where the subscript, 0, refers to the reference standard (subscript x has been omitted for convenience). Equation 2′ reduces to the following cases after applying rate-limiting assumptions.

$$K_{m(app)} = k_{-1}/k_1 \text{ when } k_3 \gg k_2 \text{ and } k_{-1} \gg k_2 \quad (4')$$

$$K_{m(app)} = k_2/k_1 \text{ when } k_3 \gg k_2 \text{ and } k_2 \gg k_{-1} \quad (5')$$

$$K_{m(app)} = k_3/k_1 \text{ when } k_2 \gg k_3 \text{ and } k_2 \gg k_{-1} \quad (6')$$

Expressing eq 4′–6′ as the logarithm of the relative $K_{m(app)}$ values results in the following equations.

$$\log [K_{m(app)}/K_{m(app)0}] = \log [k_{-1}/(k_{-1})_0] - \log [k_1/(k_1)_0] \quad (7')$$

$$\log [K_{m(app)}/K_{m(app)0}] = \log [k_2/(k_2)_0] - \log [k_1/(k_1)_0] \quad (8')$$

$$\log [K_{m(app)}/K_{m(app)0}] = \log [k_3/(k_3)_0] - \log [k_1/(k_1)_0] \quad (9')$$

An analogous treatment can be applied to the $V_{m(app)}$ values. Accordingly, the $V_{m(app)}$ values obtained graphically are written as

$$V_{m(app)} = [k_2k_3/(k_2 + k_3)]E_t \quad (10')$$

where E_t is the total enzyme concentration. Taking the logarithm of the relative $V_{m(app)}$ value yields

$$\log [V_{m(app)}/V_{m(app)0}] = \log [k_2/(k_2)_0] + \log [k_3/(k_3)_0] - \log [(k_2 + k_3)/(k_2 + k_3)_0] \quad (11')$$

After applying rate-limiting assumptions to eq 10′

$$V_{m(app)} = k_2E_t \text{ when } k_3 \gg k_2 \quad (12')$$

$$V_{m(app)} = k_3E_t \text{ when } k_2 \gg k_3 \quad (13')$$

Expressing eq 12′ and 13′ as the logarithm of the relative $V_{m(app)}$ values yields

$$\log [V_{m(app)}/V_{m(app)0}] = \log [k_2/(k_2)_0] \quad (14')$$

$$\log [V_{m(app)}/V_{m(app)0}] = \log [k_3/(k_3)_0] \quad (15')$$

Separation of the observed rate coefficients into a single relative rate term or into a sum or difference of relative rate terms provides justification for the correlation of rates with substituent parameters. For the latter case (eq 7′–9′) when both rate terms contribute significantly to the observed rate, the observed reaction constant will necessarily be a difference quantity for the two reaction steps (*i.e.*, for σ contributions to eq 7′, $\rho_{obsd} = \rho_{-1} - \rho_1$).

Notes

Structure–Activity Relationships Having a Basis in Regular Solution Theory

ARTHUR CAMMARATA,* SUH JEN YAU,

Temple University, School of Pharmacy, Laboratory of Physical Medicinal Chemistry, Philadelphia, Pennsylvania 19140

AND KENNETH S. ROGERS

Department of Biochemistry, Medical College of Virginia, Health Sciences Division of Virginia Commonwealth University, Richmond, Virginia 23219

Received June 21, 1971

Lipophilic properties of drug molecules are well known to limit the magnitude of a biological response by governing the penetrability of the molecules through tissues and by affecting "hydrophobic" interactions between drug agents and biomacromolecules. Partition coefficients have been a favorite measure of lipophilicity used in the correlation of drug effects,[1] but other measures such as polarizabilities[2] or molar attraction constants[3] have also been used. Leo, *et al.*,[4] prefer partition coefficients over other lipophilic measures on the grounds that better correlations with biological activities are obtained. In this preliminary communication we wish to point out the relationship that exists between partition coefficients and other measures of lipophilicity, notably polarizability and the molar attraction constant. Correlations of biological activity involving these indexes may thus be interpreted as reflections of the solubility properties of the compounds involved, irrespective of the context in which the correlations were originally presented.

From a consideration of the chemical potentials μ_a and μ_o for a substance in an aqueous and in an organic environment, respectively, it can readily be shown that when the reference state is taken as the pure substance the partition coefficient P is determined by the ratio of the activity coefficients γ_a and γ_o for the substance in each of the respective phases. This relationship can be given as

$$\log P = \log (S_o/S_a) = \log \gamma_a - \log \gamma_o \quad (1)$$

where S_o and S_a are the solubilities of a substance in an organic and in an aqueous phase. Equation 1 is known to relate partition coefficients with relative solubilities[5] and also forms a basis for the determination of activity coefficients from partitioning experiments.[6]

(1) (a) C. Hansch, *Farmaco Ed. Sci.*, **23**, 293 (1968); (b) C. Hansch, *Accounts Chem. Res.*, **2**, 232 (1969).

(2) (a) L. Pauling and D. Pressman, *J. Amer. Chem. Soc.*, **67**, 1003 (1945); (b) J. A. Clements and K. M. Wilson, *Proc. Nat. Acad. Sci. U. S.*, **48**, 1008 (1962); (c) D. Agin, L. Hersh, and D. Holtzman, *ibid.*, **53**, 952 (1965); (d) A. Cammarata, *J. Med. Chem.*, **10**, 525 (1967).

(3) (a) L. J. Mullins, *Chem. Rev.*, **54**, 289 (1954); (b) J. A. Ostrenga, *J. Med. Chem.*, **12**, 349 (1969).

(4) A. Leo, C. Hansch, and C. Church, *ibid.*, **12**, 766 (1969).

(5) (a) C. K. Hancock, J. N. Pawloski, and J. P. Idoux, *J. Org. Chem.*, **31**, 3801 (1966); (b) C. Hansch, J. E. Quinlan, and G. L. Lawrence, *ibid.*, **33**, 347 (1968).

(6) I. M. Klotz, "Chemical Thermodynamics," W. A. Benjamin, New York, N. Y., 1964, p 372.

To the extent that the solution of a substance in each phase can be considered regular, the activity coefficient for a not too highly polar nonelectrolytic solute, *i.e.*, one which is not highly associated with itself due to its charge characteristics, is given by Hildebrand–Scott solubility theory[7] as

$$\log \gamma_2 = \frac{\Phi_1^2 V_2}{2.303RT} (\delta_1 - \delta_2)^2 \quad (2)$$

where V_2 is the molal volume of solute in solution, Φ_1 is the volume fraction of solvent, and δ_1, δ_2 are the "internal pressures" of the solvent and solute, respectively. In dilute solution $\Phi_1 \approx 1$ and, if this condition applies to each of the phases for a distribution system, then since $\delta = F/V$ where F is the molar attraction constant equation 1 can be written[7c] (following substitution of eq 2 into eq 1, expansion and cancellation of terms, and factoring) as

$$\log P = \frac{(\delta_a - \delta_o)}{2.303RT} \times [(\delta_a + \delta_o)V_2 - 2F_2] \quad (3)$$

From eq 3 it can be said that a distribution system each of whose phases form a regular solution with a series of compounds should yield a linear relationship in a plot of log P *vs.* F when the molar volumes V_2 do not vary greatly from substance to substance or if they vary in the same direction as does F_2. Biological activities which are predominantly influenced by lipid–water partitioning should also be linearly related to F subject to the same restrictions. Equation 3 thus accounts for the relationship found by Ostrenga[3b] between the *in vitro* bacteriostatic activities of penicillins and the molar attraction constants for the side chain.

According to Hildebrand and Scott[8] the molar attraction constant F corresponds to the constant a in the van der Waals equation of state, or something very similar, and is related to this constant by the equation

$$F_2^2 = a_2 = -2\pi N^2 \int_d^\infty \epsilon \rho(r) r^2 dr \quad (4)$$

where ϵ is the bimolecular interaction energy, $\rho(r)$ is a distribution function giving the probability of having a center-to-center distance r between the molecules and N is Avagadro's number. The integration is carried out over all values of r beginning from the most favorable intermolecular separation d. Substitution of eq 4 into eq 3 provides a basis for the correlation reported by Wulf and Featherstone[9] between the narcotic potencies of gaseous anesthetics and their van der Waals a constants.

For simplicity the interaction energy between two not too highly polar molecules may be given by the Lennard-Jones "6–12" potential

$$\epsilon = -\frac{k}{r^6} + \frac{j}{r^{12}} \quad (5)$$

Under most conditions the repulsion part may be considered negligible in comparison with the attraction part in eq 5. With this understanding, and assuming that $\rho(r)$ can be taken as a constant, say unity, in first approximation, the substitution of eq 5 into eq 4 followed by integration leads to the relationship

$$F_2^2 = \frac{2\pi N^2}{3d^3} \times k \quad (6)$$

where k in eq 6 differs depending on the formalism used to express the intermolecular attraction energy. With only slightly polar molecules involved in an interaction the dispersion energy[10] will make the largest stabilizing contribution. The most widely used estimate of dispersion energy, that due to London,[10a] when substituted into eq 6 [*i.e.*, $k = {}^3/_4(\alpha^2 I)$] leads to the relationships

$$F_2^2 = \frac{\pi N^2}{2d^3} \alpha^2 I = \frac{9}{32\pi d^3} \times P_E^2 I \quad (7)$$

in which I is the ionization potential and α, P_E are the molecular and molar polarizabilities, respectively, for a substance [$P_E = {}^4/_3(\pi N\alpha)$]. To a good approximation the ionization potential can be considered essentially constant for a variety of molecules,[2a,c] hence the appropriate substitution of eq 7 into eq 3 can account for the distribution processes which are related to polarizability measures. This can be taken as a basis for the correlation often observed between anesthetic potencies and polarizability[2b,c,3a]

The finding[4] that experimental partition coefficients often provide better correlations with biological activities than do polarizabilities or molar attraction constant indicates the "solution" of drugs in biological phases is frequently not regular, *i.e.*, the solution process has identified with it a positive heat of mixing, a nonideal entropy of mixing, and/or an appreciable difference in the molal volumes of the associated substances. For those cases where regular solution theory does hold, however, the relations which have been developed can provide considerable insight into dissolution processes involving biological materials. Additional verification of the relations developed and explicit applications to biological systems will be reported in detail at a later date.

(7) (a) J. H. Hildebrand and R. L. Scott, "The Solubility of Nonelectrolytes," Dover Publications, New York, N. Y., 1964; (b) A. N. Martin, J. Swarbrick, and A. Cammarata, "Physical Pharmacy," 2nd ed., Lea and Febiger, Philadelphia, Pa., 1969, pp 299–303. (c) Equation 3 applies only if the molal volumes of solute in each phase are equal, *i.e.*, $V_a = V_o$. In the event that the molal volumes are not equal eq 3 may be written as log $P = 1/(2.303RT)\{(V_a\delta_a^2 - V_o\delta_o^2) - 2[V_a\delta_a - V_o\delta_o)/V]F + [(V_a - V_o)/V^2]F^2\}$ where V is the *molar* volume of pure solute. Parabolic fits of log P (or quantities related to log P) to F are thus predicted.

(8) J. H. Hildebrand and R. L. Scott, ref 7a, pp 94–96, 124–129.

(9) R. J. Wulf and R. M. Featherstone, *Anesthesiology*, **18**, 97 (1957).

(10) (a) F. London, *Z. Physik.*, **63**, 245 (1930); (b) J. G. Kirkwood, *Phys. Z.*, **33**, 57 (1932); (c) J. H. Van Vleck, "Electric and Magnetic Susceptibilities," Oxford University Press, New York, N. Y., 1932, p 91.

Further Evaluation of *N,N'*-Polymethylene-Bridged 2-Aminoethanethiol Derivatives and Related Compounds as Radioprotective Agents[1]

JAMES R. PIPER,* CARL R. STRINGFELLOW, JR., AND THOMAS P. JOHNSTON

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205

Received June 23, 1971

Good radioprotective activity observed with two members (A, $n = 3, 4$) of a limited series of *N,N'*-poly-

(1) This investigation was supported by the U. S. Army Medical Research and Development Command under Contract No. DA-49-193-MD-2028.