Interdependence between Physical Parameters and Selection of Substituent Groups for Correlation Studies

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Received December 29, 1970

To test whether physical parameters frequently used in correlations with biological activity are independent, correlations for various pairs of π, σ, E_R, E_s, F, R, molecular volume, parachor, and group refractivity constants were examined. As expected, σ, π, and E_R values are not linearly related, although the interdependence of E_R and σ^2 values is confirmed. A significant correlation was found between aliphatic π values and Taft's aliphatic E_s values. Significant correlations were also found for π and molecular volume, and for refractivity and molecular volume values. Some overlap was shown for π and group refractivity data. The term "nonpolar parameters" is proposed for these 5 interrelated parameters. These relationships must be considered in using structure–activity correlations as guides to understanding biological mechanisms of action. They also explain why so many of the successful structure–function correlations have involved partition and polar factors, since these are truly independent. Two-dimensional maps are given for π *vs.* σ and π *vs.* E_R. These serve as guides for the synthesis of derivatives designed to cover wide ranges of values for these parameters.

The extrathermodynamic approach to structure–function correlation has been extensively developed by Hansch and coworkers.[1] In a comparative study of parameters, Leo, *et al.*,[2] found that the partition coefficient gave better correlations over several series of compounds than those obtained by the use of polarizability, parachor, or molar attraction constants.

A more comprehensive study is now reported concerning the relative independence or interdependence of the following parameters: σ and σ^+ constants (meta and para),[3] F and R constants,[4] E_s[5,6] E_R constants,[7] π,[8,9] molecular volume,[10] parachor,[11] and group refractivity[12] data. The need for this study was anticipated by Ferguson some 30 years ago.[13]

Methods

Compilations of substituent constants were assembled in order to study simple linear correlations between pairs. Jaffe's set of meta and para Hammett σ constants[3] was augmented by the addn of 2 groups detd since 1953 (see footnotes in Table III). Brown and Okamoto's values for σ^+ constants were used.[11,14] Swain and Lupton's values for F and R,[4] which were developed by regression analysis, were used, but no other versions of σ constants were studied. Yamamoto and Otsu's[7] values for E_R were used. Bond refractivities[12] were used to calc substituent group refractivities, which were used to explore polarizability factors. Since the dipole moment is a vector quantity, and hence is not strictly additive, it was considered best not to use this parameter. Exner's tabulations were used for both the mol vol[10] and parachor[11] data. Taft's values for E_s constants[6] were augmented with values calcd by Kutter and Hansch.[5]

Correlations were detd by regression analysis, using a time-sharing computer terminal with the STATPACK statistical programs (IBM Call/360 system). Results were expressed in terms of the equation for the best-fitting straight line (or plane), together with the standard error of estimate, correlation coefficient, and F test value.[15] F and R were used both singly and as a pair

TABLE I

COMPARISON OF E_R WITH OTHER PARAMETERS

Group	E_R[a]	σ[b]	σ^2	σ^+[c]	σ^{-2}	π[d]
H	0	0	0	0	0	0
4-CH_3	0.03	−0.17	0.0289	−0.311	0.0967	0.52
4-*i*-Pr	0.03	−0.151	0.0228	−0.280	0.0784	1.40
4-*tert*-Bu	0.03	−0.197	0.0388	−0.256	0.0655	1.68[e]
4-OH	0.17	−0.357	0.127	−0.92	0.846	−0.61
4-OCH_3	0.11	−0.268	0.0718	−0.778	0.605	−0.04
4-NMe_2	0.24	−0.600	0.360	−1.7	2.89	0.18[f]
4-$COCH_3$	0.24	0.516	0.266			−0.37
4-C≡N	0.24	0.628	0.394	0.659	0.434	−0.32
4-NO_2	0.41	0.778	0.605	0.790	0.624	0.24
4-Cl	0.10	0.227	0.0496	0.114	0.0130	0.70
4-Br	0.12	0.232	0.0538	0.150	0.0225	1.02
4-I	0.12	0.276	0.0762	0.135	0.0182	1.26
4-OC_6H_5	0.13	−0.028	0.000784	−0.500	0.250	

[a] T. Yamamoto and T. Otsu, *Chem. Ind. (London)*, 787 (1967). [b] H. H. Jaffé, *Chem. Rev.*, **53**, 191 (1953). [c] H. C. Brown and Y. Okamoto, *J. Amer. Chem. Soc.*, **80**, 4980 (1958). [d] Values from para-substituted phenoxyacetic acid series [T. Fujita, J. Iwasa, and C. Hansch, *ibid.*, **86**, 5175 (1964)] except where otherwise noted. [e] Values for meta substitution used (see footnote *b*). [f] Value for benzene substituent used (same ref footnote *d*).

(1) C. Hansch, *Accounts Chem. Res.*, **2**, 232 (1969).
(2) A. Leo, C. Hansch, and C. Church, *J. Med. Chem.*, **12**, 766 (1969).
(3) H. H. Jaffé, *Chem. Rev.*, **53**, 191 (1953).
(4) C. G. Swain and E. C. Lupton, Jr., *J. Amer. Chem. Soc.*, **90**, 4328 (1968).
(5) E. Kutter and C. Hansch, *J. Med. Chem.*, **12**, 647 (1969).
(6) R. W. Taft, Jr., "Steric Effects in Organic Chemistry," M. S. Newman, Ed., Wiley, New York, N. Y., 1956, p 598.
(7) T. Yamamoto and T. Otsu, *Chem. Ind. (London)*, 787 (1967).
(8) T. Fujita, J. Iwasa, and C. Hansch, *J. Amer. Chem. Soc.*, **86**, 5175 (1964).
(9) J. Iwasa, T. Fujita, and C. Hansch, *J. Med. Chem.*, **8**, 150 (1965).
(10) O. Exner, *Collect. Czech. Chem. Commun.*, **32**, 1 (1967).
(11) O. Exner, *ibid.*, **32**, 24 (1967).
(12) A. I. Vogel, W. T. Cresswell, G. H. Jeffery, and J. Leicester, *J. Chem. Soc.*, 514 (1952).
(13) J. Ferguson, *Proc. Roy. Soc., Ser. B*, **127**, 387 (1939).
(14) H. C. Brown, and Y. Okamoto, *J. Amer. Chem. Soc.*, **80**, 4980 (1958).
(15) G. W. Snedecor, "Statistical Methods," Iowa State University Press, Ames, Iowa, 1966.

TABLE II
META AND PARA SUBSTITUENT VALUES

Group	σ_m[a]	σ_p[c]	σ_m^+[b]	σ_p^+[b]	π_m[c]	π_p[d]
CH_3	−0.069	−0.170	−0.066	−0.311	0.51	0.52
C_2H_5	−0.043	−0.151	−0.064	−0.295	0.97	
tert-Bu	−0.120	−0.197	−0.059	−0.256	1.68	
CF_3	0.415	0.551	0.520	0.612	1.07	
OCH_3	0.115	−0.268	0.047	−0.778	0.12	−0.04
COOH	0.355	0.265	0.322	0.421	−0.15	
C≡N	0.678	0.628	0.562	0.659	−0.30	−0.32
NO_2	0.710	0.778	0.674	0.790	0.11	0.24
F	0.337	0.062	0.352	−0.073	0.13	0.15
Cl	0.373	0.227	0.399	0.114	0.76	0.70
Br	0.391	0.232	0.405	0.150	0.94	1.02
I	0.352	0.276	0.359	0.135	1.15	1.26
SCH_3	0.144	−0.047	0.158	−0.604	0.62	

[a] Footnote *b*, Table I. [b] Footnote *c*, Table I. [c] T. Fujita, J. Iwasa, and C. Hansch, *J. Amer. Chem. Soc.*, **86**, 5175 (1964). [d] Where no value was available,[c] the meta value was used.

TABLE III
AROMATIC SUBSTITUENT VALUES

Group	Formula	σ_m[a]	σ_p[a]	F[b]	R[b]	π[c]	Mol vol[d]	Refractivity[e]
Acetylamino	CH_3CONH	0.210	−0.010	0.470	−0.274	−0.79	48.99	14.56
Acetoxy	CH_3COO	0.390	0.310	0.679	−0.071	−0.64[f]	48.43	12.74
Acetyl	CH_3CO	0.376	0.502	0.534	0.202	−0.55[f]	41.69	11.13
Amino	H_2N	−0.160	−0.660	0.037	−0.681	−1.23[f]	17.67	6.06
Bromo	Br	0.391	0.232	0.727	−0.176	0.86[f]	26.19	9.49
tert-Butyl	$(CH_3)_3C$	−0.100	−0.197	−0.104	−0.138	1.68	81.22	19.70
Carboxy	HOOC	0.370	0.450	0.552	0.140	−0.28[f]	27.24	7.96
Chloro	Cl	0.373	0.227	0.690	−0.161	0.71[f]	22.96	6.64
Cyano	NC	0.560	0.660	0.847	0.184	−0.57[f]	22.67	7.08
Ethoxy	C_2H_5O	0.100	−0.240	0.363	−0.444	0.50[g]	54.80	12.78
Ethoxycarbonyl	C_2H_5OCO	0.370	0.450	0.552	0.140	0.51[g]	65.01	18.94
Ethyl	C_2H_5	−0.070	−0.151	−0.065	−0.114	1.04	48.06	11.00
Fluoro	F	0.337	0.062	0.708	−0.336	0.14[f]	15.11	2.78
Hydrogen	H	0	0	0	0	0	14.90	1.68
Hydroxy	HO	0.121	−0.370	0.487	−0.643	−0.67	10.25	3.20
Iodo	I	0.352	0.180	0.672	−0.197	1.12[h]	32.93	14.61
Methoxy	CH_3O	0.115	−0.268	0.413	0.500	−0.02[f]	38.52	8.12
Methyl	H_3C	−0.069	−0.170	−0.052	0.141	0.52[i]	31.48	6.34
Methylsulfonyl	CH_3SO_2	0.600	0.720	0.900	0.215	−1.60[h]		
Methylthio	CH_3S	0.150	0.000	0.332	0.186	0.62	42.26	14.26
Nitro	O_2N	0.710	0.778	1.109	0.155	−0.28[f]	24.51	7.35
Phenyl	C_6H_5	0.060[j]	−0.010[j]	0.139	−0.088	2.13	74.65	26.66
Sulfamoyl	H_2NSO_2	0.460[j]	0.570[j]	0.679	0.188	−1.82[f]		
Trifluoromethyl	F_3C	0.430	0.540	0.631	0.186	1.16[h]	32.11	5.62

[a] Footnote *b*, Table I. [b] C. G. Swain and E. C. Lupton, Jr., *J. Amer. Chem. Soc.*, **90**, 4328 (1968). [c] Values from meta-substituted phenoxyacetic acids (footnote *c*, Table II) unless otherwise noted. [d] O. Exner, *Collect. Czech. Chem. Commun.*, **32**, 1 (1967). [e] Calcd using bond refractivities given in A. I. Vogel, W. T. Cresswell, G. H. Jeffery, and J. Leicester, *J. Chem. Soc.*, 514 (1952); includes value for a bond to carbon. [f] Benzene series, footnote *c*, Table II. [g] Calcd from benzene values, footnote *c*, Table II. [h] Unpublished experimental value obtained by C. Hansch. [i] *p*-Phenoxyacetic acid value, footnote *c*, Table II. [j] D. H. McDaniel and H. C. Brown, *J. Org. Chem.*, **23**, 420 (1958).

of constants. From their derivation,[4] it is evident that they were designed to be used together.

Results and Discussion

The data used are listed in Tables I through V. The correlations obtained are given in Table VI.

The correlations show that π is independent of polar terms such as F and R, σ constants, and E_R. However, highly significant correlations were found between π and molecular volume or parachor. Poorer, but still significant, correlations were found for π and E_s or group refractivities. Within the limits of the number and kind of groups studied, these 5 parameters appear to have a great deal in common. The generic tag "nonpolar parameters" is proposed for these related constants. It would appear of little value to use more than 1 of the 3 parameters (π, mol vol, and parachor) at the same time. Leo, *et al.*,[2] have demonstrated the superiority of the π parameter over several of these nonpolar parameters and also over the molar attraction constant.[16] It would appear to be useless to perpetuate the parachor as a physical parameter, since the mol vol has about a 99% overlap with it.[11] The availability of a large number of π values,[8,9] and the demonstrated additivity of π constants, strongly support their use instead of parachor or mol vol.

Consideration of polarizability data (in the form of bond or group refractivities) and E_s values in this type of correlation is justified. The overlap between π

(16) J. A. Ostrenga, *J. Med. Chem.*, **12**, 349 (1969).

TABLE IV
ALIPHATIC SUBSTITUENT VALUES

Group	π[a]	E_s[b]	Group refractivity[c]
H	0	1.24	1.68
F	−0.17	0.78	1.44
OH	−1.16	0.69*	3.20
CH_3O	0.47	0.69*	8.12
Cl	0.39	0.27*	6.51
Br	0.60	0.08*	9.39
CH_3	0.50	0.00	6.34
CH_3CH_2	1.00	−0.07	11.00
n-Pr	1.50	−0.36	15.66
n-Bu	2.00	−0.39	20.32
n-Am	2.50	−0.40	24.98
i-Am	2.37*	−0.35	24.98
n-Oct	4.00	−0.33	38.96
$(CH_3)_3C—CH_2CH_2$	2.68*	−0.34	29.64
$C_6H_5CH_2$	2.69	−0.38	31.32
$C_6H_5CH_2CH_2$	3.19	−0.38	35.98
$C_6H_5(CH_2)_3$	3.69	−0.45	40.64
i-Pr	1.37	−0.47	15.66
Cyclopentyl	1.97	−0.51	22.92
Cyclohexyl	2.39	−0.79	27.58
$CH_3OCH_2CH_2$	0.53*	−0.77	17.44
i-Bu	1.87	−0.93	20.32
Cyclohexylmethyl	2.89*	−0.98	32.24
Me(Et)CH	1.87	−1.13	20.32
CF_3	1.07	−1.16	5.62
$C_6H_5(Me)CH$	3.06*	−1.19	35.98
$C_6H_5(Et)CH$	3.56*	−1.50	40.64
tert-Bu	1.68	−1.54	20.32
$(CH_3)_3C—CH_2$	3.18	−1.74	24.98
$(C_6H_5)_2CH$	4.63	−1.76	56.30
Et_2CH	2.37*	−1.98	24.98
Pr_2CH	3.37*	−2.11	34.30
$i\text{-}Bu_2CH$	4.11*	−2.47	43.62
Me_2(neopentyl)CH	4.55*	−2.57	38.96
C_6H_5	2.13	−2.58*	25.36
Neopentyl$_2$CH	6.73*	−3.18	55.54
Me(*tert*-Bu)CH	2.55*	−3.33	29.64
Et_3C	3.18*	−3.8	34.30
Me_2(*tert*-Bu)C	2.86*	−3.9	34.30
Me(*tert*-Bu)(neopentyl)C	5.36*	−4.0	52.94

[a] Experimental data reported in J. Iwasa, T. Fujita, and C. Hansch, *J. Med. Chem.*, **8**, 150 (1965), and unpublished values obtd by Hansch. Values calcd from experimental data by additivity method are indicated by asterisk. [b] R. W. Taft, Jr., "Steric Effects in Organic Chemistry," M. S. Newman, Ed., Wiley, New York, N. Y., 1956, p 598, except asterisk indicates calcd by method in E. Kutter and C. Hansch, *J. Med. Chem.*, **12**, 647 (1969). [c] Ref from footnote *e*, Table III. Values shown include value for a bond to C.

and E_s is marginal (R = 0.701) and, with a selected number of substituent groups, could be even much less. Therefore, a term in E_s should be considered for addition to (or replacement for) a term in π in such correlations (see ref 5, 17, and 18). This possibility is even greater when π and refractivities are compared. However, in any such case, statistical data should be given to show that the use of terms of this type in addition to π is justified.

The various "polar" factors do not correlate well with any of the 5 "nonpolar" parameters just mentioned. No significant correlations were found for any of the pairs consisting of one "nonpolar" and one "polar" constant. No attempt was made to study

(17) C. Hansch, *J. Org. Chem.*, **35**, 620 (1970).
(18) C. Hansch and E. Coats, *J. Pharm. Sci.*, **59**, 731 (1970).

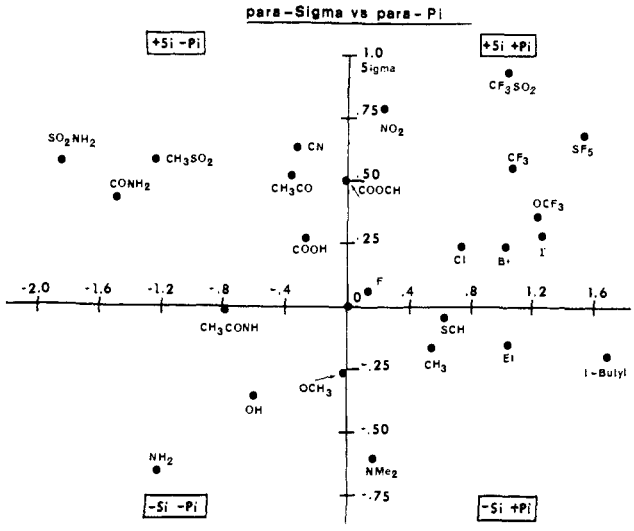

Figure 1.

TABLE V
MOLECULAR VOLUME AND PARACHOR VALUES

Group	Mol vol[a]	Parachor[b]	Aliphatic π[c]	Aromatic π[d]	Aliphatic E_s[e]
H	14.90	16.8	0	0	1.24
CH_2	16.58	39.7	0.52	0.52	
CH_3	31.48	56.5	0.52	0.52	0.00
CH_2=CH	42.48	84.6	0.68	0.68	
	79.17		1.97	1.97	−0.51
	93.92	224.5	2.39	2.39	−0.79
C_6H_5	74.65	187.2	2.13	2.13	−2.58
Naphthyl	108.4	287.8	3.37	3.37	
F	15.11	26.5	−0.17	0.14	0.78
Cl	22.96	56.0	0.39	0.71	0.27
Br	26.19	69.5	0.60	0.86	0.08
I	32.93	90.5		1.12	−0.16
OH	10.25	29.7	−1.16	−0.67	0.69
NH_2	17.67	43.7	−1.19	−1.23	
C≡N	22.67	63.5	−0.84	−0.57	
NO_2	24.51	72.9		−0.28	−1.28
Pyridyl	66.82	176.4	0.65	0.65	
	65.58	167.9	1.81	1.81	
C_6H_5O	79.20	206.3			
COOH	27.24	72.9	−0.67	−0.28	
OCH_3	38.22	76.2	−0.47	−0.02	0.69
CF_3	32.11	85.6	1.07	1.07	−1.16

[a] Footnote *d*, Table III. [b] O. Exner, *Collect. Czech. Chem. Commun.*, **32**, 24 (1967). [c] Ref from footnote *a*, Table IV. [d] See footnote *c*, Table II. [e] Refs from footnote *c*, Table IV.

cross correlations among σ constants or other polar parameters, since Swain and Lupton's elegant work (which resulted in F and R) and Hansch and Kerley's study of σ^+ and E_R[19] adequately cover this field. One exception was made—the correlation between E_R and σ^2 reported by Cammarata[20] was confirmed using 14 pairs of values.

A recent publication by Cammarata, *et al.*, reports that a significant relationship exists between group dipole moments and σ constants.[21]

(19) C. Hansch and R. Kerley, *Chem. Ind. (London)*, 294 (1969).
(20) A. Cammarata and S. J. Yau, *J. Med. Chem.*, **13**, 93 (1970).
(21) A. Cammarata, R. C. Allen, J. K. Seydel, and E. Wempe, *J. Pharm. Sci.*, **59**, 1496 (1970).

TABLE VI
CORRELATIONS

Equation		Data in Table	N^a	s^b	R^c	F^d
1	π (meta values) = 0.903 − 1.135 σ_m	II	13	0.520	0.514	3.948
2	π (meta values) = 0.823 − 0.855 σ_m^+	II	13	0.563	0.370	1.749
3	π (para values) = 0.668 − 0.438 σ_p	II	13	0.606	0.246	0.709
4	π (para values) = 0.603 − 0.207 σ_p^+	II	13	0.616	0.169	0.324
5	π (arom) = 0.469 − 1.436 σ_m	III	24	0.958	0.341	2.89
6	π (arom) = 0.196 − 0.597 σ_p	III	24	0.992	0.228	1.20
7	π (arom) = 0.752 − 1.275F + 0.392R	III	24	0.958	0.395	1.94
8	Refractivity = 12.42 − 4.21F + 2.49R	III	22	6.21	0.238	0.570
9	π (arom) = 0.649 − 1.154F	III	24	0.942	0.383	3.78
10	π (arom) = 0.0817 − 0.212R	III	24	1.017	0.0569	0.071
11	π = 0.848 − 2.568E_R	I	13	0.657	0.430	2.492
12	E_R = 0.1314 + 0.1465σ	I	14	0.0997	0.518	4.40
13	E_R = 0.0561 + 0.566σ^2	I	14	0.0419	0.933	80.8**
14	π (arom) = −0.530 + 0.0771 refractivity	III	22	0.742	0.543	8.37**
15	π (aliph) = 0.501 − 0.0626 refractivity	IV	40	0.980	0.685	33.6**
16	π (aliph) = 1.365 − 0.861 E_s	IV	40	1.18	0.701	36.6**
17	Mol vol = 34.91 − 15.57 E_s	V	13	22.27	0.607	6.43*
18	π (arom) = −0.733 + 0.0347 mol vol	V	19	0.569	0.881	58.94**
19	π (aliph) = −0.987 + 0.0376 mol vol	V	19	0.617	0.881	59.05**
20	π (aliph) = −0.842 + 0.0140 parachor	V	18	0.668	0.858	44.5**
21	π (arom) = −0.605 + 0.013 parachor	V	18	0.609	0.861	46.0**
22	Mol vol = −0.890 + 0.383 parachor	*e*	38	4.887	0.989	1599**
23	Refractivity = −0.330 + 0.287 mol vol	III	22	2.56	0.911	98.05**
24	E_s = 0.501 − 0.0626 refractivity	IV	40	0.980	0.685	33.6*

[a] Number of data sets studied. [b] Standard error of estimate = $[\Sigma(y_i - \bar{y})^2/(N-1)]^{-1/2}$. [c] R = Correlation coefficient. [d] F = F Test value = (mean square attributable to regression)/(mean square deviation from regression). The subscripts for F are 1, $N-2$, for all equations except 7 and 8, where they are 2, $N-3$. F values significant at the 5% level are given one asterisk; two asterisks indicate significance at the 1% level. See G. W. Snedecor, "Statistical Methods," Iowa State University Press, Ames, Iowa, 1966. [e] See ref in footnote *a*, Table IV.

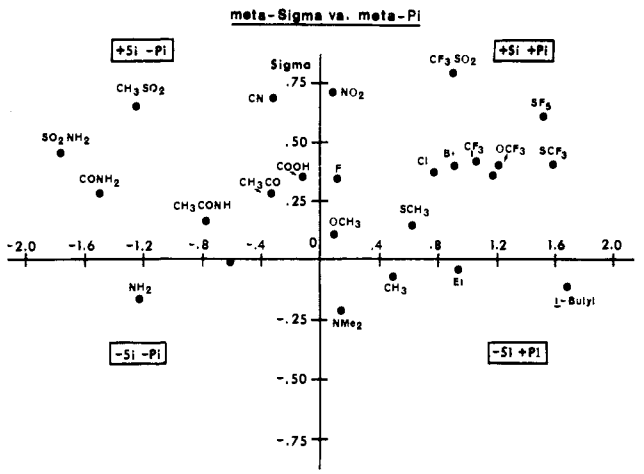

Figure 2.

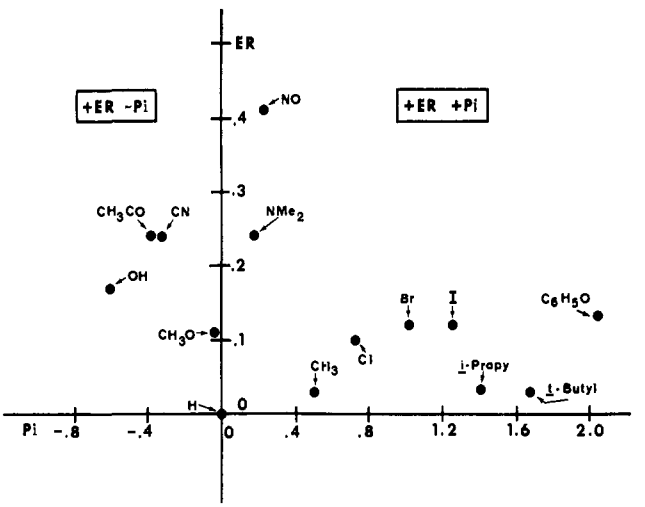

Figure 3.

Structure–activity studies which have been analyzed by the multiple parameter (extrathermodynamic) procedure must be reconsidered in light of the present findings. It will be very difficult to determine that a unique relationship exists between one "nonpolar" parameter and biological activity. It usually will be more correct to state that a relationship exists between "nonpolar" factor(s) and biological activity, or between "polar" factor(s) and biological activity.

The demonstrated independence of polar and nonpolar factors is based solely upon the lack of a linear correlation. It is not intended to imply that there can be no more complex physical relationship between them. Indeed, Rogers and Cammarata have demonstrated a relationship which describes partition data in terms of total charge density and induced polarization, both of which were calculated by molecular orbital methods.[22]

Selection of Substituent Groups.—The statistical independence of π and σ constants reinforces efforts to correlate structure and biological function with these 2 parameters. Selection of appropriate substituent groups can lead to a wide range of both π and σ or E_R values; a guide to this selection can be found in the 2-dimensional "maps" which are listed in Figures 1–3. By careful selection of substituent groups one can avoid misleading assumptions resulting

(22) K. S. Rogers and A. Cammarata, *Biochim. Biophys. Acta*, **193**, 22 (1969).

from inadequate ranges of π, σ, and E_R values. One should be careful to avoid the use of only those substituents which lie on or near a straight line in Figures 1–3; *i.e.*, those which are highly correlated.

Acknowledgment.—The author would like to acknowledge helpful discussions with Professor Corwin Hansch. This study was supported by the U. S. Army Medical Research & Development Command under Contract No. DADA-17-69-C-9106. The paper is Contribution No. 896 to the Army Research Program on Malaria.

Inhibitors and Stimulators of Cholesterolgenesis Enzymes. A Structure–Activity Study *in Vitro* of Amino and Selected N-Containing Analogs of 5α-Cholestane-3β,5α,6β-triol[1a-c]

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Received December 9, 1970

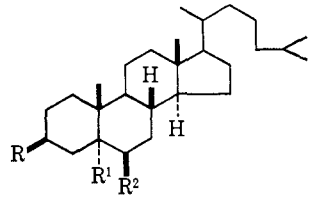
The stereoselective synthesis and biological evaluation *in vitro* of the 3β-, 3α-, 5α- and 6β-monoamino and 3β,6β-diamino analogs of 5α-cholestane-3β,5α,6β-triol and selected azido and oximino intermediates are discussed. Compounds were studied for their inhibitory action on acetate-2-^{14}C and mevalonate-2-^{14}C incorporation into nonsaponifiable products catalyzed by a rat liver homogenate preparation and for their inhibitory or stimulatory action on two semipurified liver enzymes, Δ^7-sterol Δ^5-dehydrogenase and $\Delta^{5,7}$-sterol Δ^7-reductase. Some of our preliminary studies designed to probe the mechanism of action of three inhibitors and one stimulator of the Δ^7 reductase enzyme are also described. The results suggest that the analogs exert their actions by direct effect on the microsomal enzyme and by altering the function of a sterol carrier protein (SCP) required for full activity of the enzyme.

Studies with oxo analogs and esters of 5α-cholestane-3β,5α,6β-triol (1) suggested the free 5α-OH function to be important for lowering serum cholesterol levels in the cholesterol-fed hypercholesterolemic rabbit.[2] Triol 1 also inhibits cholesterol biosynthesis *in vitro*, causing accumulation of a previously undetected 29–30 C atom intermediate.[1c,2] We anticipated therefore that replacement of the 5α-OH with a 5α-NH_2 would render the compound a more potent inhibitor of cholesterol biosynthesis; *i.e.*, the NH_2 function, either protonated or unprotonated, would bind strongly to a specific enzyme system. In this regard, examination of Dreiding molecular models[3] shows the topographical relationship between the 5α-NH_2 and 3β-OH functions of **2** to be similar to the relationship between the 4α-Me and 3β-OH groups of lanosterol, in which the A ring probably exists in a flattened chair conformation.[4] Further, it is known that removal of the 4α-Me represents the first step in the enzymatic conversion of lanosterol to cholesterol.[5]

For these stereochemical reasons we proposed[1a,1d] **2** would block the biosynthesis of cholesterol after or during squalene cyclization. Such a block may enable isolation of presently unidentified intermediates in cholesterol biosynthesis and elucidate mechanisms of specific cholesterolgenesis enzymes. In this communication the biological effects on various cholesterolgenesis enzymes *in vitro* of **1** and **2** are compared with results obtained for the 3β-, 3α-, and 6β-monoamino and 3β,6β-diamino analogs of **1**, as well as with some selected synthetic intermediates.

Results and Discussion

Synthesis.—LAH reduction of 5α-azido-5α-cholestane-3β,6β-diol (**3**) afforded the known 5α-amino-5α-cholestane-3β,6β-diol (**2**).[6a] The 5α-azido intermediate **3** was prepared from cholesterol β-epoxide[7] by a



1, R = R¹ = R² = OH
2, R = R² = OH; R¹ = NH_2
3, R = R² = OH; R¹ = N_3
4, R = OAc; R¹ = N_3; R² = OH
5, R = OAc; R¹ = N_3; R² = *keto*

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