Predictability of Correlations between *in Vitro* Tetracycline Potencies and Substituent Indices[1]

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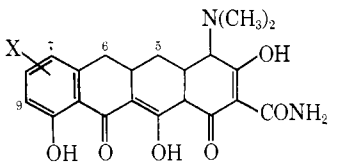
Eleven structurally related tetracyclines having a single substitution on the D ring have had their inhibition potencies against *Escherichia coli* W correlated with substituent indices using three alternative analytical approaches. A compound not included in one of the analyses had its *in vitro* activity predicted satisfactorily. The three approaches taken are shown to be equivalent and the results of the correlations were applied to predict activities for tetracyclines having multiple substitutions on the D ring. Many of the predicted inhibition potencies against *Escherichia coli* W are of the same relative order of potencies as is observed with *Staphylococcus aureus* as the test organism.

In a recent communication[2a] the bactericidal potencies of three and the bacteriostatic potencies of six monosubstituted tetracyclines of general structure I against synchronous and nonsynchronous cultures of *Escherichia coli*, respectively, were correlated with the square of the Hammett substituent index (σ^2). The



I

unprecedented use of σ^2 alone[2b] in order to gain a correlation with biological potencies was clarified at least partially by the observation of a correlation between E_r and σ^2. While E_r was initially defined as a free-

$$E_r = 0.50\sigma^2 + 0.07 \quad n = 12 \quad s = 0.06 \quad r = 0.94 \qquad (1)$$

radical index,[3] and correlations of biological activities with this index have been reported,[4] the observation of a correlation between E_r and σ^2 suggests the possibility that this index may also be a suitable measure of a frontier-controlled interaction in which the desolvation requirement is dominant.[5,6] Hence, correlations of biological potencies with either E_r or σ^2 may or may not imply the participation of a free-radical-like species in giving rise to the observed biological activity.

Recognizing that the actual physical significance of E_r and σ^2 is obscure at present (see conjecture at end of paper), we have used these physically indefinite parameters as indices of convenience in correlating the bacteriostatic potencies of 11 tetracyclines. The activities of these tetracyclines againt *Escherichia coli* W were determined using bacterial growth kinetics and a comparison of viable with total cell counts was made to show that these activites are suitable inhibition potencies.[7] Three alternative, but demonstrably equivalent, approaches were taken in correlating the data and tests of the predictability of the correlations obtained were made.

Method 1.—The compounds in Table I of structure I were grouped depending on whether the substitution was at the 7 or the 9 position, and at no other position. *The unsubstituted compound was considered common to each set.* A number of trial analyses in which the model equation for each set was a linear combination of *meta* and *para* substituent constants were not found suitable for correlating the data. Considering the aromatic D ring as a simple substituted phenol, however, led to a correlation for the 7-substituted set

$$\log k = 0.64\sigma^2 + 1.87 \quad n = 4 \quad s = 0.17 \quad r = 0.96 \qquad (2a)$$

$$\log k = 2.48E_r + 1.73 \quad n = 4 \quad s = 0.30 \quad r = 0.87 \qquad (2b)$$

and for the 9-substituted set

$$\log k = \underset{(\pm 0.31)}{0.57\sigma^2} - \underset{(\pm 0.09)}{0.46r_D} + 2.56 \quad n = 4 \quad s = 0.11 \quad r = 0.98 \qquad (3a)$$

$$\log k = \underset{(\pm 0.28)}{0.85E_r} - \underset{(\pm 0.05)}{0.47r_D} + 2.59 \quad n = 4 \quad s = 0.09 \quad r = 0.97 \qquad (3b)$$

where r_D is the van der Waals contact distance for a 9 substituent on the D ring.[8] The parameter r_D is constant with the 7-substituted set and was therefore not included. Combining the two sets provided the relation

$$\log k = \underset{(\pm 0.38)}{1.33\sigma^2} - \underset{(\pm 0.11)}{0.57r_D} + 2.51 \quad n = 7 \quad s = 0.21 \quad r = 0.93 \qquad (4a)$$

$$\log k = \underset{(\pm 1.64)}{2.70E_r} - \underset{(\pm 0.34)}{0.55r_D} + 2.51 \quad n = 7 \quad s = 0.45 \quad r = 0.67 \qquad (4b)$$

Based on standard deviations the use of σ^2 in place of E_r leads to better correlations with the 7-substituted and the combined sets of data. No great physical significance should be attached to this apparent statistical discrepancy since with the limited number of data points a wide separation between two or three points on the E_r scale relative to the σ^2 scale can lead to statistically disappointing correlations in one instance but not in the other (see footnote *g*, Table II). The statistically more satisfying correlations show a definite negative

(1) This work was supported under NIH Grant AI-09199.

(2) (a) A. Cammarata, S. J. Yau, J. H. Collett, and A. N. Martin, *Mol. Pharmacol.*, accepted for publication; (b) a correlation which is essentially an arbitrary linear combination of σ and σ^2 has been reported: C. Hansch, A. R. Steward, and J. Iwasa, *J. Med. Chem.*, **8**, 868 (1965).

(3) T. Yamamoto and T. Otsu, *Chem. Ind.* (London), 787 (1967).

(4) (a) C. Hansch, *J. Med. Chem.*, **11**, 920 (1968); (b) C. Hansch, E. Kutter, and A. Leo, *ibid.*, **12**, 746 (1969).

(5) A. Cammarata, *ibid.*, **11**, 1111 (1968).

(6) A. Cammarata, *ibid.*, **12**, 314 (1969).

(7) G. H. Miller, S. Khalil, and A. N. Martin, *J. Pharm. Sci.* in press.

(8) M. Charton, *J. Amer. Chem. Soc.*, **91**, 615 (1969).

influence of large 9 substituents on the bacteriostatic potencies of these tetracyclines. With the data at hand, however, there is no indication as to whether steric interactions between the 9 substituent and the adjacent hydroxyl group or between the 9 substituent and the biological receptor substance (or both) is the detrimental factor.

To take into account the effect of the 6-CH_3 and 6-OH substitutions on the C ring and the 5-OH substitution on the B ring it was assumed that either only the 6-CH_3 and 5-H or only the 6-OH and 5-OH came in contact with the receptor surface. Only the latter assumption led to a definite contribution for the aliphatic substituents in the regression equation

$$\log k = 0.93\sigma^2 - 0.42r_D + 3.67r_C - 1.99 \quad (\pm 0.17)\ (\pm 0.12)\ (\pm 0.76)$$

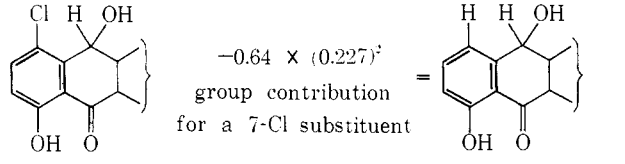
$$n = 11 \quad s = 0.20 \quad r = 0.93 \qquad (5a)$$

$$\log k = 2.76E_r - 0.51r_D + 3.81r_C - 2.17 \quad (\pm 1.30)\ (\pm 0.27)\ (\pm 1.43)$$

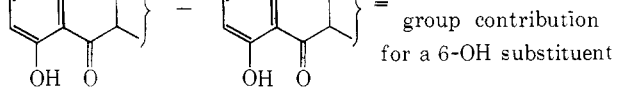
$$11 \quad 0.36 \quad 0.77 \qquad (5b)$$

In this instance, however, there are too few substituent variations at the 6 position to come to any definitive conclusions regarding the validity of the assumption and of the contribution of the van der Waals contact distance r_C in the correlation.

Method 2.—In this approach compounds of general structure I were considered as having their activities modified in an additive manner by substituents placed on the C and B rings. The term $0.64\sigma^2$ appearing in eq 2a was considered as representing the effect of a D-ring 7 substituent in determining the potency of a tetracycline (I). Successive differences were then used to determine the activity contribution characteristic for an aliphatic substituent.



$\log k = 2.714$ (obsd) $\log k = 2.681$ (calcd)



$\log k = 2.681$ (calcd) $\log k = 1.975$ (obsd)

In a similar manner, the group contribution for a 6-CH_3 substituent can be calculated from the observed activity of 7-chlorotetracycline ($\log k = 2.604$) and the derived group contribution of a 6-OH substituent.

$$[2.604 - 0.64(0.227)^2 - 0.706] - 1.975 = -0.110$$

Likewise using the observed activity of 5-hydroxytetracycline the group contribution for a 5-OH substituent is found to be −0.170.

A test of the validity of the approach taken in this instance may be made by calculating the activity expected for tetracycline in the assay used. Based on this single test case the general approach seems verified.

TABLE I
INHIBITION CONSTANTS FOR SOME TETRACYCLINES AGAINST *Escherichia coli* W

Substituents				Inhib constants,[a]
R_5	R_6	R_7	R_9	$\log k$
H	H	NO_2	H	2.874
H	OH	Cl	H	2.714
H	CH_3, OH	Cl	H	2.604
H	CH_3, OH	H	H	2.434
OH	CH_3, OH	H	H	2.400
H	H	NH_2	H	2.259
H	H	H	NH_2	2.161
H	H	H	H	1.975
H	H	Br	H	1.714
H	H	H	NO_2	1.647
H	H	H	$N(CH_3)_2$	1.374

[a] Data of ref 7.

[unsubstituted I] + [6-OH] + [6-CH_3] = [tetracycline]

(1.975) + (0.706) + (−0.110) =

2.571 (calcd)
2.434 (obsd)

Since the group parameters are defined relative to the unsubstituted parent molecule I, they should be related to the more usual substituent parameters. In fact the derived group parameters may be used as a substituent index in the usual fashion, as can be seen by the correlation obtained for those tetracyclines having a 7-substituted D ring

$$\log k = 1.53\sigma^2 + 1.28\Sigma G + 1.79 \quad (\pm 0.30)\ (\pm 0.24)$$

$$n = 8 \quad s = 0.16 \quad r = 0.94 \qquad (6a)$$

$$\log k = 2.25E_r + 1.08\Sigma G + 1.79 \quad (\pm 0.62)\ (\pm 0.28)$$

$$8 \quad 0.21 \quad 0.89 \qquad (6b)$$

In these correlations ΣG is the sum of the aliphatic group contributions.

The group indices used in arriving at eq 6 may be related to the inductive (field) influences due to the respective substituents

$$\Sigma G = 0.69\Sigma\sigma_I + 0.07 \quad n = 8 \quad s = 0.20 \quad r = 0.79 \qquad (7)$$

or they may be related to the contact distance r_C for a 6-OH in the natural configuration.

$$\Sigma G = 2.90r_C - 3.48 \quad n = 8 \quad s = 0.08 \quad r = 0.97 \qquad (8)$$

For the latter case the contact distance r_C for a 6-CH_3 in the natural configuration leads to

$$\Sigma G = 0.49r_C - 0.45 \quad n = 8 \quad s = 0.26 \quad r = 0.64 \qquad (9)$$

A wider range in 6 substitutions will have to be investigated before these alternatives can be resolved.

Method 3.—A procedure similar to that described by Free and Wilson[9] was used to determine group contributions for all of the respective substituents on the 11 tetracyclines. In this instance, however, all activities were expressed as $\log k$, and μ was assigned the value of the unsubstituted tetracycline I. As a limiting condition, the group contribution for a hydrogen substituent

(9) S. M. Free and J. W. Wilson, *J. Med. Chem.*, **7**, 395 (1964).

at any position was assigned a value of zero; in this way the group contributions become defined in an equivalent manner as are other linear free-energy substituent parameters. The group parameters calculated by this approach are given in Table II.

TABLE II
GROUP PARAMETERS FOR TETRACYCLINES[a]
(*Escherichia coli* W AS THE TEST SYSTEM)

Substituent[b]	Act. contrib, G	E_r[b]	σ^2	σ_I	r_V[c]
7-NO_2	0.899	0.41	1.613[d]		1.20[e]
6-OH	0.569			0.48	1.40
7-NH_2	0.284	0.24	0.436		1.20[e]
9-NH_2	0.186	0.24	0.436		1.55
7-Cl	0.170	0.10	0.051		1.20[e]
5-OH	−0.034			0.48	1.40
6-CH_3	−0.110			−0.04	2.00
7-Br	−0.261	0.12	0.054		1.20[e]
9-NO_2	−0.328	0.41[f]	0.609		2.59
9-$N(CH_3)_2$	−0.601	0.24	0.360		3.11

[a] Defined for [H] = 0 and μ = 1.975. [b] From Table I. [c] From M. Charton, *J. Amer. Chem. Soc.*, **91**, 615 (1969). [d] "Enhanced" value. [e] For 9-H. [f] A value of 0.15 could be said to be more appropriate. The larger *para* value could then be indicative of "enhanced" conjugation.

Those group parameters referring to D-ring substitutions are correlated by the equation

$$G = 0.87\sigma^2 - 0.57r_D + 0.84 \quad (\pm 0.18) \quad (\pm 0.16) \qquad n = 7,\ s = 0.22,\ r = 0.93 \qquad (10a)$$

$$G = 2.38E_r - 0.52r_D + 0.58 \quad (\pm 1.82) \quad (\pm 0.37) \qquad n = 7,\ s = 0.47,\ r = 0.62 \qquad (10b)$$

The formal agreement between these relations and eq 4a and b (method 1) is very good and indicates that the two methods are equivalent. This equivalence of the two approaches should not be surprising as it was implied but not demonstrated in a reported analysis of biological linear free-energy relationships.[6] Similar correlations involving the C- and B-ring substituents were not sought since any agreement or lack of agreement with eq 7–9 leads to no conclusion of significance if one considers the limited variety of substitutions in this case.

Predictability.—The group contributions found in Table II were used to calculate the activities of some tetracyclines having two substitutions in the D ring. The compounds were those whose minimum inhibitory concentrations relative to tetracycline against *S. aureus* have already been determined.[10] Unfortunately due to uncertainty regarding the most suitable r_D value for a 9-CH_3CONH substituent two compounds are omitted in this comparison. Table III presents the calculated relative order of inhibition potencies against *E. coli* W and the corresponding activities observed against *S. aureus*. Considering the difference in test organism and assay procedure the general agreement, at least with respect to order, is satisfactory. Three compounds, most notably the 7,9-dinitro and the 7-chloro-9-nitro derivatives, have their place in the order of activities poorly predicted. Either a lack of additivity of the group parameters, experimental uncertainties (*e.g.*, solubilities), or the difference in the test organisms may be factors contributing to the large disparities in these two instances.

TABLE III
RELATIVE ORDER OF TETRACYCLINE ACTIVITIES CALCULATED FOR *Escherichia coli* W AS THE TEST ORGANISM AND OBSERVED WITH *Staphylococcus aureus* AS THE TEST ORGANISM

Substituents			*In vitro* act.	
R_6	R_7	R_9	*E. coli* W (calcd)[a]	*S. aureus* (obsd)[b]
H	Cl	NH_2	2.331	525
H	Br	NH_2	1.900	320
H	NO_2	NH_2	1.833	275
CH_3	NO_2	NH_2	1.723	160
CH_3	Br	NH_2	1.790[c]	140[c]
H	NO_2	NO_2	2.546[c]	60[c]
H	Cl	NO_2	1.817[c]	21[c]
H	Br	NO_2	1.386	15

[a] Based on the parameters, G, found in Table II. [b] Data of ref 10 expressed as minimum inhibitory concentration relative to tetracycline. [c] Deleting these data points the agreement is found to be $\log k(\text{calcd}) = 0.50 \log \text{MIC(obsd)} + 0.73$ $n = 5$, $s = 0.19$, $r = 0.89$

Possible Origins of σ^2.—Because of the novelty associated with the finding that the use of σ^2 can provide a correlation with and lead to reasonable predictions of at least the order for the *in vitro* activities of tetracyclines, it may be desirable to speculate into the possible physical significance of this parameter. Which, if any, of these speculations is correct will only be established by appropriate studies on additional systems. A correlation based on the tetracycline data using quantum chemical indices does, however, support the possibilities. The significance of the present results rests on one fundamental principle: any statistical argument bearing on real data must submit to the test of prediction. In the current instance, method 2 correctly predicts (within, as it is found, one standard deviation) the bacteriostatic activity of tetracycline. Further, method 3 gives the correct order of activities for at least five tetracyclines. To appreciate these facts, it is necessary to recognize that methods 1–3 are *operationally* independent; the methods are equivalent because they have been demonstrated to be so.

A possible way for σ^2 to arise is through a coupling mechanism. Consider, initially, the situation where a tetracycline bearing a substituent X on its D ring is capable of interacting with a second tetracycline having a substituent Y on its D ring. Tetracycline chelates MT_2 which can dissociate into an ionic form MT^{n+} provide a possible example. An essentially neutral chelate, each of whose tetracycline ligands is substituted differently, might be presumed to pass through the lipophilic bacterial cell wall, and once inside the cell to dissociate into an ionic form

$$\text{XT–M–TY} \rightleftharpoons \text{XT–M}^{n+} + \text{TY or YT–M}^{n+} + \text{TX} \qquad \text{(A)}$$

The bacteriostatic activity of a tetracycline may be considered as being determined by the ability of the neutral chelate to dissociate to an ionic form[11] which

(10) J. L. Spencer, J. J. Hlavka, J. Petisi, H. M. Krazinski, and J. H. Boothe, *J. Med. Chem.*, **6**, 405 (1963).

(11) (a) A. Albert, *Nature*, **172**, 201 (1953); (b) J. T. Dolvision, and A. N. Martin, *J. Med. Chem.*, **6**, 16 (1963).

can subsequently lead to inhibition of protein synthesis by interfering with the addition of an aminoacyl-tRNA to the 30S ribosomal subunit.[12]

Say that a tetracycline with the substituent X1 always remains bound to the metal ion. The second tetracycline may have its substituent Y varied, and since it is always the incoming or departing ligand, observed biological potencies might be correlated by the equation.

$$\log k = a_{x_1}\sigma_y + d \tag{11}$$

If the substituent X1 is changed to X2, a correlation similar to eq 11, but differing in slope, should be obtained.

$$\log k = a_{x_2}\sigma_y + d \tag{12}$$

Thus, for N variations of the substituent X, there is a set of relations which can be represented as

$$\log k = a_{x_j}\sigma_y + d \; (j = 1, 2, \ldots, N) \tag{13}$$

However, such a systematic change in the substituent X suggests the correlation

$$a_x = \rho\sigma_x + c \tag{14}$$

and, since all N substituent variations are taken into account by eq 14, it may be substituted into eq 13 to give

$$\log k = \rho\sigma_x\sigma_y + \rho c\sigma_y + d \tag{15a}$$

or for the more general case where both ligands can dissociate:

$$\log k = \alpha\sigma_x\sigma_y + \beta\sigma_x + \gamma\sigma_y + d \tag{15b}$$

For the particular case where X = Y, as in the usual experimental situation where only one substituted tetracycline is used, eq 15 becomes

$$\log k = \rho\sigma^2 + \rho c\sigma + b \tag{16}$$

In certain instances, it may happen that the intercept, c, of eq 14 or the product, ρc, of eq 15 is close to zero. When this is the case, the substitution of eq 14 into eq 13 leads to

$$\log k = \rho\sigma_x\sigma_y + d \tag{17}$$

or, for the limiting situation where X = Y

$$\log k = \rho\sigma^2 + d \tag{18}$$

Chelation was chosen to illustrate substituent coupling effects primarily because of the ease of conceptualization. The participation of ionic tetracycline chelates has been suggested, however, as a binding mode for the tetracyclines in leading to their bacteriostatic effects.[11b]

An alternative is for a tetracycline to bind either to aminoacyl-tRNA or to the 30S ribosomal subunit. Coupling effects could then arise because of the addition of a second tetracycline. The coupling mechanism could be an electronic or a structural perturbation of a linked hydrogen-bonding network to which each tetracycline becomes attached. Quantum chemical indices have been shown to correlate with the activities of those tetracyclines found in Table I.[13] The total net charge, Q^T, and the electrophilic π-delocalizability, S^E, for each of the conjugated oxygen atoms (10, 11, and 12) are required to gain a correlation. This observation is consistent with either chelation or hydrogen bonding as a mechanism leading to the effects of the tetracyclines.

A second possible basis for σ^2 takes note of arguments already advanced as to the meaning of the parameters E_r,[3] Q,[14] and β[15] with respect to monomer reactivity in copolymerization. That these arguments pertain is evidenced by the correlation between E_r and σ^2. Similar correlations are also found for Q and for β.

	n	s	r	
$Q = 2.436\sigma^2 + 1.059$	7	0.172	0.925	(19)
$\beta = 1.525\sigma^2 + 4.853$	9	0.087	0.962	(20)

The correlations represented by eq 1, 19, and 20 are of practical significance in polymer chemistry, and a more detailed account will be presented elsewhere. Only the potential significance of these correlations with regard to tetracycline activity will be discussed here.

A chelation mechanism similar to that described previously will be postulated as the controlling factor in leading to the antibiotic effects of the tetracyclines. In this instance, however, it is the reaction between a metal ion and tetracycline which leads to the presumed biologically active ionic chelate

$$M^{n+} + HO\text{-}AR\text{-}X \rightleftharpoons M^{n+}\text{—}O\text{-}AR\text{-}X + H^+ \tag{B}$$

The ability of a metal ion to bind to a conjugated ligand (tetracycline) can be said to be controlled partly by the nature of the Coulombic interactions between the reactants and partly by the tendency of the metal ion to come into conjugation with the ligand. With little or no conjugation possible between the ligand and the metal ion, the stability of a chelate is determined primarily by Coulombic effects. A change in substituent on the aromatic ligand affects the stability of the ligand, in this instance, by varying the electronic charge distribution within the ligand. If tetracycline potency were determined by this type of species, then a simple Hammett-type relationship might be expected to hold.

$$\log k = \rho\sigma + d \tag{21}$$

On the other hand, the tendency of a metal ion to come into conjugation with a ligand should depend on the extent of delocalization (resonance) already present within the ligand. Substituents which resonate with a ligand equivalently should, in this instance, cause a conjugating metal ion to have essentially the same affinity toward the ligand. The parameters E_r, Q, and β were originally established to provide a measure of the resonance stabilization due to a substituent. Since these correlate with σ^2, one may say that when conjugation between a ligand and a metal determines the stability of the resulting chelate, and if such a chelate is biologically active, a relationship for the observed biological activity could take the form

$$\log k = \rho'\sigma^2 + d \tag{22}$$

Groups such as NH_2 or NO_2 appear to resonate with an aromatic nucleus almost equivalently, either by virtue of donating ($\sigma^p_{NH_2} = -0.66$) or withdrawing

(12) B. Weisblum and J. Davies, *Bacteriol. Rev.*, **32**, 493 (1968).

(13) F. Peradejordi and A. N. Martin, private communication, April 1968.

(14) T. C. Schwan and C. C. Price, *J. Polymer Sci.*, **40**, 457 (1959).

(15) C. H. Bamford and A. D. Jenkins, *Trans. Faraday Soc.*, **59**, 530 (1963).

($\sigma^{p}_{NO_2}$ = +0.78) electrons. Indeed, seven resonance forms can be drawn for 4-nitrochlorobenzene, for example, and five resonance forms can be drawn for 4-aminochlorobenzene. Thus, while the delocalization energy for conjugation of a substituent with an aromatic nucleus is proportional to σ,[16] it appears that the resonance energy of the substituted molecule should be proportional to σ^2.

A general relationship taking into account both Coulombic and resonance effects in the formation of a chelate is obtained by linearly combining σ and σ^2. The result is operationally equivalent to eq 16. Proper caution should be exercised in dealing with relations such as 16. While there are at least three arguments that can be advanced to explain a nonlinear dependance of biological activity on σ (ref 6 and the two already detailed) there is always the possibility that another parameter exists that is essentially independent of σ. Use of this parameter in combination with σ could cause an otherwise parabolic trend in σ to become linear.

With chelates as an active species, for example, a knowledge of the association constants for reactions A and B enables a calculation of the fraction of ionic chelate present at a specified pH. The observed activity could be "corrected" by multiplying the rate constants by the calculated fraction. The use of this corrected biological activity could then result in a linear dependence on σ. For the tetracyclines involved in this study, insufficient information is available to attempt this type of correction.

The basis for the precaution is essentially the same as for the linear free-energy approach taken by Hansch[17] in his account of electronic and lipophilic factors which influence biological activity. It must be concluded that correlations based on eq 16 are most probably physically significant, but the origin of σ^2 may take many forms.

(16) F. L. J. Sixma, *Rec. Trav. Chim.*, **72**, 673 (1953).

(17) C. Hansch in "Annual Review of Medicinal Chemistry," C. K. Cain, Ed., Academic Press, New York, N. Y., 1966.

Antiprotozoal Quinones. II. Synthesis of 4-Amino-1,2-naphthoquinones and Related Compounds as Potential Antimalarials[1]

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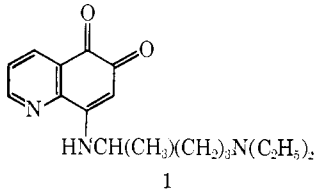
A wide variety of 4-amino-1,2-naphthoquinones, related to the presumed active metabolite of pamaquine, has been prepared and evaluated as potential antimalarials in *Plasmodium berghei* infected mice, *Plasmodium gallinaceum* infected chicks, and against the sexual phase of *P. gallinaceum* in mosquitoes. A few new 2-amino-1,4-naphthoquinones and 4-alkoxy-1,2-naphthoquinones have also been prepared and evaluated. None of the new quinones was curative but 4-(3-dipentylaminopropylamino)-1,2-naphthoquinone showed some activity in all three primary screens. In this series there seems to be a relationship between lipophilicity of the side chain and antimalarial activity as has been observed for other antiprotozoal quinones. Procedures for reaction of 1,2-naphthoquinones with enamines to give a new class of naphthalenediol derivatives are also described.

The supporting evidence for the postulate that the antimalarial action of 8-amino-6-methoxyquinolines such as pamaquine may be due to their *in vivo* oxidation to quinonoid products such as **1** has been detailed elsewhere.[2] The observation[3] that the presumed quinonoid metabolite of pamaquine has 16-fold greater *in vitro* activity than the parent drug against *Plasmodium gallinaceum* is in curious contrast to the report of Drake[2] of negligible *in vivo* activity of related 5,6-dihydroxy- and 5-hydroxy-6-methoxyquinolines in monkeys. These compounds were sensitive to oxidation and were expected to be converted to quinones *in vivo*. Our efforts have been directed toward a search for new quinones related to **1** which might possess *in vivo* antimalarial activity. This paper reports the investigation of a series of naphthoquinones **3a–c** and some related derivatives. Some 4-amino-1,2-naphthoquinones have been prepared previously by Fieser and others,[4] but the series has not been explored for possible antiprotozoal drugs.



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Chemistry.—4-Ethoxy-1,2-naphthoquinone (**2**) was prepared from the Ag salt of 2-hydroxy-1,4-naphthoquinone[5] with EtI (Scheme I). The related 4-alkoxy-1,2-naphthoquinones **59** and **60** (Table I) were also prepared in this way. The orthoquinone structure was confirmed by the ready formation of phenazine derivatives.

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(2) F. Schöenhöfer, *Z. Physiol. Chem.*, **274**, 1 (1942); K. C. Blanchard in F. W. Wiselogle, "A Survey of Antimalarial Drugs, 1941–1945," Vol. I. J. W. Edwards, Ann Arbor, Mich., 1946, p 129 ff; B. B. Brodie and S. Udenfriend, *Proc. Soc. Exptl. Biol. Med.*, **74**, 845 (1950); N. L. Drake and Y. T. Pratt, *J. Am. Chem. Soc.*, **73**, 544 (1951); E. S. Josephson, J. Greenberg, D. J. Taylor, and H. L. Bami, *J. Pharmacol. Exptl. Ther.*, **103**, 7 (1951); R. R. Holmes, J. Conrady, J. Guthrie, and R. McKay, *J. Am. Chem. Soc.*, **76**, 2400 (1954); P. B. Russell in "Medicinal Chemistry," A. Burger, Ed., Interscience Publishers, Inc., New York, N. Y., 1960, p 814.

(3) E. S. Josephson, D. J. Taylor, J. Greenberg, and A. P. Ray, *Proc. Soc. Exptl. Biol. Med.*, **76**, 700 (1951).

(4) (a) L. F. Fieser and M. Fieser, *J. Am. Chem. Soc.*, **57**, 491 (1935); (b) L. F. Fieser and J. L. Hartwell, *ibid.*, **57**, 1484 (1935); (c) L. F. Fieser and M. Fieser, *ibid.*, **61**, 596 (1939); (d) H. E. Fierz-David and E. Mannhart, *Helv. Chim. Acta*, **20**, 1024 (1937); (e) R. A. Henry and W. M. Dehn, *J. Am. Chem. Soc.*, **74**, 278 (1952); (f) W. Brackman and E. Havinga, *Rec. Trav. Chim.*, **74**, 937 (1955).

(5) L. F. Fieser, *J. Am. Chem. Soc.*, **48**, 2922 (1926).