sion. In some cases, however, the chloronitro alcohols were prepared by addition of aldehydes to 1-chloro-1-nitroethane (commercially available) in the presence of potassium carbonate. A typical example is **3-chloro-3-nitrobutan-2-ol**. An aqueous solution of acetaldehyde (10 g in 20 ml) was added to a mixture of 1-chloro-1-nitroethane (21.9 g), water (20 ml), and potassium carbonate (1 g). The homogeneous mixture became warm, the temperature rising spontaneously to 50°. After standing overnight, the reactants were acidified with hydrochloric acid, and the product was isolated by ether extraction and purified by fractional distillation: colorless oil; bp 56° (1.2 mm); yield, 18.5 g (60%). *Anal.* ($C_4H_8ClNO_3$) C, H, N. Related new compounds are listed in Table V.

Esters and Ethers of Halogenonitro Alcohols. The acyl derivatives were all obtained by heating the free alcohol or glycol in chloroform solution with a moderate excess of the appropriate acyl chloride (*cf.* Tindall[16]). See Table VI.

The ethers were prepared by treating an α-nitroalkene with sodium methoxide; this gave an intermediate sodium salt of the methoxynitroalkane which, on treatment with chlorine or bromine, yielded the desired halogenonitro ether. A typical example is **1-chloro-2-methoxy-1-nitrobutane**. 1-Nitrobut-1-ene (20 g) was added slowly to a stirred mixture of methanol (50 ml) and a methanolic sodium methoxide solution (50 ml of 4.4 *N*) cooled to 0°. The resulting solution was diluted with ice-water (300 ml) and chlorine bubbled into the liquid until no more oil was precipitated. This oil was isolated by ether extraction and purified by fractional distillation: colorless oil; bp 51–52° (2.0 mm); yield, 18.1 g (54%). *Anal.* ($C_5H_{10}ClNO_3$) N.

The following compounds were prepared similarly by adding a slight excess of bromine in place of the stream of chlorine. **1-Bromo-3,3,3-trichloro-2-methoxy-1-nitropropane**: colorless oil; bp 105° (1.6 mm); yield, 34%. *Anal.* ($C_4H_5BrCl_3NO_3$) N. **1,1-Dibromo-2-methoxy-1-nitrobutane**: Colorless prisms; mp 26°; yield, 75%. *Anal.* ($C_5H_9Br_2NO_3$) N, Br.

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Reinvestigation of a "Nonadditive" Quantitative Structure–Activity Relationship

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The toxicities toward white mice for a series of disubstituted benzenes have earlier been reported as being correlated by an interaction model of the form $\log A_{XY} = b_X + b_Y + e_Xe_Y$ where b_X and b_Y are the toxicities of the corresponding monosubstituted benzenes and e_X and e_Y are interaction parameters. It is shown in this report that these compounds can also have their toxicities correlated using the Free and Wilson additive model approach. All of the regression models developed for the correlation of biological activities are therefore interrelated.

Additive and linear multiple regression models used for the derivation of quantitative structure–activity relationships have been shown, in many instances, to be theoretically,[1] statistically,[2,3] and practically[1,2,4-6] equivalent so long as the derived parameters are defined relative to a structurally similar parent nucleus. An apparent exception to this generalization would seem to be found in the work of Boček, Kopecký, and coworkers.[7-9] In this instance the toxicities of meta- and para-substituted benzenes toward white mice are reported as best correlated by an interaction model

$$\log A_{XY} = b_X + b_Y + e_Xe_Y \quad (1)$$

where A_{XY} is the LD_{50} for a disubstituted benzene derivative relative to that of benzene, b_X and b_Y (or $\log A_{XH}$ and $\log A_{HY}$) are the corresponding measures of toxicity for monosubstituted benzenes, and e_X and e_Y are parameters characterizing the effect on the LD_{50} due to substituent interaction in the disubstituted benzenes.

Singer and Purcell[10] have made an effort to assess the implications of eq 1 in relation to the additive and linear multiple regression models. Their conclusion was that substituent interactions lead to a breakdown of the additive model, giving rise to a parabolic form of the linear multiple regression model. This conclusion is valid so long as, in taking an additive approach to obtaining structure–activity relationships, additivity means replicating the observed activity for a multisubstituted compound in terms of the activities for the corresponding monosubstituted derivatives. However, additivity in the Free and Wilson[11] sense, which is the additive model Singer and Purcell sought to relate to the linear multiple regression model, is not defined in this manner. Rather, additivity in the Free and Wilson approach means that for two or more compounds in a series, mono- *and* multisubstituted, corresponding substituents at equivalent positions of a molecular nucleus affect the observed biological activity, on average, in an identical manner. This is the criterion of additivity used by Cammarata[1] in his assessment of the relation between the additive and the linear multiple regression models.

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Table I. Data Matrix for the Toxicities of Meta- and Para-Disubstituted Benzenes toward White Mice

Substituent X						Substituent Y						Log $[(LD_{50})_{HH}/(LD_{50})_{XY}]^a$	
H	NO_2	Cl	OH	CH_3	NH_2	H	NO_2	Cl	OH	CH_3	NH_2	Para	Meta
1						1						0.012	0.011
1							1					0.546	0.553
1								1				0.336	0.281
1									1			0.302	0.271
1										1		0.220	0.166
1											1	0.014	0.043
	1					1						0.546	0.553
	1						1					1.478	2.003
	1							1				0.852	0.409
	1								1			1.196	0.748
	1									1		0.806	0.556
	1										1	0.026	0.249
		1				1						0.336	0.281
		1					1					0.852	0.409
		1						1				0.661	0.738
		1							1			0.609	0.570
		1								1		0.542	0.504
		1									1	0.363	0.465
			1			1						0.302	0.271
			1				1					1.196	0.748
			1					1				0.609	0.570
			1						1			0.917	0.536
			1							1		0.556	0.436
			1								1	−0.169	0.327
				1		1						0.220	0.166
				1			1					0.806	0.556
				1				1				0.542	0.504
				1					1			0.556	0.436
				1						1		0.436	0.345
				1							1	0.156	0.253
					1	1						0.014	0.043
					1		1					0.026	0.249
					1			1				0.363	0.465
					1				1			−0.169	0.327
					1					1		0.156	0.253
					1						1	0.705	0.198

[a] Estimated using eq 1.

In view of these differing criteria for additivity, it is appropriate to establish whether the data of Boček and his associates[7-9] can be correlated in a manner consistent with the Free and Wilson approach. However, the original toxicity data from which eq 1 was derived[7-9] have not been reported, the literature reports showing agreement between observed and estimated activities by way of a graph. Hence, in making this assessment toxicities for the disubstituted benzenes were estimated from eq 1 using the reported[8,9] values for b_X, b_Y, e_X, and e_Y. The excellent agreement shown in graphs of observed toxicities *vs.* toxicities estimated from eq 1[7-9] indicates that the original data can be well approximated in this manner.

Interaction Model in Relation to Additive Model. If one were to make use of eq 1 to define *de novo* substituent constants in a Hammett-like manner, one would obtain two sets of *de novo* constants, the values for which would depend upon the choice of parent structure. Hence, in one instance a *de novo* constant a_Y' can be defined relative to the parent structure XH, as shown by eq 2a, while in another instance a *de novo* constant a_X' can be defined relative to the parent structure YH, as shown by eq 2b. Considered in these terms eq 1 can be expressed in an additive manner by a partitioning of terms (eq 3a). Hence, one may expect an additive model (eq 3b) to apply to each of the individual disubstituted compounds. Since additivity in the Free and Wilson sense means that values for a_X and a_Y may apply to all disubstituted compounds in a series,[1,11] it would appear that eq 1 or eq 3b could, under certain circumstances,[1] correlate the same data.

$$\log (A_{XY}/A_{XH}) = \log A_{HY} + e_X e_Y \equiv a_Y' \quad (2a)$$

$$\log (A_{XY}/A_{YH}) = \log A_{XH} + e_X e_Y \equiv a_X' \quad (2b)$$

$$\log A_{XY} = (\log A_{XH} + 0.5 e_X e_Y) + (\log A_{HY} + 0.5 e_X e_Y) \quad (3a)$$

$$\log A_{XY} = a_X + a_Y \quad (3b)$$

Test of Free and Wilson Additivity. The disubstituted benzenes considered by Boček and his associates[7-9] constitute six congeneric series (based on benzene, aniline, toluene, phenol, chlorobenzene, and nitrobenzene), each of which contains six differing substituents (H, NH_2, CH_3, OH, Cl, and NO_2). Taken in terms of congeneric series there are thus represented a total of 36 para- and 36 meta-substituted benzene derivatives, as shown in Table I. Classifying the compounds in this manner results in disubstituted compounds being identified with either of two differing congeneric series, as is specified by eq 2a and 2b. Considered in more explicit terms, if one has a compound such as *m*-chlorophenol, this compound may belong either to a series of phenols or to a series of chlorobenzenes. A second compound such as *m*-nitrophenol may belong either to a series of phenols or to a series of nitrobenzenes. This dichotomy continues for every substitution made on phenol. The data of Boček and his associates are complete in the sense that each series to which a compound may belong is well represented. Table I shows the interrelated congeneric series under consideration.

De novo constants appropriate to each of the substituents are readily obtained if it is first noted that a molecule is symmetric when X and Y are identical (C_{2h} if para and C_{2v} if meta). In this instance the condition $a_X = a_Y$ can be applied *in writing the normal equations* and, since all toxicities are measured relative to benzene, the estimates for the *de novo* substituent constants can be gained directly with no additional restrictions. Here it is stressed that any conditions applied in solving an additive model by a least-squares approach must correctly be used in conjunction with the normal equations derived from the data matrix and not with the data matrix itself. Doing the latter results in a "reduced" data matrix from which a set of normal equations must still be derived in order to obtain *de novo* constants. Conditions are applied to the normal equations to ensure that they are an independent set of simultaneous equations. Applying the same conditions to obtain a "reduced" data matrix in no way guarantees that the normal equations for the "reduced" matrix will be an independent set of simultaneous equations. Nonindependence of the normal equations may cause the determinant for the equations to vanish or, in the absence of a vanishing determinant, will give rise to "ill-behaved," *i.e.*, nonunique, *de novo* parameters.

The six congeneric series represented in Table I consist of 21 independent compounds for each pattern of substitution, para and meta. A comparison of the toxicities estimated using eq 1 to the toxicities estimated by a Free and Wilson approach, shown in Table II, indicates that an additive model can correlate the data, with the exception of certain compounds. The few compounds deviating most strongly from additivity cause the statistics for the regression to be somewhat disappointing. Hence, for the para-substituted derivatives (N = 21), R = 0.773 and s = 0.216, while for the meta-substituted derivatives (N = 21), R = 0.805 and s = 0.166. Close inspection of the deviations shows that the compounds in greatest disagreement with additivity are, in the para series, X = NH_2, Y = NH_2; X = NO_2, Y = NH_2; and X = OH, Y = NH_2, while in the meta series they are X = NO_2, Y = NO_2; X = NO_2, Y = Cl; and X = NO_2, Y = NH_2. Deleting these compounds in calculating new *de novo* substituent constants gives much more satisfying statistics for the regression. Hence, for the para-substituted derivatives (N = 18), R = 0.976 and s = 0.083, while for the meta-substituted derivatives (N = 18), R = 0.983 and s = 0.039. Thus, an additive model does correlate the majority of the data.

Linear Multiple Regression Interaction Model. Cammarata and Yau[2] have proposed an approach that may be used in deriving linear multiple regression relations when an interaction between substituents seems likely. The disubstituted benzenes whose toxicities were determined by Boček and coworkers[7-9] meet the criteria for the application of this approach; hence, attempts were made to relate the toxicities of these compounds to substituent indices.

Cammarata and Yau[2] considered a situation where a substituent X1 is bound to a molecular nucleus and a second substituent Y is varied. Designating the substituent parameter for Y as S, one may expect in the simplest instance that a Hammett-like relationship will correlate the biological activities.

$$\log A_{YX1} = m_{X1}S_Y + n_{HX1} \quad (4a)$$

In this expression, m_{X1} represents the slope and n_{X1} represents the intercept for the congeneric series based on the compound HX1.

If the substituent X1 is changed to an alternative substituent X2 and substitutions are made on the resulting nucleus with substituents Y, a relationship similar to eq 4a, but differing in slope and intercept, may be obtained.

$$\log A_{YX2} = m_{X2}S_Y + n_{HX2} \quad (4b)$$

Thus, for N variations of the substituent X a set of congeneric series involving the substituents Y may be correlated by the set of relations

$$\log A_{YXi} = m_{Xi}S_Y + n_{HXi} \quad (i = 1, 2, \ldots, N) \quad (4c)$$

However, such a systemic change in the substituent X suggests that the slopes m may be correlated with the substituent index S

$$m_X = kS_X + k' \quad (5)$$

which, upon substitution into eq 4c, gives the relation

$$\log A_{YXi} = aS_Y + cS_YS_{Xi} + n_{HXi} \quad (i = 1, 2, \ldots, N) \quad (6a)$$

But, in making substitutions on a common nucleus, the choice of which substituents are to be designated X and which are to be designated Y is essentially arbitrary, the selection being made from a fixed set of possible substituents. This arbitrariness is removed by expressing eq 6a in the form

$$\log A_{YX} = aS_Y + bS_X + cS_XS_Y + d \quad (6b)$$

where S_Y is a substituent parameter defined relative to X and S_X is a substituent parameter defined relative to Y. This equation is suitable for a linear multiple regression analysis.

Test of the Linear Multiple Regression Interaction Model. A variety of substituent constants was investigated in attempting to relate the toxicities of congeneric series of the disubstituted benzenes in a simple linear manner. Only the substituent parameter E_R[12,13] proved useful in this regard. The use of σ^2, which is linearly related to E_R,[14,15] did not lead to as good correlations as did E_R. Consequently, E_R was used as the substituent parameter in assessing the suitability of eq 6b. Limitations inherent to the use of E_R, however, are that values are reported primarily for para substituents and that of these a value for NH_2 has not been determined. Table III shows the data for which the analysis was possible.

Two regression equations were derived (eq 7a and 7b).

$$\log A_{XY} = 1.46\ (\pm 0.40)\ E_{R,Y} + 1.46\ (\pm 0.40)\ E_{R,X} + 0.20 \quad (7a)$$

$$(N = 25;\ R = 0.916;\ s = 0.14;\ F_{2,22} = 57.57)$$

$$\log A_{XY} = 1.07\ (\pm 0.51)\ E_{R,Y} + 1.07\ (\pm 0.51)\ E_{R,X} + 2.82\ (\pm 2.50)\ E_{R,Y}E_{R,X} + 0.258 \quad (7b)$$

$$(N = 25;\ R = 0.934;\ s = 0.13;\ F_{3,21} = 47.74)$$

In these relations, N is the number of data points, R is the multiple correlation coefficient, s is the standard error of the estimate, F is the computed F ratio, and the quantities in parentheses following the coefficients are 95% confidence intervals.

A comparison of the statistics for eq 7a and 7b shows that the interaction term is not of any significance in correlating the data. Hence, eq 7a is sufficient to account for the toxicities of the para-substituted benzenes. The suggestion gained from this correlation is that there is no effect on toxicity due to substituent interaction. However,

Table II. Free and Wilson Estimates of Toxicity

		Para			Meta		
X	Y	Eq 1	Estd[a]	Estd[b]	Eq 1	Estd[a]	Estd[b]
H	H	0.012	0.004	−0.044	0.011	0.012	0.000
H	NO_2	0.546	0.583	0.688	0.553	0.544	0.480
H	Cl	0.336	0.335	0.278	0.281	0.279	0.342
H	OH	0.302	0.335	0.385	0.271	0.272	0.260
H	CH_3	0.220	0.219	0.170	0.166	0.168	0.155
H	NH_2	0.014	−0.051	−0.001	0.043	0.047	0.088
NO_2	NO_2	1.478	1.162	1.334	2.003	1.076	
NO_2	Cl	0.852	0.914	0.967	0.409	0.811	
NO_2	OH	1.196	0.914	1.074	0.748	0.804	0.740
NO_2	CH_3	0.806	0.798	0.859	0.556	0.700	0.635
NO_2	NH_2	0.026	0.528		0.249	0.579	
Cl	Cl	0.661	0.666	0.600	0.738	0.546	0.684
Cl	OH	0.609	0.666	0.707	0.570	0.539	0.602
Cl	CH_3	0.542	0.550	0.492	0.504	0.425	0.497
Cl	NH_2	0.363	0.280	0.321	0.465	0.314	0.430
OH	OH	0.917	0.666	0.814	0.536	0.532	0.520
OH	CH_3	0.556	0.550	0.599	0.436	0.428	0.415
OH	NH_2	−0.169	0.280		0.327	0.307	0.348
CH_3	CH_3	0.436	0.434	0.384	0.345	0.324	0.310
CH_3	NH_2	0.156	0.164	0.213	0.253	0.203	0.243
NH_2	NH_2	0.705	−0.106		0.198	0.082	0.176

[a] $(H)_p = 0.002$, $(NO_2)_p = 0.581$, $(Cl)_p = 0.333$, $(OH)_p = 0.333$, $(CH_3)_p = 0.217$, $(NH_2)_p = -0.053$; $(H)_m = 0.006$, $(NO_2)_m = 0.538$, $(Cl)_m = 0.273$, $(OH)_m = 0.266$, $(CH_3)_m = 0.162$, $(NH_2)_m = 0.041$. [b] $(H)_p = -0.022$, $(NO_2)_p = 0.667$, $(Cl)_p = 0.300$, $(OH)_p = 0.407$, $(CH_3)_p = 0.192$, $(NH_2)_p = 0.021$; $(H)_m = 0.000$, $(NO_2)_m = 0.480$, $(Cl)_m = 0.342$, $(OH)_m = 0.260$, $(CH_3)_m = 0.155$, $(NH_2)_m = 0.088$.

Table III. Structure–Activity Parameters for Para-Disubstituted Benzenes

Substituents					Log $[(LD_{50})_{HH}/(LD_{50})_{YX}]$	
X	Y	$E_{R,X}$	$E_{R,Y}$	$E_{R,X}E_{R,Y}$	Eq 1	Estd[a]
NO_2	NO_2	0.41	0.41	0.1680	1.478	1.407
NO_2	OH	0.41	0.17	0.0697	1.196	1.055
NO_2	Cl	0.41	0.10	0.0410	0.852	0.952
NO_2	CH_3	0.41	0.03	0.0123	0.806	0.849
NO_2	H	0.41	0	0	0.546	0.800
OH	NO_2	0.17	0.41	0.0697	1.196	1.054
OH	OH	0.17	0.17	0.0289	0.917	0.215
OH	Cl	0.17	0.10	0.0170	0.609	0.599
OH	CH_3	0.17	0.03	0.0051	0.542	0.467
OH	H	0.17	0	0	0.302	0.452
Cl	NO_2	0.10	0.41	0.0410	0.852	0.951
Cl	OH	0.10	0.17	0.0170	0.609	0.599
Cl	Cl	0.10	0.10	0.0100	0.661	0.496
Cl	CH_3	0.10	0.03	0.0030	0.556	0.394
Cl	H	0.10	0	0	0.336	0.350
CH_3	NO_2	0.03	0.41	0.0123	0.806	0.848
CH_3	OH	0.03	0.17	0.0051	0.542	0.394
CH_3	Cl	0.03	0.10	0.0030	0.556	0.496
CH_3	CH_3	0.03	0.03	0.0009	0.436	0.261
CH_3	H	0.03	0	0	0.220	0.240
H	NO_2	0	0.41	0	0.546	0.804
H	OH	0	0.17	0	0.302	0.452
H	Cl	0	0.10	0	0.336	0.349
H	CH_3	0	0.03	0	0.220	0.240
H	H	0	0	0	0.012	0.203

[a] From eq 7a.

considering that the physical significance of E_R is not well understood,[15] it seems appropriate to refrain from drawing such inferences at this time.

Conclusions

The interaction model used by Boček, Kopecký, and coworkers[7-9] in correlating the toxicities of disubstituted benzenes has been shown to correspond to a definition of additive substituent contributions taken individually for each compound in a series (eq 3b and assumption 1 of ref 1). It has also been shown that the Free and Wilson criterion of additivity (assumption 2 of ref 1) is generally applicable to the same data. Hence, the interrelationships between the regression models used in constructing quantitative structure–activity relationships are fully accounted for by Cammarata.[1] Within this context, substituent interactions give rise to a parabolic structure–activity relationship in the respect that they cause *de novo* constants for substituents at a particular site of substitution to first increase, reach an optimum, and subsequently decrease.

A possible reason for having had to exclude certain compounds in gaining a correlation by the Free and Wilson approach may be that a physical property not reflected by E_R may be controlling the LD_{50} values. The deviations in the para series were systematic, the compounds all being substituted anilines, and the deviations in the meta series were also systematic, the compounds all being

substituted nitrobenzenes. Each of these series will have to be extended and their LD_{50} values determined before it can be established whether a parameter in addition to E_R, or an alternative parameter combination, is necessary in order to arrive at a correlation.

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Carbon-13 Magnetic Resonance Study. Structure of the Metabolites of Orally Administered Quinidine in Humans

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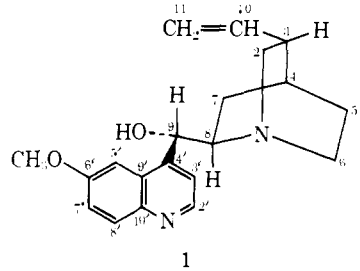
Carbon-13 pulsed Fourier transform nuclear magnetic resonance spectra of DMSO-d_6 solutions of the major metabolites of orally administered quinidine were obtained. The resonances were assigned by chemical shift comparisons and single-frequency off-resonance decoupling. The data have been used to establish the structure of the metabolites as 3-hydroxyquinidine and 2′-quinidinone.

The important cinchona alkaloid, quinidine (1), gives two major biotransformation products when administered orally to humans.[1] An analysis of the mass spectra of these metabolites showed that one of the metabolites (metabolite 5)† resulted from oxidative modification of the quinoline ring of 1, whereas the second metabolite (metabolite 4)† resulted from hydroxylation of the quinuclidine ring of 1.[1] An analysis of the mass spectrum and ir and uv properties of metabolite 5 indicated that it was the 2′-oxo derivative of quinidine; however, it was not possible to assign a structure to metabolite 4. In the present paper we report our results on the application of carbon-13 nuclear magnetic resonance (^{13}C nmr) spectroscopy for the structure elucidation of these metabolites of quinidine (1).



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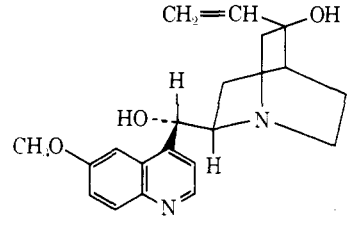
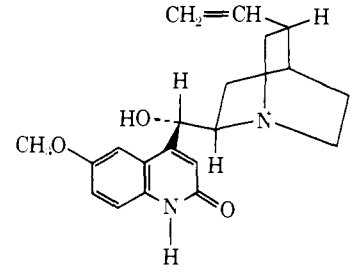
The structure assignments are based on an analysis of the natural abundance, proton noise decoupled and single frequency off-resonance decoupled ^{13}C nmr spectra of these metabolites and the parent drug. The chemical shift and multiplicity of the carbons for metabolite 4, metabolite 5, quinidine, and the maleate salts of quinidine and metabolite 4 are listed in Table I. Specific assignments were made with the use of reported chemical shift parameters for quinidine and other cinchona alkaloids[2] as well as the model compounds listed in Table I.



metabolite 4



metabolite 5

Metabolite 4. The observation that the mass spectrum of metabolite 4 gives a molecular ion at m/e 340 and gives a quinuclidine fragment at m/e 152 compared to m/e 136 for quinidine is conclusive evidence that the metabolite contains a hydroxyl group in the quinuclidine ring.[1] Thus, the problem is to establish the position of attachment and stereochemistry of the hydroxyl group. Both quinidine and metabolite 4 and their maleate salts show nine signals in

†Metabolite 4 and metabolite 5 are the designation used by Palmer and coworkers for these metabolites.[1]