Quantum Chemical Approach to Structure–Activity Relationships of Tetracycline Antibiotics

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Abstract □ Bacteriostatic activities for tetracyclines, determined *in vitro* by microbial growth kinetics, should be related to the perturbation energy, ΔE_i, which characterizes the interaction between these antibiotics and their receptor substance. Quantum perturbation molecular orbital theory, combined with multiple-regression techniques, are applied in gaining estimates of the relative variations in ΔE_i for a family of tetracyclines which act in an identical way on a receptor region of unknown structure. The results of this theoretical approach are in good agreement with a number of independent observations relating to the bacteriostatic effects of tetracyclines and are consistent with the hypothesis that bacterial growth inhibition by tetracyclines is a consequence of an inhibition in protein synthesis due to the association of the antibiotic with a ribosomal site. The derived relationships between the bacteriostatic activities and the electronic structure of the tetracycline molecules implicate the oxygens at positions 10, 11, and 12 of the tetracycline nucleus as being intimately involved in leading to the bacteriostatic effects. This relationship also leads to satisfactory estimates for the inhibition rate constants of other tetracyclines, provided that specific effects such as intramolecular hydrogen bonding are taken into account. The effect of substituents on the electronic properties of the C_6 and phenoldiketone tetracycline regions is to produce a gradation in the inhibitory properties of this family of antibiotics.

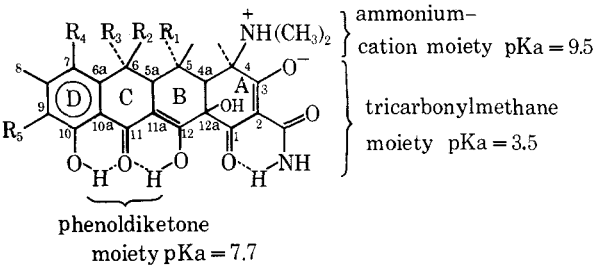
Keyphrases □ Tetracycline antibiotics—structure–activity relationships □ Quantum chemical approach—structure–activity relationships, tetracyclines □ Bacterial protein growth inhibition hypothesis—tetracyclines □ Molecular orbital perturbation theory—tetracycline activity □ Ribosomes, aminoacyl site—tetracycline binding

In both the past and the present, most clinically useful drugs have been discovered empirically or through synthesis of congeners of preexisting successful types. It was estimated (1) that only about one out of every 3000–5000 compounds synthesized is found to be a useful therapeutic agent. For this reason, even an imprecise or qualitative knowledge of molecular structure–activity relationships and mode of action of each family of compounds can be very useful in developing a more rational and direct approach to the synthesis of new drugs.

Since the isolation in 1947 (2) of the first known member of the tetracycline family, the clinical importance of these compounds as therapeutic and prophylactic agents against a wide range of infections has stimulated efforts (3–6) to define their mode of action as inhibitors of bacterial reproduction. Because of the advances to date in unraveling the mode of action of tetracyclines, this class of drugs is particularly well adapted for such investigations. This paper uses quantum chemical methods in an attempt to advance the knowledge of the relationship between electronic structure and the action of tetracyclines at the receptor site. An analysis of the possibilities and shortcomings of applying principles of quantum chemistry to the study of biological properties of drugs can be found in *Reference 7*.

EXPERIMENTAL DATA AND MODELS

Tetracyclines are derivatives of an octahydronaphthacene hydrocarbon system composed of four rings (Structure I). For useful



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clinical activity, previous work has shown that they clearly depend more upon the presence of their phenoldiketone, tricarbonylmethane, and ammonium-cation moieties than upon the presence of substituents at carbons 5 to 9. Modifications of the tricarbonylmethane structure, which is not readily reversible by simple aqueous hydrolysis, lead to drastic reductions in biological activity.

The oxygen functions at positions 10, 11, and 12 appear to be essential for biological activity, since alteration of this chromophoric group leads to compounds that are nearly or totally inactive. These functions are also among the most likely sites for metal chelation. The binding of tetracyclines to several macromolecules in the presence of metal ions was reported (8–10). The ability of the tetracyclines to chelate divalent cations is probably involved in their biological action. Alterations of the dimethylamino group at carbon 4 strongly reduce antibacterial activity. Substituent changes at carbons 5 to 9 produce derivatives with quantitatively different activities, affording a series of derivatives that offers a good possibility for studies of structure–activity relationships (11). The relative significance of these structures toward the mechanism of the biological action of these compounds has not yet been precisely determined and needs to be clarified in order to relate fully the structural characteristics to the activities.

For tetracyclines, the close similarity among the chemical reactions, their similar antibacterial spectra, the fact that the bacteriostatic effects of the most active compounds occur at similar concentrations, and the common occurrence of cross-resistance have led to the general assumption (4) that the mechanisms by which they inhibit the growth of microbial cells are similar if not identical. Although other effects may play a part in tetracycline bacteriostasis, many investigators presently feel that a general inhibition of protein biosynthesis appears to be the primary mechanism by which the tetracyclines exert their antibiotic activity. Tetracycline probably exerts its inhibitory effects on protein synthesis by binding to the ribosomes, thus interfering with the formation of the necessary complex between ribosome, messenger-RNA, and aminoacyl-*t*-RNA. This idea, widely accepted, is the basis for most of the recent research in this area (9, 12–15).

Quantitative biological activities of a large number of compounds, obtained under identical conditions in a precise manner, are required to establish structure–activity relationships. Ideally, these activities should be directly related to the mechanism of action of the compounds and be free of extraneous competing equilibria. Most of the available activities for tetracycline antibiotics have been summarized by Barrett (16), Boothe (17), and Plakunov (18). Many of these activities have been determined under conditions such that the results parallel clinical activity. This type of activity may be different than activity at the receptor site.

In a recent work (6, 15), activities suitable for the study of structure–activity relationships were established for a series of com-

Table I—Experimental Inhibitory Rate Constants

Number, i	Compound, T_i	k_i^I
1	7-NO_2-6-Demethyl-6-deoxytetracycline	748 ± 55
2	7-Cl-6-Demethyltetracycline	517 ± 34
3	7-Cl-Tetracycline	401 ± 15.9
4	Tetracycline	271 ± 11.3
5	5-Oxytetracycline	257 ± 24.5
6	7-NH_2-6-Demethyl-6-deoxytetracycline	181 ± 7.82
7	9-NH_2-6-Demethyl-6-deoxytetracycline	145 ± 8.79
8	6-Demethyl-6-deoxytetracycline	94.4 ± 3.06
9	7-Br-6-Demethyl-6-deoxytetracycline	51.8 ± 3.14
10	9-NO_2-6-Demethyl-6-deoxytetracycline	44.3 ± 1.51
11	9-$N(CH_3)_2$-6-Demethyldeoxytetracycline	23.6 ± 1.52
12	5*a*(6)-Anhydrotetracycline	15.5 ± 1.23
13	12*a*-Deoxytetracycline	2.54
14	7-Cl-5*a*(11*a*)-Dehydrotetracycline	0.34
15	4-Dedimethylaminotetracycline	24.1 ± 1.19
16	4-CH_3I-Tetracycline	2.18 ± 0.22
17	2-CN-Tetracycline	0.00
18	7-Cl-Isotetracycline	0.00
19	7-Cl-6-Demethyl-6-deoxytetracycline	(84.52)[a]
20	7-Dimethylamino-6-demethyldeoxytetracycline	(145.49)[a]

[a] Calculated based on Eq. 17.

pounds, including both clinically active and "inactive" tetracycline antibiotics. These activities were obtained by three types of kinetic measurements on exponentially growing *Escherichia coli* W inhibited by the antibiotics. Inhibition of cell division, protein synthesis, and nucleic acid synthesis was simultaneously studied so that activities might be more directly related to the mode of action. Generation rate constants were determined in the presence of low, graded concentrations of tetracyclines. The effect of the action of each antibiotic, T_i, is to cause a reduction in the generation rate constants:

$$[N_i]_i = [N_0]e^{k_i t} \qquad k^0 > k_i > 0 \qquad \text{(Eq. 1)}$$

where $[N_i]$ and $[N_0]$ are concentrations of cells, proteins, or nucleic acids in the culture; k_i is the corresponding generation rate constant in the presence of antibiotic T_i; and k^0 is the generation rate constant in the absence of antibiotic. The constants k^0 were found to be experimentally identical. The rate constants k_i were obtained by application of regression analysis to Eq. 1. Rate constants k_i were found to be related to antibiotic concentration $[T_i]$ by the equation:

$$k_i = k^0 - k_i^I [T_i] \qquad \text{if } k_i > 0 \qquad \text{(Eq. 2)}$$

where k_i^I are the inhibitory rate constants. Within experimental accuracy, the inhibitory rate constants for cell division, for protein synthesis, and for nucleic acid synthesis are practically identical for each antibiotic. The constants k_i^I are accurate measures of the potency of the antibiotics under the test conditions and should be useful in structure–activity relationship studies. Values of k_i^I for the compounds studied are given in Table I.

From the experimental results on the kinetics of inhibition, kinetic models for the interference of tetracycline antibiotics with protein synthesis in the individual cell can be constructed (6, 19). These models are presented in terms of reactions involved in protein synthesis in a bacterial culture. The models consider that tetracyclines bind to the aminoacyl site of ribosomes and thereby prevent the binding of aminoacyl-*t*-RNA. They also assume that there is a reversible passage of tetracyclines from outside to inside the cells. Tetracyclines inside the cell bind to ribosome-RNA (RIB) and form a complex (RIB–T_i) which is not capable of synthesizing protein. This may be written as follows:

$$[T_i'] \overset{K_i^p}{\rightleftharpoons} [T_i] \qquad \text{(Eq. 3)}$$

$$[T_i] + [\text{RIB}] \overset{K_i^c}{\rightleftharpoons} [\text{RIB}\text{–}T_i] \qquad \text{(Eq. 4)}$$

The stoichiometry of this equation is that suggested by Day (8) and Maxwell (3). As a starting point, the simplest model involving the least number of reactions is postulated. Considering the limiting cases of this model, a simple expression for the inhibitory constants can be written (19):

$$k_i^I = k^0 K_i^p K_i^c \qquad \text{(Eq. 5)}$$

This approach is, of course, an oversimplification and is used here because of this simplicity. Several variations of this model have been discussed (6). Equation 5 shows the principal factors responsible for the variation of k_i^I when passing from one molecule to another in a series of tetracycline antibiotics. It affords a basis for the theoretical study of structure–activity relationships.

THEORETICAL APPROACH

The present theoretical work is based on several considerations and assumptions supported by the experimental results:

1. The unusual structural groupings in the tetracyclines produce three macroscopic acidity constants in aqueous solution which, it is generally agreed (20–22), are approximately equal to 3.5, 7.7, and 9.5. The functional groups responsible for the thermodynamic pKa values are located in the tricarbonyl–methane system, the phenoldiketone moiety, and the ammonium cation, respectively. On this basis, and from studies on the effect of broth pH changes on tetracycline-inhibited cultures (15, 23), the authors adopted the dipolar ion molecular model in Structure I.

2. It is assumed that all the investigated compounds act in the same way by binding either directly or through metal chelates to the site on the ribosome which normally binds the transfer-RNA molecules (4, 6, 12, 15).

3. If the locus of action of an antibiotic lies within the microbial cell, the ability to permeate the cells, represented in Eq. 5 by the factor K_i^p, is an important factor in antibiotic activity; it is difficult to distinguish between structural changes that alter the ability of the tetracyclines to react at the receptor site and changes that alter the tetracycline's ability to permeate the cell. Removal or specific alteration of the functional groups responsible for the macroscopic acidity constants significantly alters the acidic characteristics of the molecules. This alteration could lead to important modifications of the ability to permeate the cells (24, 25). To reduce, as much as possible, the influence of the K_i^p factor, and as a first step to gain insight on action of the tetracycline antibiotics at the molecular level, only those members of the tetracycline family (normal tetracyclines) that have intact 4-dimethylamino, tricarbonylmethane, and phenoldiketone structures (Compounds 1–14, Table I) were included in this study. It is then assumed (15, 24, 25) that, for the compounds of the series considered here, the factor essentially responsible for the variation in k_i^I values is the variation of the equilibrium constant, K_i^c, due to differences in electronic molecular structures.

In accord with these assumptions, Eq. 5 can be written, for normal tetracyclines, in the following approximative form:

$$\log k_i^I = \text{constant} + \log K_i^c \qquad \text{(Eq. 6)}$$

This relation can be utilized to estimate the k_i^I values, for molecules belonging to this series, from K_i^c calculations. From Eq. 4 and according to the principles of statistical thermodynamics, the follow-

Table II—Empirical Parameters for π-Structure Calculations (39, 40)

Atom, p	δ_p	Bond, p—q	η_{pq}
C	0.00	C—C	1.00
O (carbonyl)	0.70	C—O (carbonyl)	2.00
O (hydroxyl)	2.00	C—O (hydroxyl)	0.90
O (nitro)	1.00	C—N	0.80
N (amino)	1.80	C—Br	0.30
N (nitro)	2.00	C—Cl	0.40
Br	1.60	N—O	0.70
Cl	2.10		

ing set of equations can be written:

$$K_i^c = \frac{f_{RT_i}}{f_R f_{T_i}} e^{-\Delta\epsilon/kT} \quad i = 1, 2, \ldots, 14 \qquad \text{(Eq. 7)}$$

where $\Delta\epsilon_i = \epsilon_{RT_i} - (\epsilon_R + \epsilon_{T_i})$. The quantity $\Delta\epsilon_i$ is the difference in ground-state energies between the complex RIB–T_i and the reactants RIB and T_i; the f terms are partition functions; and k and T stand, respectively, for Boltzman's constant and the absolute temperature. The set of equations in Eq. 7 can be written in the more convenient form:

$$\log K_i^c = \log \frac{f_{RT_i}}{f_R f_{T_i}} + \text{constant} \cdot \Delta\epsilon_i \qquad i = 1, 2, \ldots, 14 \qquad \text{(Eq. 8)}$$

To separate the solvation energy contribution, ΔE_i(solv.), the term $\Delta\epsilon_i$ can be approximated by the sum (26):

$$\Delta\epsilon_i = \Delta E_i + \Delta E_i(\text{solv.}) \qquad \text{(Eq. 9)}$$

where the quantity ΔE_i(solv.) is given by the equation:

$$\Delta E_i(\text{solv.}) = E_{RT_i}(\text{solv.}) - \{E_R(\text{solv.}) + E_{T_i}(\text{solv.})\} \qquad \text{(Eq. 10)}$$

Since the equilibrium reactions were carried out under the same experimental conditions for all the compounds, and because of the close structural similarity between the selected tetracyclines, the usual simplifications can be utilized; *i.e.*, the terms $\log (f_{RT_i}/f_R f_{T_i})$ and ΔE_i (solv.) do not significantly vary with respect to the variation of ΔE_i. The assumption that the partition function terms are constant appears to be generally valid (27), provided that no specific effects (such as steric hinderance) are involved. The assumption of constant solvation energy terms is supported (26) by the fact that each of the two compound series, RT_i and T_i, are composed of molecules of the same size, having similar structure and a similar charge distribution in their zwitterionic moieties. The set of Eq. 8 can then be written in the following form:

$$\log K_i^c = a + b\,\Delta E_i \qquad i = 1, 2, \ldots, 14 \qquad \text{(Eq. 11)}$$

where a and b are assumed to be practically constant. Substituting Eq. 11 in Eq. 6, one has the set of equations

$$\log k_i^I = \text{constant}_1 + \text{constant}_2\,\Delta E_i \qquad i = 1, 2, \ldots, 14 \qquad \text{(Eq. 12)}$$

Then, within the limitations of the theory and the assumed simplifications, k_i^I activities can be related to differences in the molecular electronic ground-state energies, ΔE_i. To study the ΔE_i variation in the present case, by using the familiar molecular orbital theory (28), the perturbation energy of the two systems, T_i and RIB, is evaluated when they weakly interact in the complex RIB–T_i. As a basis for a general theory of organic chemistry, Dewar (29) developed a method wherein, instead of calculating the coefficients of the molecular orbitals of a mesomeric system AB by the usual variational procedure, he showed that they can be obtained to a sufficiently good approximation by regarding formation of the bonds linking A to B as a perturbation and applying perturbation theory.

The formation of charge-transfer complexes and the reactivity of conjugated molecules, for a definite type of reaction with a definite reagent, were similarly discussed, as a function of the magnitude of the perturbation energy, by Fukui *et al.* (30, 31) and Morokuma *et al.* (32). From an approximate expression of the perturbation energy, the delocalizability indexes of reactivity were derived by these authors. More recently, Klopman and Hudson (33–35) described a more general perturbation treatment of chemical reactivity, in which allowance is made for ionic interaction and the theory is not restricted to π-conjugated molecules.

If T_i and RIB approach such that an atom t of T_i and an atom r of RIB interact, being weakly linked in the RIB–T_i complex, this interaction produces a change in energy ΔE_i, the magnitude of which depends on the reactants, the atoms t and r, and their distance d_{tr}. If, as indicated, one is only concerned with electronic effects, then perturbation theory as applied by Klopman and Hudson (33–35) to weakly interacting compounds gives, for p pairs of t and r bonding atoms, the following set of expressions[1]:

$$\Delta E_i = \Sigma_p \left\{ Q_{i_{t_p}} Q_{r_p}(tt|rr) + \Sigma_{ki}\Sigma_m \frac{2c_{ki}^2{}_{t_p}}{\epsilon_{ki} - \epsilon_m} c_{m r_p}^2 \beta_{t_p r_p}^2 - \Sigma_{1i}\Sigma_n \frac{2c_{1i}^2{}_{t_p}}{\epsilon_{1i} - \epsilon_n} c_{n r_p}^2 \beta_{t_p r_p}^2 \right\} \qquad i = 1, 2, \ldots, 14 \qquad \text{(Eq. 13)}$$

where Σ_p holds for the p pairs, t_p–r_p, of interacting atoms in the complex; $Q_{i_{t_p}}$ and Q_{r_p} are the net charges of atoms t_p and r_p, respectively; $(tt|rr)_p$ is the coulombic electron-repulsion energy: $(tt|rr)_p = \int \chi_{t_p}{}^2\,(1)\,(1/d_{12})\,\chi_{r_p}{}^2\,(2)\,dz$; the c_{us}'s are the atomic orbital coefficients in the molecular orbital development: $\phi_u = \Sigma_s c_{us}\chi_s$; ϵ_{ki} and ϵ_{1i}, respectively, stand for the energies of the occupied and unoccupied molecular orbitals of T_i; ϵ_m and ϵ_n have the same significance for RIB; and β_{tr} represents the resonance integral associated with the t_p–r_p bond.

By combining Eqs. 12 and 13, one can write the following set of equations:

$$\log k_i^I = c + d\Sigma_p \left\{ Q_{i_{t_p}} Q_{r_p}(tt|rr)_p + \Sigma_{ki}\Sigma_m \frac{2c_{ki}^2{}_{t_p}}{\epsilon_{ki} - \epsilon_m} c_{m r_p}^2 \beta_{t_p r_p}^2 - \Sigma_{1i}\Sigma_n \frac{2c_{1i}^2{}_{t_p}}{\epsilon_{1i} - \epsilon_n} c_{n r_p}^2 \beta_{t_p r_p}^2 \right\} \qquad i = 1, 2, \ldots, 14 \qquad \text{(Eq. 14)}$$

c and d being constants.

The complex RIB–T_i probably does not involve (19) covalent bonding, and it is expected to be charge controlled. That is to say, the differences $\epsilon_{ki} - \epsilon_m$ and $\epsilon_{1i} - \epsilon_n$ for all pairs of orbitals will be large, with very little transfer of charge. The small differences between the various ϵ_m and between the various ϵ_n values can then be neglected (35). Thus, equations in Eq. 14 can be written in the following simplified form:

$$\log k_i^I = c + d\Sigma_p \left\{ Q_{i_{t_p}} Q_{r_p}(tt|rr)_p + \left(\Sigma_{ki} \frac{2c_{ki}^2{}_{t_p}}{\epsilon_{ki} - \bar{\epsilon}_m}\right)(\Sigma_m c_{m r_p}^2) \times \beta_{t_p r_p}^2 + \left(\Sigma_{1i} \frac{2c_{1i}^2{}_{t_p}}{\epsilon_{1i} - \bar{\epsilon}_n}\right)(\Sigma_n c_{n r_p}^2)\beta_{t_p r_p}^2 \right\} \qquad i = 1, 2, \ldots, 14 \qquad \text{(Eq. 15)}$$

where $\bar{\epsilon}_m$ and $\bar{\epsilon}_n$ are average values. The terms $\Sigma_{ki}[2c_{ki}^2{}_{t_p}/(\epsilon_{ki} - \bar{\epsilon}_m)]$ and $\Sigma_{1i}[2c_{1i}^2{}_{t_p}/(\epsilon_{1i} - \bar{\epsilon}_n)]$ are very closely related to Fukui's *et al.* (30, 31) electrophilic ($E_{i_{t_p}}$) and nucleophilic ($N_{i_{t_p}}$) delocalizabilities, $E_{i_{t_p}} \approx \Sigma_{ki}(2c_{ki}^2{}_{t_p}/\lambda_{ki}\beta_{cc})$ and $N_{i_{t_p}} = \Sigma_{1i}(2c_{1i}^2{}_{t_p}/\lambda_{1i}\beta_{cc})$, where λ_k is the energy of the k^{th} molecular orbital in units of β_{cc} with reference to α_c, α_c and β_{cc} being the Coulomb and the resonance integrals of benzene. This circumstance, as well as the fact that the quantities $Q_{r_p}(tt|rr)_p$, $(\Sigma_m c_{m r_p}^2)\beta_{t_p r_p}^2/\beta_{cc}$, and $(\Sigma_n c_{n r_p}^2)\beta_{t_p r_p}^2/\beta_{cc}$ are essentially constant for all the equations of the set, transforms the equations in Eq. 15 into the following set of simultaneous equations:

$$\log k_i^I = c + \Sigma_p\{f_p Q_{i_{t_p}} + g_p E_{i_{t_p}} + h_p N_{i_{t_p}}\} \qquad i = 1, 2, \ldots, 14 \qquad \text{(Eq. 16)}$$

where c, f_p, g_p, and h_p are constants. This expression is formally similar to expression 14 in *Reference 36*.

In principle, this system of simultaneous equations holds for the atoms p of the tetracyclines directly perturbed by bonding in the complex with ribosomes. Combined with the usual multiple-regression techniques, these equations can be usefully applied to estimate the relative variation of ΔE_i for the family of tetracyclines which act in an identical way on the RIB of unknown structure. They can also be used to determine which tetracycline atoms, p, are directly concerned in complex formation.

[1] It is assumed in this work that there is no degeneracy between molecular orbital energies. See following discussion.

Table III—Empirical Parameters for Electronic Localized Structure Calculations (3, 9, 41, 43, 44)

$\delta°$ Values

H	C(Ternary)	C(Quaternary)	N(Ternary)	N(Quaternary)
0.00	0.12	0.07	0.24	0.31

O(Hydroxyl)	O(Carbonyl)	$O^{-1/2}$(Nitro)	Cl	Br	O . . .	H—O
0.40	0.28	0.33	0.35	0.30	+0.04	−0.04

ϵ_{AB} Values

C—C	C—H	C—N	C—O	C=O	C—Cl	C—Br	N—H	O—H	$C—O^{-1/2}$	$C—N^+R_3$	$N^+—R_3$	O . . . H
1.00	1.00	1.00	0.95	0.70	0.65	0.58	0.45	0.45	0.80	1.33	0.60	0.07

$\gamma_{A(B)}$ Values

	A					
B	H	C	N	O	Cl	Br
H	—	0.3	0.3	0.3	—	—
C	0.4	0.1	0.1	0.1	0.4	0.5
N	0.4	0.1	—	0.1	—	—
O	0.4	0.1	0.1	—	—	—
Cl	—	0.2	—	—	—	—
Br	—	0.23	—	—	—	—

Table IV—Calculated Atomic Charge Indexes, $Q_p(\sigma + \pi)$, for Atoms 6, O_{10}, O_{11}, and O_{12}

Compound, T_i	Q_6	Q_{O10}	Q_{O11}	Q_{O12}
1	−0.0610	−0.2866	−0.2719	−0.2955
2	+0.0951	−0.3191	−0.2759	−0.2955
3	+0.1415	−0.3191	−0.2759	−0.2955
4	+0.1393	−0.3183	−0.2760	−0.2955
5	+0.1264	−0.3183	−0.2759	−0.2955
6	−0.0610	−0.3221	−0.2758	−0.2955
7	−0.0610	−0.3213	−0.2760	−0.2955
8	−0.0610	−0.3183	−0.2760	−0.2955
9	−0.0590	−0.3188	−0.2759	−0.2955
10	−0.0614	−0.2869	−0.2997	−0.2955
10′	−0.0614	−0.2869	−0.2760	−0.2955
11	−0.0614	−0.3381	−0.2751	−0.2955
11′	−0.0614	−0.3213	−0.2760	−0.2955
12	−0.0716	−0.3144	−0.3515	−0.1596
13	+0.1393	−0.3184	−0.2646	−0.3213
14	+0.1524	−0.3189	−0.2061	−0.2773
19	−0.0590	−0.3189	−0.2760	−0.2955
20	−0.0614	−0.3221	−0.2760	−0.2955

CALCULATIONS

The general molecular structure (I) assumed for tetracyclines is based on the experimental data of Donohue *et al.* (37). The two unsaturated phenoldiketone and tricarbonylmethane moieties can be considered as being practically planar. The σ–π separability has been assumed for these two local molecular regions. The two local π-electronic structures were calculated by using the conventional Hückel method as modified by Wheland and Pauling (38). The empirical parameters utilized (39, 40) are given in Table II. Since the number of valence atomic orbitals in the electronic localized structure of tetracyclines is exceedingly large, the localized electronic properties were evaluated by use of Del Re's method (41) as modified by Berthod and Pullman (39, 42). The latter modification was used to take into account the σ–π interaction and hydrogen-bond contributions. The empirical parameters utilized (39, 41, 43, 44) are reproduced in Table III. The calculated atomic net charge indexes Q_{it_p} $(\sigma + \pi)$, for atoms C_6, O_{10}, O_{11}, and O_{12}, and their π-delocalizability indexes, E_{it_p} and N_{it_p}, are shown, respectively, in Tables IV and V[2]. The sigma part of the delocalizability indexes was neglected.

Equation 16, which relates the inhibitory activity of the tetracyclines to their electronic structure, was analyzed by multiple-regression models (45) involving the inhibitory rate constants $k_i{}^I$ and the terms Q_{it_p}, E_{it_p}, and N_{it_p}. Ideally, all possible combinations of the variables should be examined, but this rapidly becomes impracticable. The difficulty can be resolved by an appreciation of the technical background of the problem. A few general rules exist for the handling of problems involving many variables. It is a common practice to begin by fitting a regression on the variable that is thought, on informed opinion, most likely to be related to the dependent variable and then to introduce other variables in turn, retaining those that significantly increase the regression sum of squares. Following this procedure, the authors considered a great number of possible combinations of variables, as many as were judged necessary to avoid the possibility that a significant contribution might be overlooked. A total of 356 regressions was investigated. Some of the regression equations tested are given in Table VI[3].

RESULTS AND DISCUSSION

The multiple-regression equation, relating the tetracycline inhibitory index, $k_i{}^I$, to the perturbation energies, ΔE_i, that was found to best satisfy statistically the simultaneous system of Eqs. 16 is

$$\log k^I = 18.3975 + 56.1733Q_{O10} + 16.9155E_{O10} + 48.7956Q_{O11} - 1.1063E_{O11} + 71.3254Q_{O12} + 18.3655E_{O12} + 3.3880Q_{C_6} \quad \text{(Eq. 17)}$$

Equation 17 has: (*a*) a multiple-correlation coefficient, $R = 0.993$, which is highly significant [the 0.1% point for 12 degrees of freedom being 0.78 (45)]; (*b*) an estimated standard deviation, SD, of 0.1627; and (*c*) a standard error of the mean, SEM, of 0.0434.

The *t*-test for the significance of the coefficients is shown in Table VII. The analysis of variance for the multiple regression is shown in Table VIII. This type of analysis gives the value $F_6{}^7 = 61.068$ for the significance of the regression equation. This value is highly significant ($p < 0.001$). Table IX compares experimental and predicted values for $\log k_i{}^I$. Figure 1 gives a graphic representation of experimental *versus* predicted $\log k_i{}^I$ values. The result is very satisfactory when one considers the number of approximations used.

Within the accuracy of the approximations used for the determination of ΔE_i, the results obtained in this study are consistent both with the existence of a direct dependence between the variation in activity and the equilibrium constant $K_i{}^c$ and, within the assumed mode of action, the existence of a direct inhibition of protein synthesis by ribosome–tetracycline interaction. These results are consistent, more generally, with any mode of action of the general type described in the model, Eqs. 3–5. Equation 17 indicates that the variation in activity in normal tetracyclines is directly related with the electronic characteristics of the phenoldiketone and C_6 molecular regions as a function of the effects of substituents. The introduction in the regression of any variable other than those appearing in

[2] The charge and delocalizability indexes for atoms other than C_6, O_{10}, O_{11}, and O_{12} will be sent to the interested reader on request.

[3] The analysis of all tested regressions will be sent on request.

Table V—Calculated π-Electronic Delocalizability Indexes, E_p and N_p, for Atoms 6, O_{10}, O_{11} and O_{12}

Compound, T_i	E_6	N_6	$E_{O_{10}}$	$N_{O_{10}}$	$E_{O_{11}}$	$N_{O_{11}}$	$E_{O_{12}}$	$N_{O_{12}}$
1	—	—	+0.9946	+0.0547	+0.8185	−0.5831	+1.0451	−0.1101
2	—	—	+1.0592	−0.0680	+0.8277	−0.5949	+1.0453	−0.1103
3	—	—	+1.0592	−0.0680	+0.8277	−0.5949	+1.0453	−0.1103
4	—	—	+1.0532	−0.0694	+0.8273	−0.5948	+1.0452	−0.1103
5	—	—	+1.0532	−0.0694	+0.8273	−0.5948	+1.0452	−0.1103
6	—	—	+1.0832	−0.0634	+0.8294	−0.5952	+1.0453	−0.1104
7	—	—	+1.0863	−0.0665	+0.8273	−0.5932	+1.0452	−0.1103
8	—	—	+1.0532	−0.0694	+0.8273	−0.5948	+1.0452	−0.1103
9	—	—	+1.0577	−0.0684	+0.9276	−0.5948	+1.0453	−0.1103
10	—	—	+1.0043	−0.0499	+0.8270	−0.5916	+1.0452	−0.1103
10′	—	—	+1.0043	−0.0449	+0.8270	−0.5916	+1.0452	−0.1103
11	—	—	+1.0863	−0.0665	+0.8273	−0.5932	+1.0452	−0.1103
11′	—	—	+1.0863	−0.0665	+0.8273	−0.5932	+1.0452	−0.1103
12	+1.2601	−0.8270	+1.0776	−0.0852	+1.0655	−0.0958	+0.6633	−0.5550
13	—	—	+1.0570	−0.0679	+1.4467	−0.4570	+1.0326	−0.0606
14	—	—	+1.0588	−0.0687	+0.7274	−0.6773	+0.6131	−0.6131
19	—	—	+1.0592	−0.0680	+0.8277	−0.5949	+1.0452	−0.1103
20	—	—	+1.0832	−0.0634	+0.8294	−0.5952	+1.0453	−0.1104

Eq. 17 reduces the significance of the regression equation as measured by F- and t-tests.

Consider the two regression equations:

$$\log k^I = 7.23 + 52.41Q_{O_{10}} + 19.54E_{O_{10}} + 27.55Q_{O_{11}} - 1.80E_{O_{11}} + 47.27Q_{O_{12}} + 14.02E_{O_{12}} + 3.37Q_6 - 0.79Q_9 + 19.65Q_{10a} \quad \text{(Eq. 18)}$$

and

$$\log k^I = -333.75 + 184.59Q_{6a} + 65.52E_{6a} - 4.38Q_7 - 180.11E_7 - 1491.56Q_{10a} + 178.73E_{10a} + 657.51Q_{11} + 206.62E_{11} + 124.77Q_{11a} - 9.11E_{11a} \quad \text{(Eq. 19)}$$

Regression 18, which differs from Eq. 17 only by including Q_9 and Q_{10a}, has a multiple-correlation coefficient $R = 0.994$; but it has a significance given by $F_4^9 = 37.53$ ($p < 0.005$), which is less significant than the F-test for Eq. 17. The t-tests for the significance of the coefficients in Eq. 18 are shown in Table X; this test shows that the newly introduced variables, Q_9 and Q_{10a}, are not significant. Regression 19 has a multiple-correlation coefficient $R = 0.981$ but this equation is not acceptable statistically, $F_3^{10} = 7.683$ ($p < 0.1$). The t-test for significance of coefficients is shown in Table XI. Both the F-test and the t-test show the nonsignificance of this regression. Similar conclusions are obtained from the analysis of the other tested regressions (see Table VI).

It is important to realize that it is the dependence of the variation of the inhibitory ability, Δk_i^I, on the variation of the perturbation energy, $\Delta\Delta E_i$, as a whole, that has a real physical meaning. The quantity ΔE_i is made up of a combination of several more or less arbitrarily defined "reactivity indexes," charges, and so on, of which the nature depends on the particular method used in evaluating ΔE_i. In dealing with chemical reactivity problems, it is necessary to analyze the whole variation of ΔE_i. The simultaneous analysis of the different reactivity indexes making up ΔE_i indicates the type of "controlling effect" in each type of reaction. As pointed out by Hudson and Klopman (33), it is intrinsically incorrect to relate reactivity to a particular reactivity index. In the case of this series of tetracycline compounds, all component terms of ΔE_i that do not significantly contribute to the $\Delta\Delta E_i$ variation do not explicitly appear in Eq. 17. The ones that appear, Q_6, $Q_{O_{10}}$, $Q_{O_{11}}$, $Q_{O_{12}}$, $E_{O_{10}}$, $E_{O_{11}}$, and $E_{O_{12}}$, are not significant considered in isolation. In fact, each one frequently does not vary, or varies little, when passing from one molecule to another in the series (Tables IV and V).

Table VI—Some Regression Equations Tested

1. $\log k^I = 22.8809 + 34.0946Q_{O_{10}} + 6.7370E_{O_{10}}$ (1.3) (0.5) $+ 51.6236Q_{O_{11}} - 0.5822E_{O_{11}}$ (2.6) (−0.7) $+ 69.6836Q_{O_{12}} + 17.5739E_{O_{12}}$ (3.3) (4.3)
   $R = 0.9534$, $F_7^6 = 11.673$ ($p < 0.005$)
2. $\log k^I = 18.3975 + 56.1733Q_{O_{10}} + 16.9155E_{O_{10}}$ (4.9) (3.1) $+ 48.7956Q_{O_{11}} - 1.1063E_{O_{11}}$ (5.9) (−3.1) $+ 71.3254Q_{O_{12}} + 12.3655E_{O_{12}}$ (8.1) (10.7) $+ 3.3880Q_6$ (5.8)
   $R = 0.9930$, $F_6^7 = 61.068$ ($p < 0.001$)
3. $\log k^I = 11.1498 + 0.0818Q_9 + 12.5640E_9$ (0.0) (0.5) $+ 45.3364Q_{O_{10}} + 2.8236E_{O_{10}}$ (1.6) (0.1) $- 19.4518Q_{O_{11}} - 3.6663E_{O_{11}}$ (−0.2) (−0.9) $- 24.0750Q_{O_{12}} + 3.1213E_{O_{12}}$ (−0.2) (0.2)
   $R = 0.9668$, $F_5^8 = 8.9546$ ($p < 0.025$)
4. $\log k^I = 31.1534 + 1.2606Q_7 + 7.4356E_7$ (1.8) (5.3) $+ 52.2854Q_{O_{10}} + 1.7786E_{O_{10}}$ (1.6) (0.1) $+ 74.6820Q_{O_{11}} - 0.6185E_{O_{11}}$ (2.4) (−0.8) $+ 78.4148Q_{O_{12}} + 22.1390E_{O_{12}}$ (3.1) (3.6)
   $R = 0.9678$, $F_5^8 = 9.2443$ ($p < 0.025$)

Table VII—t-Test for Significance of Coefficients in the Multiple-Regression Eq. 17

Variable	t-Value	p
Q_0	4.895	<0.005
$E_{O_{10}}$	3.077	>0.01
$Q_{O_{11}}$	5.89	<0.005
$E_{O_{11}}$	−3.087	>0.01
$Q_{O_{12}}$	8.095	<0.005
$E_{O_{12}}$	10.681	<0.005
Q_6	5.779	<0.005

Table VIII—Analysis of Variance for the Multiple Regression (17)[a]

Source	Sum of Squares	Degrees of Freedom	Mean Squares
Due to regression	11.3170	7	1.6167
About regression	0.1588	6	0.0265

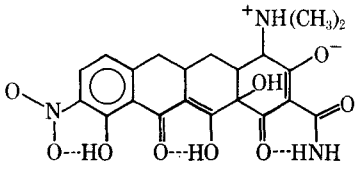
[a] $F_6^7 = 61.068$, and $p < 0.001$.

Table IX—Actual and Predicted Values for the Logarithm of the Inhibitory Rate Constants

Compound, i	log K_i^I (exp.)	log k_i^I (calcd.)	Residuals
1	2.874	2.860	+0.014
2	2.714	2.454	+0.260
3	2.604	2.611	−0.007
4	2.434	2.541	−0.107
5	2.410	2.502	−0.092
6	2.259	2.165	+0.094
7	2.161	2.252	−0.091
8	1.975	1.861	+0.114
9	1.714	1.923	−0.209
10	1.647	1.641	+0.006
11	1.374	1.352	+0.022
12	1.190	1.190	0.000
13	0.399	0.399	0.000
14	−0.469	−0.470	−0.001

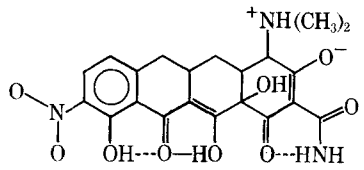
Regression 17 underlines the electrostatic or charge-controlled character of tetracycline action. The introduction of the N_{t_p} variables associated with atoms O_{10}, O_{11}, and O_{12} gives nonsignificant t-values and important lowering of the F-values for the significance of the multiple correlation (Table VI). The fact that the E_{t_p} delocalizabilities are significant indicates that the π-electronic donor character of the phenoldiketone structures are also important. This fact is consistent with the possibility that ribosome–tetracycline interaction occurs through metal chelates.

Inasmuch as Eq. 17 gives an indication of the relationship between inhibitory activity and the molecular structure for the normal tetracycline series, it should be possible to predict the influence of specific factors, such as steric hindrance, on the inhibitory index k_i^I. The phenolic hydroxyl groups in the tetracycline molecules play an essential role in the interaction process. As a consequence, when the 10-hydroxyl group is hindered by hydrogen bonding with an *ortho*-nitro substituent, the activity is greatly reduced. This is the case (46) for the 9-NO_2-6-demethyl-6-deoxytetracycline (Compound 10 in this series). In the calculations, the authors considered this type of interaction of the hydroxyl group by using the molecular model shown here for Compound 10.



Compound 10

It is easy to account for the quantitative importance of this hindering factor by considering a compound with the ideal structure (Compound 10′), where the hydroxyl group-10, as in the other molecules in the series, is not *ortho*-inhibited (Compound 10′). Equation 17 gives a rate inhibitory constant of $k_{10'}{}^I = 635.25$ for Compound 10′. As one would expect, this value for the activity is very near to that of the 7-NO_2-compound, $k_1{}^I = 748.78$. The experimental $k_{10}{}^I$ is 44.33, an activity which is well correlated with the calculated value for Compound 10 of 43.72. A similar case was observed with 9-dimethylamino-6-demethyl-6-deoxytetracycline, Compound 11. When $k_{11}{}^I$ is calculated neglecting the hydrogen bonding between the methylamino substituent and the hydroxyl group-10, the predicted value for activity is $k_{11}{}'^I = 178.81$. This value lies between the activities for the 7-NH_2 and the 9-NH_2 derivatives and it is significantly different from the experimental activity value $k_{11}{}^I = 23.67$. The calculated value for the 10-hydroxyl-hindered compound is 23.66.



Compound 10′

Table X—t-Test for Significance of Coefficients in the Multiple Regression (18)

Variable	t-Value	p
$Q_{O_{10}}$	3.788	≤ 0.01
$E_{O_{10}}$	2.825	≤ 0.025
$Q_{O_{11}}$	1.037	>0.1
$E_{O_{11}}$	1.982	<0.1
$Q_{O_{12}}$	1.584	≤ 0.1
$E_{O_{12}}$	2.573	<0.05
Q_6	4.423	<0.01
Q_9	0.641	>0.1
Q_{10a}	0.784	>0.1

Table XI—t-Test for Significance of Coefficients in the Multiple Regression (19)

Variable	t-Value	p
Q_{6a}	2.294	<0.1
E_{6a}	2.176	<0.1
Q_7	−0.624	>0.1
E_7	−2.357	$=0.05$
Q_{10a}	−2.220	<0.1
E_{10a}	1.665	≤ 0.1
Q_{11}	2.457	<0.05
E_{11}	2.377	≤ 0.05
Q_{11a}	2.146	<0.1
E_{11a}	−2.365	$=0.05$

Insofar as Eq. 17 is correct, it is possible to use it for the predictions of the activity of untested normal tetracycline derivatives. For instance, in the experimental work of Miller *et al.* (15), the rate activity constant for 7-chloro- and 7-dimethylamino-6-demethyl-6-deoxytetracyclines (Compounds 19 and 20 in the tables) were not determined. The predicted values, from Eq. 17, are, respectively, $k_{19}{}^I = 84.52$ and $k_{20}{}^I = 145.49$. The predicted activity $k_{20}{}^I$ is of the same order (*cf.*, Table I) as those for the 7-NH_2- and 9-NH_2-6-deoxytetracycline derivatives. Also, the predicted activity $k_{19}{}^I$ is of the same order as those for the 6-demethyl-6-deoxytetracycline and 7-Br-6-demethyl-6-deoxytetracycline derivatives. The latter result is in good agreement with the experimental results of Hlavka *et al.* (47) on the *in vitro* activities for these three compounds.

CONCLUSION

In spite of the large number of approximations used, the results of this theoretical approach seem to explain reasonably well a great quantity of experimental data on the inhibitory properties of normal tetracyclines by relating them to the electronic structure of the molecules.

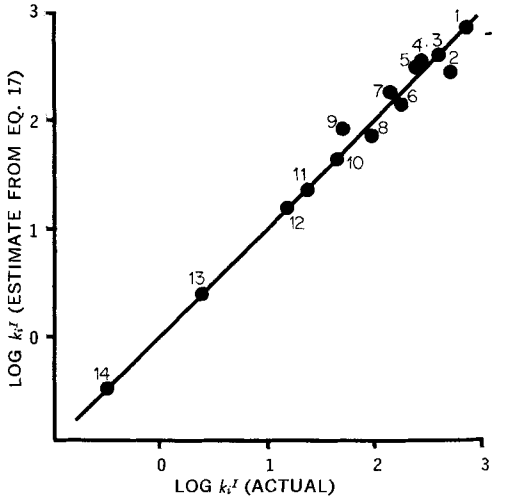


Figure 1—*Graphic representation of actual* versus *estimated log* $k_1{}^I$ *values.* $F_6{}^7 = 61.07$ ($p < 0.001$).

The theoretical results are consistent with the hypothesis of a direct inhibition of *E. coli* caused by complexation of tetracyclines with a biological structure, for example, inhibition of protein synthesis by complexation with a ribosome.

The tetracycline action seems to involve a direct interaction of C_6 and the phenoldiketone region with the proposed ribosome receptor site. The process seems to be charge controlled. The interaction of phenoldiketone oxygen functions with the ribosome may be achieved through metal chelates, since this interaction is a function of the donor character of the oxygen atoms.

The effect of substituents on the electronic properties of the C_6 and phenoldiketone regions is to produce a graduation of the inhibitory properties of normal tetracyclines.

Equation 17 permits a satisfactory estimation of rate inhibitory constants k_i^I for normal tetracyclines, provided that specific effects such as steric hindrance are taken into account. Furthermore, it allows the estimation of activities of tetracyclines not yet tested *in vitro* or clinically for inhibitory activities.

This paper serves as an example of the use of quantum chemical techniques in elucidating biological action and provides an indication of the changes one can make in a drug molecule to alter biological action. These methods could also be used to suggest molecular changes needed for greater stability, enhanced penetration through membranes, and general solubility and partitioning characteristics.

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