Monoamine Oxidase Inhibition by β-Carbolines: A Quantum Chemical Approach

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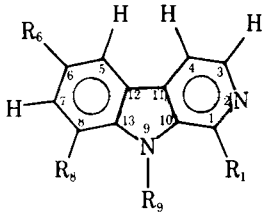
Abstract □ Monoamine oxidase inhibition by β-carboline derivatives is related to the energy change, ΔE, arising from complex formation between the inhibitor and the enzyme. The energy change was expressed in terms of electronic indexes, which were estimated for a set of aromatic β-carbolines. The electronic indexes were correlated to the experimental activity indexes by a simplified quantum chemical perturbational treatment with a multiple regression procedure. A characteristic structure for the inhibitor–enzyme complex was derived from the correlation. The molecules are linked by two kinds of bond. One involves the lone pyridine nitrogen pair of β-carbolines; the other is due to a π-electronic interaction between the inhibitor indole fragment and a suitable area of the enzyme. Such a model explains the competitive inhibition by β-carbolines compared to tryptamine and other aromatic amines that are monoamine oxidase substrates.

Keyphrases □ β-Carbolines—monoamine oxidase inhibition, quantum chemistry □ Monoamine oxidase—inhibition by β-carbolines, quantum chemistry □ Monoamine oxidase inhibitors—β-carbolines, quantum chemistry

Some β-carboline (9*H*-pyrido[3,4-*b*]indole) derivatives are inhibitors of monoamine oxidase in oxidative cerebral amine deamination. *In vitro* studies using tryptamine as the substrate in deamination found that some derivatives were potent competitive inhibitors (1–8). The common β-carboline structure is the tricyclic aromatic system. Substituents enhance or diminish activity.

The present work was designed to relate the inhibitory activity to the aromatic β-carboline derivative electronic structure. An inhibition mechanism was derived from the correlation between experimental data and estimated electronic properties.



	R_1	R_6	R_8	R_9
β-carboline (I)	H	H	H	H
1-methyl-β-carboline (II)	$-CH_3$	H	H	H
9-methyl-β-carboline (III)	H	H	H	$-CH_3$
6-methyl-β-carboline (IV)	H	$-CH_3$	H	H
8-methyl-β-carboline (V)	H	H	$-CH_3$	H
6,9-dimethyl-β-carboline (VI)	H	$-CH_3$	H	$-CH_3$
8,9-dimethyl-β-carboline (VII)	H	H	$-CH_3$	$-CH_3$
1-amino-β-carboline (VIII)	$-NH_2$	H	H	H
6-amino-β-carboline (IX)	H	$-NH_2$	H	H
8-amino-β-carboline (X)	H	H	$-NH_2$	H
6-methoxy-β-carboline (XI)	H	$-OCH_3$	H	H
8-methoxy-β-carboline (XII)	H	H	$-OCH_3$	H
6-methoxy-9-methyl-β-carboline (XIII)	H	$-OCH_3$	H	$-CH_3$

EXPERIMENTAL

Molecular Orbital Calculations—The "valence shell" molecular orbital theory and the INDO (intermediate neglect of differential overlap) semiempirical method (9) were used. Calculations were performed with the CNINDO program (10) using the parameters implicit in the program.

The molecular conformation was built according to the following criteria:

1. Molecular tricyclic structure was considered to be planar, as inferred from electronic delocalization.
2. Substituents did not alter the tricyclic system conformation significantly.
3. The tricyclic system geometry derived from that of the glycyl-L-tryptophan (11) and pyridine (12) cyclic fragments.

The substituent bond lengths are given in Table I. The geometric parameters used in the calculations are given in Table I and Fig. 1.

Theoretical Considerations on Inhibitory Activity—The inhibitory activity is expressed by I_{50}, the inhibitor concentration that diminishes the enzyme activity by 50%. To compare all β-carbolines, the I_{50} must be measured using the same experimental conditions. With the assumption that the β-carboline derivatives inhibit the catalytic action of monoamine oxidase on tryptamine oxidation by a competitive mechanism, such a mechanism was analyzed considering the formation of the inhibitor–enzyme complex. The equilibrium constant for complex formation can be derived from experimental I_{50} data (1–8) and also can be estimated from theoretical electronic structure calculations. The aim is to find a correlation between the complex equilibrium constant and the inhibitor electronic properties.

The equilibrium constant can be derived from reported I_{50} values. Literature studies (1–8) suggested that the inhibitor and the substrate (tryptamine) are competitive toward the same enzyme centers. The competitive mechanism can be expressed by:

$$S + E \underset{k_2}{\overset{k_1}{\rightleftarrows}} SE \xrightarrow{k_3} P + E$$

Scheme I

$$B + E \overset{K_i}{\rightleftarrows} BE$$

Scheme II

where *S*, *E*, *B*, *SE*, and *BE* are the substrate, the enzyme, the inhibitor, the substrate–enzyme complex, and the inhibitor–enzyme complex, re-

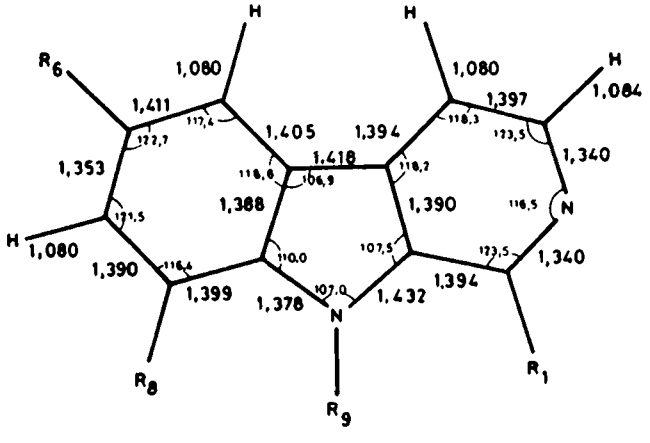


Figure 1—*Geometrical β-carboline tricyclic conformation.*

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Table I—Substituent Bond Lengths

Bond	Length, Å	Reference.
C_1-H	1.084	13
C_6-H	1.080	14
C_8-H	1.080	14
N_9-H	1.090	15
C_1-CH_3	1.500	16
C_6-CH_3	1.500	16
C_8-CH_3	1.500	16
N_9-CH_3	1.460	17
C-H	1.110	16
C_1-NH_2	1.360	18
C_6-NH_2	1.360	18
C_8-NH_2	1.360	18
N-H	1.040	18
C_6-OCH_3	1.360	19
C_8-OCH_3	1.360	19
O-CH_3	1.350	19
C-H	1.110	16

spectively; k_1, k_2, and k_3 are the rate constants; and K_i is the equilibrium constant:

$$K_i = \frac{[BE]}{[B][E]} \qquad \text{(Eq. 1)}$$

By applying the steady-state hypothesis, the degree of inhibition, i, is (20):

$$i = \frac{K_A K_i [B]}{K_A(1 + K_i[B]) + [S]} \qquad \text{(Eq. 2)}$$

where $K_A = k_2 + k_3/k_1$, the Michaelis constant, referred to the substrate; and S and B are the concentrations of the substrate and inhibitor, respectively, in the experiment.

From the I_{50} definition, Eq. 2 can be written:

$$K_i = \frac{K_A + [S]}{K_A I_{50}} \qquad \text{(Eq. 3)}$$

This expression can be simplified by suitable planning of the experiment. The value $K_A = 8.1 \times 10^{-5}$ was reported when the same initial tryptamine concentration, $[S] = 6 \times 10^{-2}$ mole/liter, was used for every experiment (1). Under these experimental conditions when $[S] > 100 \times K_A$, the oxidation kinetics can be considered to be zero order (with less than 1% deviation). Consequently, Eq. 3 reduces to:

$$K_i = \frac{K_{AS}}{I_{50}} \qquad \text{(Eq. 4)}$$

where:

$$K_{AS} = \frac{K_A + [S]}{K_A} \qquad \text{(Eq. 5)}$$

Obviously, K_{AS} depends on the experimental conditions, but it will be constant if $[S]$ is constant. In the experiments quoted, the conditions were constant for all β-carbolines. Thus, Eq. 4 or its logarithmic form:

$$\log K_i = \log K_{AS} + pI_{50} \qquad \text{(Eq. 6)}$$

where:

$$pI_{50} = -\log I_{50} \qquad \text{(Eq. 7)}$$

can be used to estimate the equilibrium constants for inhibitor–enzyme complex formation. From the experimental data (1), $\log K_{AS} = 2.870$. The values of pI_{50} and K_i derived by applying Eq. 6 to the experimental data are given in Table II.

Quantum Chemical Treatment of Inhibition—After an experimental estimate of the equilibrium constant K_i was obtained, the next step was to relate K_i to electronic and/or structural properties. No correlation was found between atomic electronic properties and β-carboline inhibitory activity in any previous work (21).

According to statistical thermodynamics, K_i is expressed by:

$$K_i = \frac{f_{BE}}{f_B f_E} \exp(-\Delta E_i/kT) \qquad \text{(Eq. 8)}$$

where ΔE_i is the difference between the complex energy and the separate enzyme and inhibitor energies, all at ground state; f is the partition function, and k is the Boltzmann constant. Structural similarities permit the simplification of Eq. 8 for the entire β-carboline set. The partition quotient can be considered nearly constant for all β-carbolines (22).

Table II—Inhibitory Activity Parameter (pI_{50}) and Equilibrium Constant for the β-Carbolines–Monoamine Oxidase Complexes

Compound	pI_{50} Experimental[a]	pI_{50} Calculated[b]	$K_i \times 10^{-6}$ liter/mole Calculated[b]
I	4.538	4.537	25.54
II	3.854	3.890	5.30
III	5.000	5.012	74.13
IV	3.921	3.844	6.18
V	4.143	4.153	10.30
VI	4.155	4.173	10.59
VII	4.699	4.696	37.07
VIII	3.824	3.796	4.94
IX	3.201	3.237	1.19
X	3.237	3.215	1.28
XI	4.367	4.366	17.22
XII	3.921	3.967	6.18
XIII	4.553	4.527	26.49
Tryptamine	—	—	3.15

[a] Reference 6. [b] This work.

The energy variation in complex formation can be developed into several components:

$$\Delta E_i = \Delta E_n^i + \Delta E_e^i + \Delta E_{st}^i + \Delta E_{sv}^i \qquad \text{(Eq. 9)}$$

where ΔE_n^i represents the difference due to nuclear rotation and vibration, ΔE_{st}^i expresses the variation from steric rearrangements, ΔE_{sv}^i concerns the solvation phenomenon, and ΔE_e^i expresses the change of electronic energy. The most important contribution to ΔE_i is ΔE_e^i (23). It is also the differential factor of each molecule in the ΔE_i estimation because the formation mechanism must be equal for all compounds. Equation 9 can be simplified to:

$$\Delta E_i = \Delta E_e^i + C \qquad \text{(Eq. 10)}$$

where C includes all contributions except the electronic ones and is constant for the β-carbolines.

From these considerations, the equilibrium constant is:

$$K_i = D(T) \exp(-\Delta E_e^i/kT) \qquad \text{(Eq. 11)}$$

where:

$$D(T) = \frac{f_{BE}}{f_B f_E} \exp(-C/kT) \qquad \text{(Eq. 12)}$$

$D(T)$ depends on temperature but is the same for all β-carbolines. In a logarithmic form, Eq. 11 can be written:

$$\log K_i = A(T) - \frac{\Delta E_e^i}{kT} \qquad \text{(Eq. 13)}$$

where $A(T) = \log D(T)$. Finally, from Eqs. 6 and 13, the inhibitory activity expressed by pI_{50} can be related to the complex formation electronic energy by:

$$pI_{50}^i = a + b\,\Delta E_e^i \qquad \text{(Eq. 14)}$$

where $a = A(T) - \log K_{AS}$ and $b = -1/kT$; a and b are constants for a temperature and for the entire β-carboline set.

Unfortunately, the direct ΔE_e^i calculation is not feasible. The monoamine oxidase molecular structure is unknown, and the electronic energy cannot be calculated. A greater difficulty appears in the electronic energy calculation of the inhibitor–enzyme complex, because the nature of the complex is unknown. The only energy that can be calculated is the inhibitor electronic energy in the geometrical conformations proposed.

Nevertheless, perturbational treatment (24, 25) was performed on the complex, considering its nature and stability. The important features observed in the β-carbolines were:

1. All compounds inhibited monoamine oxidase catalysis on aromatic amines and, more precisely, on tryptamine.
2. The inhibitory mechanism seemed to be common.
3. The inhibition was produced because the β-carbolines blocked the enzyme active centers on which the substrate was to be fixed.
4. The inhibitory mechanism reversibility implied molecular attraction rather than covalent bonding between molecules.
5. The pyridine nitrogen as well as the delocalized electronic system was essential for the inhibitory behavior. Carbazole, having a similar electronic structure to β-carboline but lacking the pyridine nitrogen, does not present inhibitory properties (26).
6. Substituents only altered the activity located at the delocalized tricyclic system.

Table III—Net Charges (e) of the Most Significant Atoms in Eq. 22

Compound	Q_5	Q_6	Q_9	Q_{13}
I	0.0297	−0.0054	−0.1656	0.1462
II	0.0299	−0.0054	−0.1647	0.1462
III	0.0294	−0.0047	−0.1283	0.1684
IV	0.0172	0.0056	−0.1653	0.1416
V	0.0241	−0.0016	−0.1655	0.1367
VI	0.0167	0.0060	−0.1279	0.1342
VII	0.0236	−0.0011	−0.1271	0.1286
VIII	0.0312	−0.0064	−0.1589	0.1457
IX	−0.0553	0.1619	−0.1663	0.1080
X	−0.0222	0.0297	−0.1600	0.0721
XI	−0.0523	0.2048	−0.1650	0.1208
XII	−0.0034	0.0234	−0.1564	0.0859
XIII	−0.0524	0.2046	−0.1275	0.1138
Tryptamine	0.0216	−0.0125	−0.1109	0.1359

Table IV—Superdelocalizability Indexes of the Most Significant Atoms in Eq. 22

Compound	$S_5^{(e)}$	$S_6^{(e)}$	$S_8^{(e)}$	$S_9^{(e)}$	$S_{13}^{(e)}$
I	−0.1941	−0.1950	−0.2009	−0.2682	−0.1852
II	−0.1940	−0.1951	−0.2024	−0.2676	−0.1788
III	−0.1939	−0.1928	−0.2001	−0.2683	−0.1800
IV	−0.2004	−0.1931	−0.2005	−0.2681	−0.1805
V	−0.1955	−0.1996	−0.1960	−0.2671	−0.1811
VI	−0.2028	−0.1928	−0.2003	−0.2688	−0.1813
VII	−0.2154	−0.1943	−0.1965	−0.2679	−0.1788
VIII	−0.1950	−0.1963	−0.2010	−0.2703	−0.1797
IX	−0.2164	−0.1759	−0.1988	−0.2729	−0.1881
X	−0.2079	−0.1943	−0.1801	−0.2714	−0.1918
XI	−0.2037	−0.1714	−0.1959	−0.2673	−0.1821
XII	−0.1999	−0.1921	−0.1789	−0.2675	−0.1845
XIII	−0.2035	−0.1739	−0.1957	−0.2688	−0.1834
Tryptamine	−0.2005	−0.2019	−0.2281	−0.2614	−0.1837

The electronic interaction energy between a pair of atoms, b from the inhibitor B and e from the enzyme E, can be expressed (27) by:

$$\Delta E_{be} = Q_b Q_e (bb|ee) + \sum_m \sum_{n'} \frac{d_{mb} d_{n'e}}{\epsilon_m - \epsilon_{n'}} \beta_{be}^2 + \sum_{m'} \sum_n \frac{d_{m'b} d_{ne}}{\epsilon_{m'} - \epsilon_n} \beta_{be}^2 \quad \text{(Eq. 15)}$$

where Q_b and Q_e are the net charges over the atoms and β_{be} is the resonance integral; $d_{ks} = \Sigma_j 2c_{kj}$ is the orbital charge of atom s in molecular orbital ϕ_k, where c_{kj} are the coefficients of the atomic orbitals from the atom s in ϕ_k. The indexes m' and n' denote the virtual orbitals of inhibitor and enzyme, respectively, and m and n stand for the doubly occupied orbitals of the corresponding molecules. The ϵ values are the molecular orbital energies; $(bb|ee)$ is the repulsion energy between the electrons occupying orbitals centered on b and e atoms. The hypothesis of INDO formalism gives a constant value for the repulsion integral $(bb|ee)$ among the atoms b and e valence orbitals despite the kind of such orbitals. The value of β_{be} is independent of the kind of atomic orbitals because the molecular nature of the complex does not involve covalent bonds.

By extending Eq. 15 to all pairs of interacting atoms, p, it can be written:

$$\Delta E_e^i = \sum_p \left\{ Q_b^p Q_e^p (bb|ee) + \beta_{be}^2 \sum_m \frac{d_{mb}^p}{\epsilon_m - \bar{\epsilon}_{n'}} \sum_{n'} d_{n'e}^p + \beta_{be}^2 \sum_{m'} \frac{d_{m'b}^p}{\epsilon_{m'} - \bar{\epsilon}_n} \sum_n d_{ne}^p \right\} \quad \text{(Eq. 16)}$$

This expression has been written with the consideration that the occupied molecular orbitals of one molecule and the virtual orbitals of the other have very different energies, as in a charge controlled process. The following approach may be used:

$$\epsilon_m - \epsilon_{n'} = \epsilon_m - \bar{\epsilon}_{n'} \quad \text{(Eq. 17)}$$

$$\epsilon_{m'} - \epsilon_n = \epsilon_{m'} - \bar{\epsilon}_n \quad \text{(Eq. 18)}$$

where $\bar{\epsilon}$ is the average of the corresponding energies.

Since the atoms from the tricyclic system, which are the same for all β-carbolines, are the only ones involved in complex formation and since the complexes are formed with the same atoms of the enzyme, some of the magnitudes appearing in Eq. 16 probably are constant for all β-carbolines. The integrals $(bb|ee)$, the resonance integrals β_{be}, the summations referring the enzyme coefficients, and the average enzyme orbital energies may be considered constant.

Otherwise, the summations concerning the β-carboline orbitals are closely related to the generalized superdelocalizability indexes (28, 29) and are expressed by:

$$S_b^{(e)} = \sum_m \frac{d_{mb}}{\epsilon_m} \quad \text{(Eq. 19}a\text{)}$$

$$S_b^{(n)} = \sum_{m'} \frac{d_{m'b}}{\epsilon_{m'}} \quad \text{(Eq. 19}b\text{)}$$

where $S^{(e)}$ is the electrophilic index and $S^{(n)}$ is the nucleophilic index.

Since the enzymatic terms in Eqs. 16 and 19 are constant, the following expression for the electronic energy variation can be written:

$$\Delta E_e^i = \sum_p \{A_p Q_p^2 + B_p S_b^{(e)p} + C_p S_b^{(n)p}\} \quad \text{(Eq. 20)}$$

The coefficients A_p, B_p, and C_p are constants for all β-carbolines, and the indexes Q and S are variables for each complex.

Finally, by introducing Eq. 20 in Eq. 14 and rearranging, the following expression can be found:

$$pI_{50} = a + \sum_p (c_{1p} Q_p + c_{2p} S_p^{(e)} + c_{3p} S_p^{(n)}) \quad \text{(Eq. 21)}$$

In this equation, the summation is extended over all of the atoms of the aromatic β-carboline tricyclic system, and the coefficients c_{ip} are constant. Equation 21 relates the experimentally found inhibitory activity to the theoretically estimated electronic indexes. This equation holds if the enzyme–β-carboline complex is well approximated by the proposed molecular model, *i.e.*, if it is a molecular charge-controlled complex without covalent bonds. A statistical study of the applicability of Eq. 21 to the molecules considered was performed to identify the coefficients that gave the best statistical correlation.

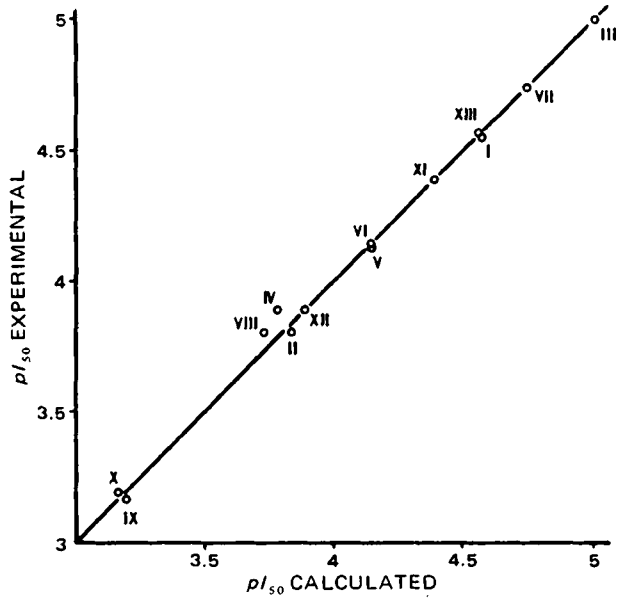

Figure 2—*Experimental* pI_{50} *values* versus *calculated values from Eq. 22.*

RESULTS AND DISCUSSION

The calculated values for the electronic indexes appearing in Eq. 21 are summarized in Tables III and IV. The statistical fitting of Eq. 21 was performed by means of stepwise linear regression techniques with pI_{50} as the dependent variable and the electronic indexes as independent variables. More than 400 combinations of electronic indexes were performed; the best correlated expression found is Eq. 2 , where the numerical values for the coefficients of Eq. 21 are shown:

$$pI_{50} = 31.806 + 11.782\, Q_9 + 140.546\, S_9^{(e)} + 14.405\, Q_6 + 18.199\, S_6^{(e)} + 42.378\, Q_5 - 28.496\, S_5^{(e)} + 22.284\, Q_{13} - 101.543\, S_{13}^{(e)} + 63.959\, S_8^{(e)} \quad \text{(Eq. 22)}$$

Table V—t-Test for Significance of Coefficients in Eq. 22

Variable	t Value	p
Q_9	13.003	<0.0005
$S_9^{(e)}$	10.307	<0.0025
Q_6	10.165	<0.0025
$S_2^{(e)}$	2.32	= 0.05
$S_8^{(e)}$	10.684	<0.0025
Q_5	10.491	<0.0025
$S_5^{(e)}$	9.291	<0.0025
Q_{13}	10.608	<0.0025
$S_{13}^{(e)}$	13.067	<0.005

Equation 22 has a multiple correlation coefficient (R) of 0.999 that represents a significance better than 99.9%, a mean standard deviation (MSD) of 0.011_7, and a residual standard deviation (RSD) of 0.042_4. A detailed statistical analysis is given in the *Appendix*.

The next best correlation is given by:

$$pI_{50} = 34.157 + 16.215\,Q_9 + 139.164\,S_9^{(e)} + 10.245\,Q_6 + 24.402\,Q_5 + 14.753\,Q_{13} - 75.452\,S_{13}^{(e)} + 32.385\,S_8^{(e)} \quad \text{(Eq. 23)}$$

with the following statistical parameters: $R = 0.972$, $MSD = 0.053_2$, and $RSD = 0.192$.

The pI_{50} values taken from the literature (1–8) and the ones estimated by Eq. 22 are summarized in Table II, which also shows the estimated equilibrium constants. The experimental pI_{50} values are fitted *versus* the calculated ones in Fig. 2.

As can be derived from Eq. 22, the inhibitory activity was not closely related to any individual atomic property but to a definite set of electronic indexes located on some tricyclic system atoms. The electrophilic index $S_9^{(e)}$ was the most important term influencing the activity. The inhibitory activity of any aromatic β-carboline could be predicted from the corresponding electronic indexes of its ring atoms using Eq. 22. The significant features from the statistical analysis were:

1. The electronic indexes corresponding to pyridine nitrogen, N_2, did not appear in any tested regression giving an acceptable statistical analysis.
2. The same circumstance was observed for C_1, C_3, and C_4 belonging to the pyridine β-carboline fragment.
3. The benzene fragment electronic indexes, as well as the pyrrole fragment indexes, showed the best correlation found for the entire β-carboline indolic part.
4. In all significant regressions studied, the Q_9 and $S_9^{(e)}$ indexes gave important contributions to the regression.

Only the indole fragment is involved in β-carboline–enzyme complex formation. The pyridine nitrogen, N_2, is essential to the inhibitory activity of β-carbolines but does not contribute to the complex formation.

From an electronic point of view, the indole group exhibited a delocalized charge with some accumulations and depletions over its atoms (Table III); it was a good system to form molecular or π-electronic complexes. The pyridine nitrogen is an electron donor system, responsible for the basicity of β-carbolines. The electronic characteristics of both molecular regions are responsible for the interaction with monoamine oxidase. β-Carbolines and monoamine oxidase form a molecular complex on which the molecules are linked by two interactions. The first one arises from the molecular electronic complex between the β-carboline indole fragment and a suitable fixing region of monoamine oxidase (Fig. 3). This

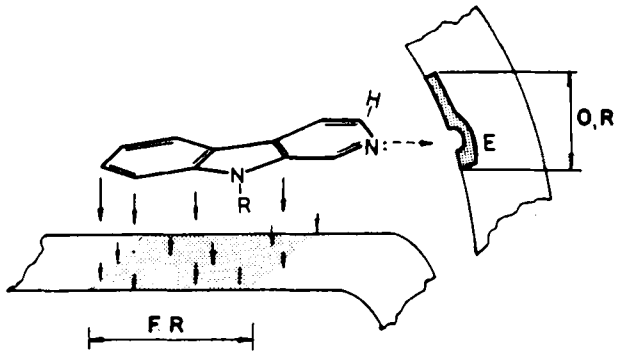

Figure 3—*Interaction model between inhibitor and enzyme. FR = fixing region, and OR = oxidizing region.*

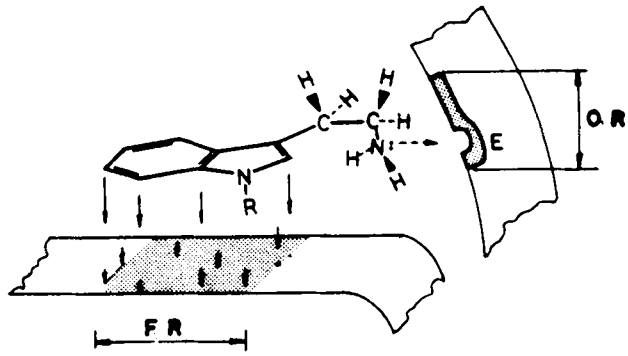

Figure 4—*Interaction model between tryptamine and the enzyme.*

complex has π-complex characteristics, like Mulliken's external complex ($b\pi$–$a\pi$) (30). The indole system acts as an electron donor by means of N_9, C_6, and C_8.

The second interaction is due to pyridine nitrogen, N_2, which acts as an electron donor over an electrophilic monoamine oxidase center located in a region identified as OR (oxidizing region) (Fig. 3). This interaction must be stronger than the former because of its electronic nature. Such a model involves specific structures at the enzyme interaction regions, the so-called FR and OR. The OR interacts with N_2 by means of a strong bond (dative covalent, hydrogen bonding, *etc.*). The FR must have a delocalized structure to match the electronic singularities of the inhibitor indole fragment. Some kind of conformational change is involved in the enzyme itself in order to bind adequately to the inhibitor.

Such a model adequately explains the competitive inhibition of tryptamine and probably of other aromatic cerebral amines. Tryptamine has an indole structure that can form a complex with the enzyme FR in a similar mode to β-carboline. Also, the tryptamine side chain can take a suitable conformation facing the nitrogen of the amino group to the enzyme OR (Fig. 4). In such a conformation, the nitrogen atom reacts, breaking the C–N bond and initiating the oxidative degradation that inactivates the amine. After the oxidation, the complex between the indole fragment and the FR region opens again, releasing the inactivated product.

The inhibitory β-carboline behavior can be explained as the result of a mimetic mechanism forming the same bonds with the same enzymatic regions; the difference is that β-carboline cannot be oxidized because of the stability of its delocalized electronic structure. If such a model works, the competition for the formation of the complex with the enzyme and, consequently, the inhibitory activity are due to the more stable complexes formed by β-carbolines with the enzyme FR than by tryptamine. Thus, the tryptamine–FR complex stability is estimated using Eq. 22, with the electronic indexes corresponding to the indole group of tryptamine (Tables III and IV). A value of 3.15×10^6 liters/mole was found for the equilibrium constant in the reaction tryptamine + FR(enzyme) ⇌ complex.

This value is similar to those found for β-carbolines (Table II). From Table II, it can be seen that the greatest inhibitory activity belongs to the greatest difference between the estimated equilibrium constants of β-carboline and tryptamine. Such a result sustains the model proposed.

APPENDIX

The analysis of variance of Eq. 22 gives $F_{9,3} = 208.53$, giving a very high significance for this equation ($p < 0.0005$). The t-test values of the coefficients of Eq. 22 are shown in Table V. All of them are significant (significance limits of 95–99.9%); consequently, the corresponding indexes influence the values of pI_{50} and K_i.

The introduction or elimination of any other index decreases substantially the regression equation significance level.

Finally, the regression equation referring to the β-carboline benzene ring atoms, together with their most important statistical parameters, are:

$$pI_{50} = 33.991 + 31.452\,S_6^{(e)} + 8.732\,Q_6 + 16.228\,S_7^{(e)} + 97.778\,Q_7 - 28.030\,S_{13}^{(e)} + 14.143\,Q_{13} - 30.809\,S_5^{(e)} + 172.652\,S_8^{(e)} + 133.849\,Q_{12} \quad \text{(Eq. A1)}$$

with $RSD = 0.100$, $R = 0.995$, $MSD = 0.028$, $F_{9,3} = 37.12$, and $p < 0.01$. The equation of regression and the statistical parameters for the atoms

of the pyrrole fragment are:

$$pI_{50} = 105.773 + 503.033\,S_9^{(e)} + 27.992\,Q_9 - 183.106\,S_{13}^{(e)} + 8.801\,Q_{13} - 62.903\,S_{11}^{(e)} - 176.198\,Q_{11} - 46.928\,Q_{12} - 63.207\,Q_{10} \quad \text{(Eq. A2)}$$

with $RSD = 0.10$, $R = 0.994$, $MSD = 0.028$, $F_{8,4} = 41.67$, and $p < 0.005$. These correlations are poorer than the one found for the indole group.

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New Biphenyl Derivatives II: 1-(4-Biphenylyl)-1-hydroxy-2-aminoethanes and 1-(4-Biphenylyl)-1-chloro-2-aminoethanes as Potential β-Adrenoceptor Blocking Agents

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Abstract □ Series of 1-(4-biphenylyl)-1-hydroxy-2-aminoethanes and 1-(4-biphenylyl)-1-chloro-2-aminoethanes were synthesized. Newly developed reaction conditions for aryl aminomethyl ketone reduction and reductive alkylation, using sodium borohydride, are described. The prepared compounds were examined for adrenergic blocking activity on an anesthetized dog blood pressure preparation and on isolated toad hearts. β-Adrenergic blockade was investigated using isoproterenol as the agonist. The benzylamino and cyclohexylamino analogs exhibited marked β-adrenoceptor blocking activity, for which the latter derivatives were more potent.

Keyphrases □ β-Adrenergic blocking activity—biphenyl derivatives, synthesis, structure–activity relationships □ Biphenyl derivatives, various—synthesized, evaluated for β-adrenergic blocking activity, structure–activity relationships □ Structure–activity relationships—biphenyl derivatives, β-adrenergic blocking activity

Optimum β-adrenoceptor blockade occurs when 1-phenyl-1-hydroxy-2-aminoethane structures (I) bear certain substituents at the phenyl 4- or 3,4-position and an isopropyl grouping on the amine head (1). 1-(3,4-Dichlorophenyl)-1-hydroxy-2-isopropylaminoethane (2) (II), 1-(2-naphthyl)-1-hydroxy-2-isopropylaminoethane (pronethalol) (3, 4) (III), and 1-(4-nitrophenyl)-1-hydroxy-2-isopropylaminoethane (5) (IV) are well-known examples.

In vitro hydrolysis of 1-aryl-1-chloro-2-aminoethanes to 1-aryl-1-hydroxy-2-aminoethanes was used to prepare a series of 1-chloro-2-aminoethanes related to III (6, 7).

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