constants of proportionality related to drug concentration partitioned into the biophase and its affinity for available receptor sites. The dependence of *E. coli* growth rate on drug concentrations is invariant with the organism population or broth concentrations. However, values for k_a increase 10-fold as the pH of broth is increased from 6.80 to 7.80 while k_0 remains constant. This indicates that the unprotonated fraction of the drug concentration contributes to the activity. Lincomycin in Phase I-affected growth has the same formal dependency on concentration as does erythromycin with a potency ratio of 6.68:1, erythromycin base to lincomycin base, on a weight basis. The combined effects of erythromycin and lincomycin in Phase I of its effect are not antagonistic on the growth rate of *E. coli* in the subinhibitory range and can be predicted on the basis of adding equivalent amounts in accordance with the coincident response–dose curves of erythromycin and lincomycin (Phase I).

Keyphrases ☐ Erythromycin effect—*Escherichia coli* steady-state growth ☐ Lincomycin growth, Phase I—erythromycin effect ☐ Reversibility—erythromycin antimicrobial activity ☐ Microbiological analysis—erythromycin action on *E. coli* ☐ Kinetics—erythromycin action on *E. coli*.

The mode of action of the macrolide erythromycin is generally ascribed to inhibition of protein synthesis (1–9). Erythromycin binds exclusively to the 50 S subunit of ribosomes from *Escherichia coli* (1, 4, 10), *Staphylococcus aureus* (11), *Bacillus subtilis* (6, 8, 12), *Bacillus megaterium* (2, 13), and *Bacillus stearothermophilus* (2) in reconstituted cell-free systems to inhibit polypeptide synthesis which has been stated to be a rapid and reversible process (8). Tanaka *et al.* (4) observed maximum binding of the 50 S ribosomal subunit of *E. coli* Q. 13 at very low concentrations of erythromycin ($\sim$0.6 mcg. ml.$^{-1}$). Only about one-tenth of that amount was found to bind to the ribosomes of resistant mutants.

It has been speculated (8) that erythromycin competes with the transport RNA carrying the peptidyl radical involved in amino acid polymerization for a common binding site on the 50 S subunit of ribosomes. An alternative proposed model (12) for erythromycin's protein inhibition is that it inhibits a "translocase" factor which is necessary for the transfer of the peptidyl t-RNA elongated by a single aminoacyl residue from a ribosomal acceptor site to a donor site.

Conflicting views have been expressed about the modes of action of those antibiotics (*e.g.*, erythromycin, lincomycin, and chloramphenicol), which are supposed to have the common 50 S ribosomal binding site (7, 8, 10, 12). In fact, combinations of erythromycin and lincomycin have been claimed to be antagonistic (2, 3, 8, 10, 12), which would not necessarily follow from an assumption of similar modes of action.

The application of microbial kinetics to the quantification and prediction of antimicrobial action has been demonstrated (14–22). The effects of subinhibitory concentrations of drugs on the growth of bacteria have been studied in simple reproducible systems with *E. coli* strain B/r as the test organism to derive kinetic parameters that may characterize antibacterial action and drug–receptor interaction and to provide insight into the possible mechanisms of drug action.

This paper presents the results of such studies on the action of erythromycin on the growth of *E. coli*. It considers the formal dependence of the kinetic constants of growth inhibition on antibiotic concentration as affected by inoculum size, composition and pH of the media, and reversibility of the erythromycin concentrations. In addition, the actions of combinations of erythromycin and lincomycin on microbial growth, as compared with their *a priori* expectation, are considered.