were shown to be configurationally homogeneous by their ir and NMR spectra.

With this procedure, but in the absence of $CaCO_3$, mixtures of the two isomeric amides V and VI were obtained with some acids. The isomerization takes place during the treatment of the acids with oxalyl chloride. Treating the residue of the acid chlorides with 10% aqueous Na_2CO_3 at 0 °C and then stirring 24 h at room temperature, mixtures of *E* and *Z* acids were obtained starting from pure *E* or *Z* acids. Only starting *E* or *Z* acids were recovered through the same procedure, but in the presence of $CaCO_3$.

Reformatsky Reaction of 3,4,5-Trimethoxyacetophenone (VII) with Ethyl α-Bromopropionate (VIII). A portion (40 ml) of a solution of VII (198.0 g, 0.94 mol) and VIII (186.0 g, 1.03 mol) in anhydrous benzene (220 ml) was added to zinc powder (66.9 g, 1.02 g-atoms) and the flask was warmed gently until the reaction started. When the reaction began, stirring was started and the remainder of the solution was added at such a rate that a gentle reflux was maintained. The reaction mixture was then refluxed for 2 h, cooled at 0 °C, and hydrolyzed by addition of ice-cold 20% sulfuric acid (300 ml). The organic layer was washed with 10% aqueous Na_2CO_3 and H_2O, filtered, and evaporated to dryness to give a mixture of the diastereoisomeric esters IX and X (229 g).

erythro-**Ethyl 2-methyl-3-(3,4,5-trimethoxyphenyl)-3-hydroxybutyrate (IX)** was isolated by crystallization from petroleum ether (bp 60–80 °C): mp 75–76 °C; ir 3425 (OH) and 1725 cm^{-1} (C=O); NMR δ 1.00 [CH_3 (a)], 1.41 [CH_3 (b)], 3.00 (H), 1.30 [CH_3 (c)], and 3.94 ppm (CH_2). Anal. ($C_{16}H_{24}O_6$) C, H.

Threo ester X was obtained by preparative TLC on silica gel plate (Merck F_{254}) from the faster moving band, using a mixture of ethyl acetate–petroleum ether (bp 40–70 °C) (80:20) as the eluent and repeating the elution four times: mp 59–60 °C; ir 3450 (OH) and 1712 cm^{-1} (C=O); NMR δ 1.33 [CH_3 (a)], 1.58 [CH_3 (b)], 2.83 (H), 0.97 [CH_3 (c)], and 4.32 ppm (CH_2). Anal. ($C_{16}H_{24}O_6$) C, H.

(*E*)- and (*Z*)-(3,4,5-Trimethoxy)-α,β-dimethylcinnamic Acids (I and II, Ar = 3,4,5-Trimethoxyphenyl). A solution of the mixture of IX and X (189.0 g, 0.60 mol) and iodine (15.0 g, 0.06 mol) in anhydrous benzene (800 ml) was refluxed for 22 days, washed with saturated aqueous $NaHCO_3$ and $Na_2S_2O_3$, and evaporated to give a residue (165.0 g) which was distilled to give a mixture of esters XI and XII (147.5 g), bp 150–156 °C (0.1 mm) (NMR). This mixture (138.1 g, 0.47 mol) was refluxed with KOH (52.6 g, 0.94 mol) in dioxane (100 ml) and H_2O (260 ml) for 8 h, washed with Et_2O, acidified with ice-cold 5 N H_2SO_4, and extracted with Et_2O. Evaporation of the dried ($MgSO_4$) Et_2O extracts afforded a residue consisting essentially of a mixture of the two acids (102.0 g) (NMR). Fractional crystallization from ligroine (bp 80–100 °C) yielded pure I and II, Ar = 3,4,5-trimethoxyphenyl. The *Z* acid is less soluble than *E* isomer.

***E* acid**: mp 134–136 °C; ir 1681 cm^{-1} (C=O); NMR δ 1.84 [CH_3 (a)] and 2.38 ppm [CH_3 (b)]. Anal. ($C_{14}H_{18}O_5$) C, H.

***Z* acid**: mp 157–158 °C; ir 1658 cm^{-1} (C=O); NMR δ 2.02 and 2.09 ppm [CH_3 (a), CH_3 (b)]. Anal. ($C_{14}H_{18}O_5$) C, H.

(*E*)-*N*-Alkyl-(3,4,5-trimethoxy)-α,β-dimethylcinnamamides (V, Ar = 3,4,5-Trimethoxyphenyl). These amides were obtained from the *E* acid I, as previously described for the *p*-phenyl-substituted ones. Starting from *Z* acid II, **2,3-dimethyl-5,6,7-trimethoxyindenone** was obtained as the final product: mp 106–108 °C from petroleum ether (bp 40–60 °C); ir 1689 cm^{-1} (C=O); NMR δ 1.75, 2.05 (CH_3), 3.80, 3.92, and 4.11 ppm (CH_3O). Anal. ($C_{14}H_{16}O_4$) C, H.

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Use of Distribution Coefficients in Quantitative Structure–Activity Relationships

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The use of distribution coefficients (log *D*) for the analysis of structure–activity relationships of ionizable compounds is described. (*D* is the ratio of the equilibrium concentration of compound in an organic phase to the total concentration of un-ionized and ionized species in the aqueous phase at a given pH.) Simpler equations, often with improved correlations, have resulted. This method has the advantage that the influence of pK_a or equivalent electronic factors on distribution can be distinguished from electronic effects related to mechanism of action. Several absorption studies are reanalyzed as well as studies on membrane conductance and uncoupling of oxidative phosphorylation.

This paper describes the use of distribution coefficients in the regression analysis of structure–activity relationships of ionizable compounds. The distribution coefficient (*D*) is defined as the ratio of the concentration of compound in the lipid phase to the concentration of all species in the aqueous phase at a given pH (the organic phase is assumed to contain only un-ionized species). The partition coefficient (*P*) refers to the ratio of un-ionized compound in each phase. All values of *P* and *D* refer to octanol as the organic phase unless otherwise stated.

When an ionizable compound is equilibrated in a two-phase system at a pH at which it is partially ionized, its concentration in the organic phase is not determined by log *P* alone. A correction has to be made based on the

Table I. Correction for Dissociation Factor, C_D[a]

pK_a − pH (acids) pH − pK_a (bases)	C_D	pK_a − pH (acids) pH − pK_a (bases)	C_D
>2	0	−0.1	−0.35
2.0	0	−0.2	−0.41
1.0	−0.04	−0.3	−0.48
0.9	−0.05	−0.4	−0.55
0.8	−0.06	−0.5	−0.62
0.7	−0.08	−0.6	−0.70
0.6	−0.10	−0.7	−0.78
0.5	−0.12	−0.8	−0.86
0.4	−0.15	−0.9	−0.95
0.3	−0.18	−1.0	−1.04
0.2	−0.21	−2.0	−2.00
0.1	−0.25	−3.0	−3.00
0.0	−0.30		

[a] For use with eq 1 and 2: $C_D = \log [1/(1 + 10^{pH-pK_a})]$ for acids; $\log [1/(1 + 10^{pK_a-pH})]$ for bases.

pK_a of the compound. On the other hand, the concentration in the organic phase *is* directly related to log D, which incorporates this correction, as indicated in eq 1 and 2. One reason for wanting to examine possible relationships using log D is to be able to assess the extent electronic factors (pK_a, σ) may be associated with various processes, or activities, apart from their influence on compound concentration in biolipid phases. Examples D and E illustrate this separation of electronic effects.

A second reason for exploring the use of log D is that regression equations such as

$$\log 1/C = a(\log D)^2 + b \log D + c$$

would be difficult to duplicate treating log P and pK_a or σ as independent variables. This can be seen by substituting log D from eq 1 or 31. Such terms as $(pK_a)^2$ and pK_a log P would have to be introduced. If, in fact, the correlations using $(\log D)^2$ are better, and perhaps more closely represent the true relationships of biological activity to physical properties, then the use of distribution coefficients should be a powerful tool in quantitative structure–activity relationships. A quadratic relationship in log D is illustrated in example A.

The relationship between distribution coefficient and partition coefficient, pK_a, and pH is given by eq 1 and 2 for acids and bases. These are developed in the Appendix. The second term in each equation represents the log of the fraction of compound *un*-ionized. It is convenient to call this second term C_D, for "correction for dissociation". Values of C_D for various pK_a and pH differences are tabluated in Table I. C_D is zero when the fraction of drug un-ionized is one (log D = log P); otherwise, C_D is negative and log D is less than log P.

$$\log D_{acids} = \log P + \log [1/(1 + 10^{pH-pK_a})] = \log P + C_D \quad (1)$$

$$\log D_{bases} = \log P + \log [1/(1 + 10^{pK_a-pH})] = \log P + C_D \quad (2)$$

The examples below illustrate the use of log D in regression analyses and compare the results with more conventional published treatments. The first three examples are concerned with absorption. The absorption of compounds should, a priori, be one of the simpler processes to examine since receptor interactions or specific biological properties are not involved (examples A–C). On the other hand, the effects of acids on membrane conductance (example D) and uncoupling of oxidative phosphorylation (example E) are related to their acidity as well as their membrane concentration (log D).

Example A. Colonic Absorption of Acids. Lien[1] has recently reviewed absorption data which has been treated by regression analysis. Much of this is his own work. Partition coefficients alone have been insufficient in many cases to adequately explain much absorption data for ionizable compounds. Lien introduced a ratio term log u/d for undissociated/dissociated forms of acids and bases which he used either alone or in combination with $(\log P)^2$ and/or log P terms. We have found substantial improvement in some of these analyses using distribution coefficients alone.

The data of Schanker[2] on the colonic absorption of acids at pH 6.8 and the physical properties used by Lien and ourselves are found in Table II. Log u/d is given by pK_a − pH. Lien approximated the absorption rate in his calculations by using log percent absorbed in a unit time.

Our calculations are compared with his in eq 3 and 4. The absorption rate is highly correlated with log D (eq 3) and is even better fitted by the inclusion of a $(\log D)^2$ term (eq 4). The latter is significant at the 99.5 percentile level (F = 22.7). From eq 4 an optimum log D for absorption can be calculated to be 1.49. The values in parentheses are 95% confidence limits. Equation 5 was reported by Lien.

$$\log \% \text{ abs} = 0.233 \log D + 1.266 \quad (3)$$
$$n = 10; r = 0.922; s = 0.186$$

$$\log \% \text{ abs} = -0.079 (\log D)^2 + 0.236 \log D + 1.503 \quad (4)$$
$$n = 10; r = 0.965; s = 0.096$$

$$\log D_0 = 1.49 \ (0.96 - 2.98)$$

$$\log \% \text{ abs} = 0.156 (pK_a - 6.8) - 0.366 \log P + 0.755 \quad (5)$$
$$n = 10; r = 0.866; s = 0.258$$

Table II. Absorption of Organic Acids from the Rat Colon and Physicochemical Constants Used for Eq 3–5

Acid	Log P[a]	pK_a − 6.8 (log u/d)[a]	C_D[b]	Log D[c]	Log % abs Obsd[d]	Log % abs Calcd[e]	Log % abs Calcd[f]
5-Nitrosalicylic acid	1.98	−4.5	−4.5	−2.52	0.30	0.41	0.78
m-Nitrobenzoic acid	1.83	−3.4	−3.4	−1.57	1.00	0.94	0.89
Salicylic acid	2.26	−3.8	−3.8	−1.54	1.08	0.95	0.99
Benzoic acid	1.85	−2.6	−2.6	−0.75	1.28	1.28	1.03
Phenylbutazone	3.22	−2.4	−2.4	0.82	1.58	1.64	1.56
o-Nitrophenol	1.79	0.2	−0.2	1.58	1.74	1.68	1.44
Thiopental	2.50	0.8	0	2.44	1.70	1.61	1.80
p-Hydroxypropiophenone	1.85	1.0	0	1.81	1.66	1.67	1.59
m-Nitrophenol	2.00	1.4	0	2.00	1.64	1.66	1.71
Phenol	1.46	3.1	0	1.46	1.55	1.68	1.77

[a] From ref 1. [b] Obtained from Table I. [c] Calculated from eq 1. [d] From ref 2. [e] Calculated from eq 4. [f] Calculated from eq 5 in ref 1.

Table III. Absorption of Organic Bases from the Rat Colon and Physicochemical Constants Used for Eq 6-8

Base	Log P^a	6.8 $-pK_a$ (log u/d)^a	$C_D{}^b$	Log D^c	Log % abs Obsd^d	Log % abs Calcd^e	Log % abs Calcd^f
Acetanilide	1.16	6.5	0	1.16	1.56	1.60	1.75
p-Nitroaniline	1.39	5.8	0	1.39	1.70	1.69	1.72
Antipyrine	0.23	5.4	0	0.23	1.30	1.27	1.31
m-Nitroaniline	1.37	4.3	0	1.37	1.68	1.68	1.64
Aniline	0.90	2.2	0	0.90	1.64	1.51	1.46
Aminopyrine	0.76	1.8	0	0.76	1.32	1.46	1.39
p-Toluidine	1.39	1.5	0	1.39	1.71	1.68	1.47
Quinine	1.83	−1.6	−1.6	0.23	1.30	1.27	1.21
Ephedrine	1.56	−2.8	−2.8	−1.24	0.95	0.73	1.21
Tolazoline	2.65	−3.5	−3.5	−0.85	0.60	0.87	0.60
Levorphan	3.02^g	−3.0^h	−3.0	0.02	1.11	1.19^i	0.29^i

^{a} From ref 1. ^{b} Obtained from Table I. ^{c} Calculated from eq 2. ^{d} From ref 2. ^{e} Calculated from eq 6. ^{f} Calculated from eq 8 in ref 1. ^{g} Calculated from D at pH 7.10 (ref 4) and eq 30. ^{h} pK_a from ref 4. ^{i} Not used to derive eq 6 and 8.

Since absorption is assumed to follow first-order kinetics, it would be more correct to derive first-order rate constants for each compound and rerun the regression analyses. This was done for this example without changing the point that the use of log D gave improved correlations. Since this was a perfusion experiment (0.2 ml/min through a 13-cm colon) we had to take an arbitrary time value (60 min) and used the nomograph of Smith and Stevens[3] for first-order rate constants. The absolute values of log k, but not the relative rates, would be affected by the time interval selected. The results are given in eq 3′–5′.

$$\log k = 0.265 \log D - 4.216 \quad (3')$$
$$n = 10; r = 0.935; s = 0.191$$

$$\log k = -0.082 (\log D)^2 + 0.268 \log D - 3.968 \quad (4')$$
$$n = 10; r = 0.986; s = 0.095$$

$$\log k = 0.177 (pK_a - 6.8) + 0.417 \log P - 4.801 \quad (5')$$
$$n = 10; r = 0.877; s = 0.277$$

From eq 4′ log D_0 = 1.63 (1.07 − 3.11).

Example B. Colonic Absorption of Bases. The colonic absorption of the bases measured by Schanker[2] is listed in Table III. The single variable log D provided a higher correlation coefficient than the two or three variable eq 7 and 8 reported by Lien. Addition of a $(\log D)^2$ term was not significant, but this is not unexpected since there are no compounds with log $D > 1.4$ in this group.

$$\log \% \text{ abs} = 0.362 \log D + 1.83 \quad (6)$$
$$n = 10; r = 0.930; s = 0.143$$

$$\log \% \text{ abs} = -0.388 (\log P)^2 + 0.821 \log P + 1.117 \quad (7)$$
$$n = 10; r = 0.814; s = 0.242$$

$$\log \% \text{ abs} = -0.330 (\log P)^2 + 0.869 \log P + 0.059 (6.8 - pK_a) + 0.817 \quad (8)$$
$$n = 10; r = 0.910; s = 0.187$$

Levorphan was not used in the derivation of eq 7 and 8 so we also omitted it. Distribution data and a new pK_a were recently reported for this compound.[4] Even though eq 6 and 8 "explain" the variation in absorption rate about equally well (r^2 = 86 and 83%, respectively), the former is predictive while the latter is not when the values for levorphan are inserted (calculated, 1.19 and 0.29; found, 1.11).

Example C. Absorption of Bases from the Small Intestine. Brodie[5] studied the absorption of compounds from the rat small intestine. One can use either the "virtual" pH value of 5.3 (calculated by Brodie to account for equilibria using intestinal membrane) or the bulk pH value of 6.6 in analyzing these data.

The "bases" analyzed include a number of compounds essentially unprotonated at pH 5.3 (Table IV). We find an excellent correlation of percent absorption with log D alone (eq 9). It is, in fact, a better correlation than reported by Lien[6] using three variables (eq 10). The $(pH - pK_a)$ term corresponds to the ratio of unprotonated to protonated species. Introduction of a $(\log D)^2$ term to eq 9 gives no significant improvement, but the highest log D is under 1.4. The correlation assuming a pH of 6.6 (eq 11) is not as satisfactory as that at pH 5.3. Assuming pH 5.3

$$\log \% \text{ abs} = 0.244 \log D + 1.478 \quad (9)$$
$$n = 11; r = 0.969; s = 0.098$$

$$\log \% \text{ abs} = -0.131 (\log P)^2 + 0.362 \log P + 0.105 (5.3 - pK_a) + 1.273 \quad (10)$$
$$n = 11; r = 0.916; s = 0.182$$

Assuming pH 6.6

$$\log \% \text{ abs} = 0.328 \log D + 1.349 \quad (11)$$
$$n = 11; r = 0.905; s = 0.170$$

Example D. Effect of Aromatic Acids on Membrane Permeability. Levitan and Barker[7] measured the ability of a series of benzoic and salicylic acids to increase the membrane potential and conductance of potassium in mollusk neurons at pH 7.8. They suggested this property might be related to the mechanism of action of arylcarboxylic acids as nonnarcotic analgesics. They had to treat benzoic and salicylic acids separately in order to obtain satisfactory correlations with log P and ΔpK_a (eq 12 and 13). In addition, nitro derivatives had to be excluded from both groups. Another consideration is that the use of three variables for ten compounds increases the likelihood of chance correlations.

We have reexamined this work. Equations 14 and 15 were obtained for the separate classes *including* the nitro compounds and eq 16 for the combination of all compounds. Equations linear in log D rather than quadratic functions of log P were obtained (eq 14–16). Both log D and pK_a terms are significant at the >99.9% level. $(\text{Log } D)^2$ cannot be included in eq 16 above the 85% confidence level. The results are in agreement with the generalization that the ability of an acid to promote potassium conductance (and inhibit chloride conductance) is directly

Table IV. Absorption of Bases from the Rat Small Intestine and Physicochemical Constants Used for Eq 9-11

Base	Log P[a]	Log u/d $(5.3 - pK_a)$[a]	C_D[b]	Log D[c]	Log % abs Obsd[a]	Log % abs Calcd[d]	Log % abs Calcd[e]
Acetanilide	1.16	5.0	0	1.16	1.62	1.76	1.57
Theophylline	−0.78	4.6	0	−0.78	1.46	1.29	1.40
p-Nitroaniline	1.39	4.3	0	1.39	1.83	1.81	1.98
Antipyrine	0.23	3.9	0	0.23	1.51	1.53	1.76
m-Nitroaniline	1.37	2.8	0	1.37	1.89	1.81	1.82
Aniline	0.90	0.7	−0.08	0.82	1.73	1.68	1.57
Aminopyrine	0.76	0.3	−0.18	0.58	1.52	1.62	1.52
p-Toluidine	1.39	0.0	−0.30	1.09	1.77	1.74	1.52
Quinine	1.83	−3.1	−3.1	−1.27	1.18	1.17	1.17
Ephedrine	1.56	−4.3	−4.3	−2.74	0.85	0.81	1.07
Tolazoline	2.65	−5.0	−5.0	−2.35	0.78	0.90	0.79

[a] From ref 6. [b] Obtained from Table I. [c] Calculated from eq 2. [d] Calculated from eq 9. [e] Calculated from eq 10, ref 6.

Table V. Membrane Conductance and Physicochemical Constants Used for Eq 12-16

	Log P[a]	pK_a[a]	$pK_a - 7.8$	Log D[b]	Obsd[a,c]	Calcd[d]	Calcd[e]
			Benzoic Acids				
Unsubstituted	1.87	4.19	−3.61	−1.74	−0.70	−0.55	−0.72
3-OH	1.50	4.06	−3.74	−2.24	−1.00	−0.86	−1.00
3-Br	2.84	3.86	−3.94	−1.10	0.48	0.36	0.36
4-Br	2.86	3.98	−3.82	−0.96	0.40	0.36	0.29
2-Cl	1.98	2.92	−4.88	−2.90	−0.70	−0.29	−0.27
3-Cl	2.68	3.82	−3.98	−1.30	0.18	0.22	0.24
4-Cl	2.65	3.98	−3.82	−1.17	0.00	0.17	0.12
3,4-Cl_2	3.46	3.66	−4.14	−0.68	0.93	0.94	0.99
3-I	3.13	4.0	−3.80	−0.67	0.60	0.60	0.64
4-I	3.02	3.91	−3.89	−0.87	0.54	0.51	0.47
4-CH_3	2.27	4.36	−3.44	−2.09	−0.52	−1.04	−0.47
3-NO_2	1.83	3.47	−4.33	−2.50	−0.70	−0.49	−0.30
4-NO_2	1.89	3.41	−4.39	−2.50	−0.52	−0.43	−0.28
			Salicylic Acids				
2-OH	2.21	2.97	−4.83	−2.62	0.00	−0.09	0.06
2,4-$(OH)_2$	1.60	4.70	−3.10	−1.50	−1.00	−0.86	−0.52
2,5-$(OH)_2$	1.72	2.97	−4.83	−3.11	−0.70	−0.53	−0.65
2,6-$(OH)_2$	2.20	2.70	−5.10	−2.90	0.18	−0.06	0.00
5-Br, 2-OH	3.21	2.62	−5.18	−1.97	1.23	0.85	1.13
5-Cl, 2-OH	3.02	2.63	−5.17	−2.15	1.00	0.68	0.95
5-I, 2-OH	3.52	2.65	−5.15	−1.63	1.43	1.12	1.39
3,5-Br_2, 2-OH	4.21	2.26	−5.54	−1.33	1.60	1.79	1.74
3,5-Cl_2, 2-OH	3.88	2.30	−5.50	−1.62	1.48	1.49	1.57
3,5-I_2, 2-OH	4.78	2.33	−5.47	−0.69	2.00	2.29	1.96
5-OCH_3, 2-OH	2.41	2.86	−4.94	−2.53	0.18	0.10	0.31
5-NO_2, 2-OH	2.21	2.32	−5.48	−3.27	0.00	−0.01	−0.08
3,5-$(NO_2)_2$, 2-OH	2.21	1.55	−6.25	−4.04	0.00	0.09	−0.26

[a] From ref 7. [b] Calculated from eq 1. [c] Activity relative to salicylic acid. [d] Calculated from eq 16. [e] Calculated from eq 12 and 13, ref 7.

Table VI. Substituent Constants and Uncoupling Activity for Phenols in the pH Range 5-8

Substitution	Log P[a]	pK_a[b]	C_D[c] pH 5	C_D[c] pH 6	C_D[c] pH 7	C_D[c] pH 8	Log D[d] pH 5	Log D[d] pH 6	Log D[d] pH 7	Log D[d] pH 8
1. 4-NO_2	1.96	7.0	0	0	−0.3	−1.0	1.96	1.96	1.66	0.96
2. 2,6-$(NO_2)_2$	1.25	3.7	−1.3	−2.3	−3.3	−4.3	−0.05	−1.05	−2.05	−3.05
3. 2,6-$(NO_2)_2$, 3,4-Me_2	2.54	4.2	−0.9	−1.8	−2.8	−3.8	1.64	0.74	−0.26	−1.26
4. 2,6-$(NO_2)_2$, 4-*i*-Bu	3.32	4.3	−0.8	−1.7	−2.7	−3.7	2.52	1.62	0.62	−0.38
5. 2,6-$(NO_2)_2$, 4-*i*-Pent	3.82	4.1	−1.0	−1.9	−2.9	−3.9	2.82	1.92	0.92	−0.08
6. 2,6-$(NO_2)_2$, 4-*i*-Oct	5.32	4.1	−1.0	−1.9	−2.9	−3.9	4.32	3.42	2.42	1.42

	pH 5			pH 6			pH 7			pH 8		
	Obsd	Calcd[e]	Calcd[f]	Obsd	Calcd[e]	Calcd[f]	Obsd	Calcd[e]	Calcd[f]	Obsd	Calcd[e]	Calcd[f]
1.	3.30	3.28	3.54	3.37	3.35	3.54	3.51	3.52	3.42	3.39	3.41	3.13
2.	4.77	4.71	4.71	4.22	4.17	4.31	3.68	3.75	3.90	3.12	3.23	3.49
3.	4.92	4.99	5.10	4.51	4.64	4.74	4.36	4.34	4.33	3.95	3.95	3.92
4.	5.16	5.24	5.40	4.88	4.97	5.03	4.71	4.72	4.63	4.48	4.40	4.22
5.	5.50	5.48	5.65	5.42	5.23	5.28	5.18	5.00	4.87	4.92	4.71	4.46
6.	6.08	6.02	6.26	5.89	5.91	5.89	5.64	5.76	5.48	5.41	5.59	5.07

[a] From ref 11. [b] From ref 9. [c] From Table I. [d] Calculated from eq 1. [e] Calculated from eq 17-20. [f] Calculated from eq 21.

proportional to the concentration of the acid in the membrane and its acidity (or the stability of its potassium salt in the membrane?). Physical constants and relative activities (RA) for the series are given in Table V.

$$\log RA_{\text{benzoic acids}} = -0.056 (\log P)^2 + 1.166 \log P - 0.633 \Delta pK_a - 1.935 \quad (12)$$
$n = 10; r = 0.992; s = 0.098$

$$\Delta pK_a \equiv pK_{a(\text{acid})} - pK_{a(\text{salicylic acid})}$$

$$\log RA_{\text{salicylic acids}} = -0.236 (\log P)^2 + 2.422 \log P + 0.231 \Delta pK_a - 4.133 \quad (13)$$
$n = 10; r = 0.992; s = 0.132$

$$\log RA_{\text{benzoic acids}} = 0.899 \log D - 0.643 pK_a + 3.810 \quad (14)$$
$n = 13; r = 0.968; s = 0.174$

$$\log RA_{\text{salicylic acids}} = 0.863 \log D - 1.020 pK_a + 5.255 \quad (15)$$
$n = 13; r = 0.973; s = 0.238$

$$\log RA_{\text{combined}} = 0.894 \log D - 1.022 pK_a + 5.289 \quad (16)$$
$n = 26; r = 0.967; s = 0.226$

Example E. Uncoupling of Oxidative Phosphorylation. Hemker[8] proposed a number of years ago that the activity of phenols in uncoupling oxidative phosphorylation was related to their distribution coefficients (between medium and miticondria) as well as their acidity. He measured uncoupling activity at four pH values to support this. These results (Table VI) have been examined by several groups. Hansch et al.[9] found the correlation eq 22 at pH 5.0. Fujita[10] examined the series at other pH's and derived equations such as 23 at pH 8 which incorporate a term to correct log $1/C$ to log $1/C_{\text{neutral}}$. He concluded that it was possible that both neutral and ionic forms are active. Tollenaere[11] more recently has reexamined the data at various pH's to try to understand the mechanism of action. He found that δ, an electronic parameter[12] (which was slightly better than σ), and π correlated well with activity. Equation 24 at pH 8 is an example. Tollenaere incorporated the data at all pH's into a single four-parameter equation, eq 25. Analysis of the value of coefficients of π and σ at various pH's, and compounds which are exceptions, led Tollenaere to consider several postulates relating to uncoupling activity—the degree of ionization becomes irrelevant at higher pH; the activity is enhanced by increasing the ionized/neutral ratio until the optimal ratio of 1:1 is reached; and there may be an opposing effect of the unfavorable alteration of the mitochondrial receptor due to an increase of the pH of the test medium. We have examined these data in terms of log D and pK_a, eq 17–21. A very simple explanation of the mechanism of action can be formulated from the resulting eq 21 covering all pH values. The log D term is proportional to the concentration of phenol in the biolipid phase. The remaining electronic effect represents a property important in the rate-limiting step of its action. This could be loss of a proton. This is in line with Hemker's proposal[8] and the conclusion of Stockdale and Selwyn[13] that phenols uncouple by mediating proton conduction across the inner mitochondrial membrane. It is interesting to note the relative constancy of the pK_a coefficient in eq 17–20. This is masked in the alternative treatments. Even using the calculations of Fujita[10] in terms of the neutral fraction at various pH's, as in eq 23 for pH 8, the coefficient of ΔpK_a varies with pH.

$$\text{pH 5: } pC = 0.361 \log D - 0.651 pK_a + 7.135 \quad (17)$$
$n = 6; r = 0.998; s = 0.081$

$$\text{pH 6: } pC = 0.449 \log D - 0.657 pK_a + 7.071 \quad (18)$$
$n = 6; r = 0.992; s = 0.145$

$$\text{pH 7: } pC = 0.506 \log D - 0.639 pK_a + 7.151 \quad (19)$$
$n = 6; r = 0.992; s = 0.132$

$$\text{pH 8: } pC = 0.587 \log D - 0.657 pK_a + 7.445 \quad (20)$$
$n = 6; r = 0.988; s = 0.178$

$$\text{pH 5-8: } pC = 0.409 \log D - 0.604 pK_a + 6.970 \quad (21)$$
$n = 24; r = 0.968; s = 0.231$

$$\text{pH 5:}^{9} \quad pC = 0.400 \pi - 0.501 pK_a + 6.607 \quad (22)$$
$n = 6; r = 0.999; s = 0.039$

$$\text{pH 8:}^{10} \quad pC + \log [([H^+] + K_a)/[H^+]] = 0.548 \pi + 0.286 \Delta pK_a + 5.770 \quad (23)$$
$n = 5; r = 0.986; s = 0.179$

$$\text{pH 8:}^{11} \quad pC = 0.585 \pi + 0.429 \delta + 3.009 \quad (24)$$
$n = 6; r = 0.988; s = 0.178$

$$\text{pH 5-8:}^{11} \quad pC = 0.073 (\pi)(\text{pH}) - 0.935 (\delta)(\text{pH}) + 7.797 \delta + 0.252 (\text{pH}) + 1.081 \quad (25)$$
$n = 24; r = 0.994; s = 0.106$

Some General Aspects. There are several general aspects of the use of distribution coefficients which, though seemingly simple, may not come readily to mind.

(a) There are situations in which one has, for example, a series of neutral compounds of desirable biological activity and optimum partition coefficient, P_0, and wishes to test ionizable analogues such as a carboxyl derivative. If the carboxyl group has an estimated pK_a of 4.2, one would have to increase the log P by about 3.2 units over log P_0 to maintain the same biolipid–aqueous ratio at pH 7.4. The additional substitution would also have to take into account the π value of the $-CO_2H$ (−0.3 for benzoic acid,[14] −1.3 for acetic acid[14]). It remains to be demonstrated that log D_0 for ionized compounds will be the same as log P_0 for neutral compounds in the same model.

(b) One cannot focus on one aspect of distribution, the degree of ionization, without considering the equilibrium situation. For example, Hansch[15] states that aliphatic amine antibacterials (log P_0 of 5.7 and 6.3) would be "completely ionized under the test conditions" which "seems to indicate that little crossing of biological membranes is necessary". But assuming a pK_a of 10.6, log D corresponding to this would be 2.5 (log P_0 − pK_a + pH = 5.7 − 10.6 + 7.4), indicating that quite reasonable biolipid levels are possible.

(c) "Log P" values for ionized molecules have often been incompletely defined. It may be that authors have always meant, and actually measured, the ratio of ionized form in the organic phase to ionized form in the aqueous phase. It is possible, though, that what actually was measured was a distribution coefficient on a highly ionized compound. For purposes of illustration such a "log P" value will be compared with the corresponding calculated values for distributions.

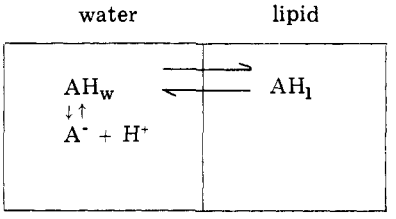
Hansch and Glave[16] studied a broad range of membrane-perturbing agents including aliphatic acids. From the log P of hexanoate and hexanoic acid they calculate a correction for ionization from "log $P_{\text{hexanoate}}$" − log $P_{\text{hexanoic acid}}$ of −4.10. The hexanoate value could refer to a log D. Since log D − log P = pK_a − pH, and pK_a for hexanoic acid is 4.88,[17] the distribution would have been determined at pH 9.0. The value for this correction at pH 7.5 (the membrane perturbation experimental conditions) is pK_a − pH = 4.88 − 7.50 = −2.62. The authors went on to use the −4.10 value to calculate the "log P" of α-bromopropionate. If "log P" is, in fact, a log D, it depends on the pK_a of the acid so neither the −4.10 nor the −2.62 value would be appropriate for α-bromopropionate.

Summary

We hope the examples of the use of distribution coefficients for the regression analysis of ionizable compounds are sufficient to encourage others to try this approach. We anticipate that further insight into mechanisms of action may be gained when electronic effects on distribution and mechanism are separated, especially when the lead of Hansch[16] and others is followed in comparing the coefficients of these terms for different classes of compounds in the same and different systems.

Appendix

Relationship of Distribution Coefficient, Partition Coefficient, pK_a, and pH. Consider the equilibration of an acid AH in a two-phase system at an aqueous phase pH such that it is partially ionized. This is illustrated below.



By the definitions given in the introduction one has eq 26 and 27 where AH and A^- represent concentrations of the respective species.

$$D = AH_l/(AH_w + A^-) \tag{26}$$

$$P = AH_l/AH_w \tag{27}$$

The fraction of AH species in the aqueous phase which is un-ionized, f_u, is given by the expression in eq 28.

$$f_u = \frac{AH_w}{AH_w + A^-} = \frac{1}{1 + 10^{pH - pK_a}} \tag{28}$$

From eq 26–28 one obtains eq 29 from which eq 30 follows.

$$D = f_u \cdot P \tag{29}$$

$$\log D = \log P + \log f_u = \log P + \log [1/(1 + 10^{pH - pK_a})] \tag{30}$$

When AH is appreciably ionized (pH − pK_a > 1), eq 30 is approximated by eq 31.

$$\log D_{\text{acids}} = \log P + pK_a - pH \tag{31}$$

For bases the following relations, eq 32–34, hold.

$$f_u = 1/(1 + 10^{pK_a - pH}) \tag{32}$$

$$\log D = \log P + \log f_u = \log P + \log [1/(1 + 10^{pK_a - pH})] \tag{33}$$

For the range pK_a − pH > 1, eq 33 is approximated by eq 34.

$$\log D_{\text{bases}} = \log P - pK_a + pH \tag{34}$$

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