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Correlation of the Potencies of Tetracyclines *in Vitro* with a D-Ring Substituent Index

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SUMMARY

Inhibitory and inactivation potencies of tetracyclines toward nonsynchronous and synchronous cultures, respectively, of *Escherichia coli* can be correlated with the same substituent index for groups placed on the D-ring. The implication gained from this pair of correlations is that the bacteriostatic and bactericidal effects of tetracyclines are controlled by a common electronic factor. Similarly, an equivalent electronic index and the lipophilic index π may be used to correlate the inhibitory potencies of a series of chloramphenicols, whose mode of action is similar to but at a site different from that of the tetracyclines.

Bacterial growth kinetics provides a means of assessing quantitatively the relative potencies of antibacterial agents. A culture of bacteria in balanced growth has its change in population with time described by a first-order rate law. Introduction of an antibiotic into this culture results in a diminished rate of growth, which is reflected by a lowered apparent first-order rate constant. The effect of the antibiotic on the culture may be to inhibit the growth of the bacteria at low concentrations of drug, or it may be bactericidal at high concentrations of drug, in which case a certain portion of the cells are inactivated, i.e., killed. An antibiotic is assumed to inhibit bacterial growth when the rate law based on total population is superposable on the rate law based on viable cell counts. With tetracycline as the antibiotic, it has been reported (1) that the inhibition and the inactivation of *Escherichia coli* cultures lead to differing forms of the apparent rate law. Inhibition of the cultures led to first-order dependence, while inactivation of the cultures followed a fractional-order dependence on tetracycline concentration. The implication gained from this observation is that tetracyclines inhibit the growth of bacteria in one way and inactivate their growth in another.

Recently bacterial growth kinetics has been applied to obtain the inhibitory potencies of a congeneric series of tetracyclines toward cultures of *E. coli* in balanced growth (2). These studies have been extended to obtain the inactivation potencies of a limited congeneric series of tetracyclines on *synchronously growing* cultures of *E. coli*. Details of the synchronization procedure and the behavior of the synchronized cultures toward the tetracyclines at various

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TABLE 1

Inhibitory potencies of D-ring substituted tetracyclines

Substituent	E_r	σ	σ^2	r_v[a]	Log k_g, observed[b]	Log k_g, calculated[c]	$\lvert\Delta\rvert$
7-NO_2	0.41	1.26[d]	1.61		2.87	2.88	0.01
7-NH_2	0.24[e]	−0.66	0.43		2.26	2.14	0.12
7-Br	0.12	0.23	0.05		1.71	1.90	0.19
H	0.00	0.00	0.00	1.20	1.97	1.87 (2.01)	0.10 (0.04)
9-NH_2	0.24[e]	−0.66	0.43	1.55	2.16	(2.09)	(0.07)
9-NO_2	0.41	0.78	0.61	2.59	1.64	(1.72)	(0.08)
9-$N(CH_3)_2$	0.24	−0.6	0.36	3.11	1.37	(1.34)	(0.03)

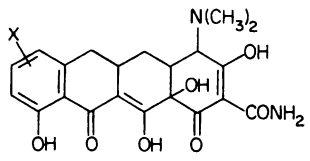
[a] From Charton (3).
[b] Data of Miller *et al.* (2).
[c] Calculated on the basis of Eq. 1a. Values in parentheses were calculated on the basis of Eq. 3a.
[d] "Enhanced" value.
[e] Assumed equal to $N(CH_3)_2$.

TABLE 2

Inactivation potencies of D-ring substituted tetracyclines

Substituent	E_r	σ	σ^2	Log k_d, observed	Log k_d, calculated[a]	$\lvert\Delta\rvert$
7-NO_2	0.41	1.26	1.61	−1.13	−1.11	0.02
9-NH_2	0.24	−0.66	0.43	−1.27	−1.32	0.05
H	0.00	0.00	0.00	−1.44	−1.40	0.04

[a] Calculated on the basis of Eq. 2a.



STRUCTURE I

times in the cycle will be reported at a later date.[2] This report shows that the inhibitory and inactivation potencies of tetracyclines toward nonsynchronous and synchronous cultures, respectively, can be correlated with the same electronic index for D-ring substituents.

The general structure of the tetracyclines considered is represented by I, where the D ring is the aromatic ring on the left. Inhibitory potencies for tetracyclines of structure I toward nonsynchronous cultures are given in Table 1, and inactivation potencies for tetracyclines of the same general structure toward synchronous cultures are given in Table 2.

It is generally agreed that tetracyclines affect bacterial growth by inhibiting the addition of aminoacyl-tRNA to the 30 S ribosomal subunit (4, 5). The inhibitory potencies listed in Table 1 parallel the relative rates of protein synthesis within the organisms when the tetracyclines are present (2), so that they may be considered as representing the efficacy of the tetracyclines in associating with a common site on the ribosomal subunit.

On the other hand, evidence has been presented suggesting that inhibition of cell wall synthesis (6), of components of the respiratory chain (7), or of other biochemical systems (8) may contribute to the bactericidal effects of the tetracyclines. Figure 1

[2] J. H. Collett, C. Collett, A. N. Martin, and A. Cammarata, manuscript in preparation.

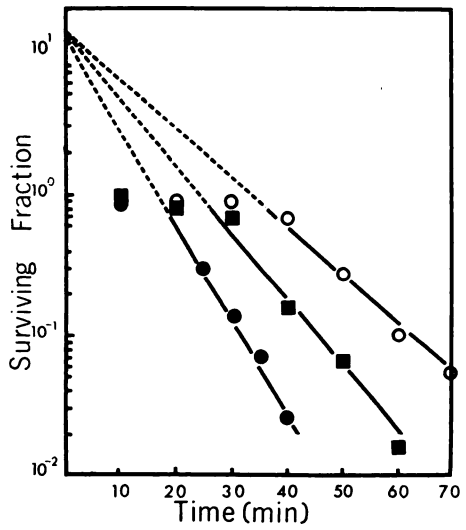


FIG. 1. *Superposition of survival curves for synchronous cultures of E. coli B/r in the presence of tetracyclines of structure I*

●, X = 7-NO_2; ■, X = 9-NH_2;
○, X = H.

shows a superposition of survival curves obtained for the synchronous cultures in the presence of 20 μg/ml of each tetracycline studied. It is representative of the survival curves obtained at any time during the growth cycle of the synchronous cultures, since the inactivation constant for each tetracycline (Table 2), which is the slope of the linear portion of the curve, is the same at all stages up to and including division. This kinetic behavior is consistent with a mechanism in which the tetracyclines inactivate a process that is continuous throughout the life cycle of a bacterial cell. The common extrapolated intercept indicates that the tetracyclines have a common mode of action at the high concentration used. These results are interpreted as suggesting the inactivation of a continuous biochemical process, such as protein synthesis, which in turn leads to the bactericidal effects of the tetracyclines. The lag period between the time when a synchronous culture is first placed in contact with a tetracycline and the ideal time when the total bacterial population first experiences the bactericidal effects of the tetracycline is taken as the time during which a pool of an essential metabolite, such as protein, may be utilized.

If it is assumed that the inhibition and inactivation of bacterial growth by tetracyclines depends upon a common process such as the interruption of protein synthesis, it might be expected that the inhibitory and inactivation potencies for the tetracyclines can be related to the same index for D-ring substituents. That this is indeed the case is shown by Eq. 1, derived for the inhibitory potencies given in Table 1 (7-substituted compounds)

$$\log k_g = 0.62\sigma^2 + 1.87, \quad n = 4,\ s = 0.17,\ r = 0.96 \tag{1a}$$

$$\log k_g = 2.48E_r + 1.73, \quad n = 4,\ s = 0.30,\ r = 0.87 \tag{1b}$$

and by Eq. 2, derived for the inactivation potencies of Table 2.

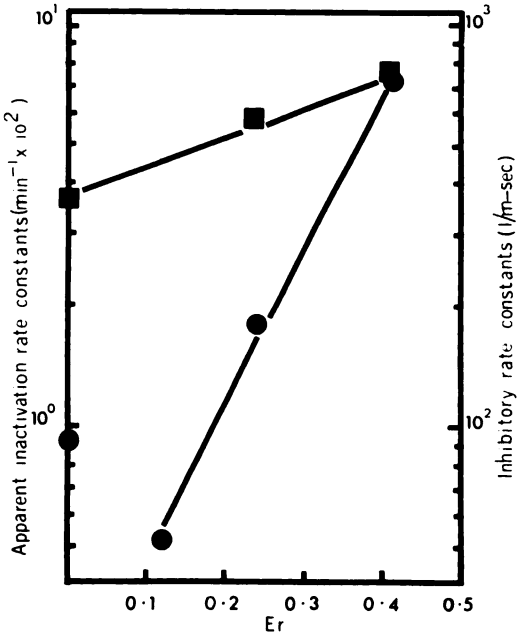


FIG. 2. *Correlation of tetracycline inhibitory* (●) *and inactivation* (■) *potencies with* E_r.

TABLE 3

Parameters used in correlating σ^2 and E_r

Substituent	σ[a]	σ^2	E_r, observed[b]	E_r, calculated	$\lvert\Delta\rvert$
4-OH	−0.37	0.137	0.17	0.14	0.03
4-NMe_2	−0.66[c]	0.435	0.24	0.23	0.01
4-OMe	−0.27	0.073	0.11	0.10	0.01
4-*t*-Bu	−0.19	0.036	0.03	0.08	0.05
4-Me	−0.17	0.029	0.03	0.08	0.05
4-Cl	0.22	0.048	0.10	0.09	0.01
4-Br	0.23	0.053	0.12	0.09	0.03
4-I	0.18	0.032	0.12	0.08	0.04
4-CN	0.66	0.436	0.24	0.23	0.01
4-NO_2	0.78	0.608	0.41	0.39	0.02
4-COMe	0.50	0.250	0.24	0.20	0.04
4-OPh	−0.32	0.102	0.13	0.12	0.12

[a] From Jaffé (10).
[b] From Yamamoto and Otsu (11).
[c] Assumed equivalent to NH_2.

$$\log k_g = 0.18\sigma^2 - 1.40, \quad n = 3, s = 0.07, r = 0.94 \tag{2a}$$

$$\log k_g = 0.76E_r - 1.44, \quad n = 3, s = 0.01, r = 0.99 \tag{2b}$$

A plot of the data is shown in Fig. 2. E_r is the parameter used in making this plot, since, with 7-substituted tetracyclines at least, this quantity led to correlations of greater statistical significance than when σ^2 was the substituent parameter. Attempts to correlate the same data using the substituent indices σ (Hammett), π (Fujita and Hansch), π^2, r_v (van der Waals contact distance), P_E (electronic polarizability), or σ_I and σ_R (Taft) alone and in linear combination were unsuccessful.

Although it may be argued that the small number of data points tends to compromise the correlations given by Eq. 1 and 2, it should be emphasized that the substituents represented are ordinarily considered as imparting extremes in electronic and lipophilic character. Thus, a 7-NO_2 group is strongly electron-withdrawing ($\sigma = 0.78$) and tends to increase lipophilic solubility ($\pi = 0.24$), while a 7-NH_2 group is strongly electron-donating ($\sigma = -0.66$) and tends to increase aqueous solubility ($\pi = -1.63$) relative to 7-H ($\sigma = \pi = 0.00$). The correlation of tetracycline activity with σ^2 tends to suggest a possible parabolic dependence of tetracycline activity on σ. Since the 7-NO_2 and 7-NH_2 tetracycline derivatives are the most active of the compounds investigated, it would seem that any parabolic trend in tetracycline activity with σ would have a minimum but, theoretically at least, no maximum. In other words, 7-substituents that are more strongly electron-withdrawing than 7-NO_2 or more strongly electron-donating than 7-NH_2 should lead to even more potent tetracyclines.

This implication should not be taken overzealously. It might be thought that by making substitutions at positions 7 and 9 of the tetracycline nucleus a more potent tetracycline might be constructed. Unfortunately, steric factors mitigate the antibacterial effects of 9-substituted tetracyclines, as can be seen by the correlation

$$\log k_g = 0.57(\pm 0.31)\sigma^2 - 0.46(\pm 0.09)r_v + 2.56, \quad n = 4, s = 0.11, r = 0.98 \tag{3a}$$

$$\log k_g = 0.85(\pm 0.28)E_r$$

TABLE 4

Inhibitory potencies of chloramphenicols substituted on the aromatic ring

Substituent	E_r	σ	σ^2	π^a	π^2	Log k_0, observed	Log k_0, calculated[b]	$\lvert\Delta\rvert$
4-SO_2Me	0.12	0.73	0.53	−1.26	1.58	0.80	0.95	0.15
4-I	0.12	0.28	0.08	1.26	1.58	1.56	1.36	0.20
4-*i*-Pr	0.03	−0.15	0.02	1.40	1.96	1.08	1.38	0.30
4-Cl	0.10	0.23	0.05	0.70	0.49	1.10	1.14	0.04
4-OMe	0.11	−0.27	0.07	−0.04	0.00	1.26	1.20	0.06
4-Br	0.12	0.23	0.05	1.02	1.04	1.32	1.10	0.22
4-NO_2	0.41	0.78	0.61	0.24	0.05	2.00	2.11	0.11
4-SMe	0.24	−0.05	0.00	0.62	0.38	1.76	1.42	0.34
4-NH_2	0.24	−0.66	0.43	−1.63	2.65	0.55	0.68	0.13

[a] From the phenoxyacetic acid system.

[b] Calculated on the basis of Eq. 5a.

$$- 0.47(\pm 0.05)r_v + 2.59, \quad n = 4,\ s = 0.09,\ r = 0.97 \tag{3b}$$

The compounds on which this correlation is based are found in Table 1. To increase the sample size, the unsubstituted compound is also included in this set.

The unprecedented use of σ^2 alone[3] (9) in order to gain a correlation with biological potencies may be at least partially understood by noting that E_r parameters (Table 3), which are intended to provide a measure of the ability of a substituent to stabilize free radicals (11), are correlated by the equation

$$E_r = 0.50\sigma^2 + 0.07, \quad n = 12,\ s = 0.06,\ r = 0.94 \tag{4}$$

At present, the correlations reported should not be interpreted as indicating that the antibacterial effects arise from a tetracycline free radical or a tetracycline complexed with a metal ion having a lone electron in one of its *d*-orbitals (feedback might give the appearance that a free radical is being stabilized). The chemical systems used to derive the E_r parameters show a parabolic dependence in rate on σ, and E_r is an empirical parameter that in combination with σ leads to a linear trend. It would thus seem that E_r must be equivalent or proportional to σ^2 for a parabolic trend with σ to be taken into account. The physical process leading to the parabolic trend, however, is obscure at present. Perhaps desolvation control of a frontier-controlled interaction (12) is a contributing factor, but further work is needed to clarify this point.

In this regard, it should be noted that a series of chloramphenicol analogues, which inhibit the addition of aminoacyl-tRNA to the 50 S ribosomal subunit rather than the 30 S ribosomal subunit, as for the tetracyclines (13), have also been studied with respect to their potencies as determined by bacterial growth kinetics (14) (Table 4). These inhibitory potencies have been correlated with electronic polarizabilities for portions of the substituents that may be in contact with a receptor surface (15) (a frontier-controlled interaction), and they have also been correlated by a linear combination of the parameters E_r and π (16). In the first instance the 4-SMe and 4-NO_2 points were deleted and in the second instance the 4-NH_2 point was omitted in arriving at a correlation. By assuming that E_r for 4-NMe_2 is close to that for 4-NH_2, all of the compounds in Table 4 can be correlated by

[3] A case related to the use of σ and σ^2 has been reported (9).

$$\log k_g - \sigma^2 = 0.34(\pm 0.08)\pi - 0.26(\pm 0.10)\pi^2 + 1.26, \quad (5a)$$

$$n = 9,\ s = 0.24,\ r = 0.92$$

$$\log k_g - E_r = 0.22(\pm 0.08)\pi - 0.23(\pm 0.09)\pi^2 + 1.30, \quad (5b)$$

$$n = 9,\ s = 0.22,\ r = 0.88$$

The left-hand side of Eq. 5 may be considered as giving the biological potency in excess of that which is a consequence of the interaction implied by σ^2 or E_r .

Whatever the physical origin of a σ^2 term, it is clear that the potencies of the tetracyclines and chloramphenicols considered should be related to the same type of substituent property. The π contribution in the case of the chloramphenicols may reflect the difference in the mechanism of action for these compounds from that for the tetracyclines. With the tetracyclines, at least, it seems probable that the change in the form of the rate law with high and with low concentrations of drug (1) is indicative of a change in the manner by which protein synthesis is interrupted, rather than of an additional contribution of one or more alternative modes of action involving different biochemical processes.

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