Biochemical Pharmacology, Vol. 34, No. 14, pp. 2413–2416, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

COMMENTARY

USES OF MOLECULAR VOLUME IN BIOCHEMICAL PHARMACOLOGY

ALAN MELLORS*† and JOHN C. MCGOWAN‡

*Guelph-Waterloo Centre for Graduate Work in Chemistry, Department of Chemistry and Biochemistry, University of Guelph, Guelph, Ontario N1G 2W1, Canada; and ‡"Quantock", 13 Moreton Ave, Harpenden, Herts, AL5 2EU, U.K.

One of the basic concepts taught in undergraduate or high school chemistry is the use of atomic masses (atomic weights) to determine the molecular mass (molecular weight) of a compound. The analogous operation, the summation of atomic volumes to obtain the molecular volume of the compound, is rarely encountered. Under what circumstances can useful information be obtained from comparisons of molecular volumes? The major applications of molecular volumes are where the size of the molecule is more important than its chemical properties. Such applications include solubility and partition behavior, where the size of the solute determines the work that must be done to create a cavity within the solvent. Special applications of solubility and partition behavior in biochemistry include hydrophobicity, general anesthesia and narcosis, physical toxicity and some drug–receptor and enzyme–substrate interactions. In this review, we show one method for the estimation of molecular volumes, and some applications to chemical–biological interactions.

It has been known for over a century that partition coefficients for general anesthetics correlate well with anesthetic potency, whether the partition is between water and olive oil [1], or 1-octanol [2], or some other organic phase. This observation is central to the hypothesis that anesthesia is achieved when a drug reaches a certain concentration (C_B) in some biophase [3–5]. As the partition coefficient for biophase/water increases with molecular size of compounds, anesthetic potency increases up to a maximum, and then decreases sharply, the "anesthetic cut-off". Above this maximum size, the solubility of the drug in the biophase is insufficient for the drug to reach the necessary anesthetic concentration§. Thus, the partition coefficient for biophase/water and solubility in the biophase for the drug are the two major determinants of general anesthetic potency. A number of other biological activities resemble anesthesia and narcosis in that they are determined primarily by the partitioning of drug between biophase and water, and its solubility in the biophase. Among these effects are the toxicities of many organic vapors and liquids for many organisms [3, 6–8, §], inhibition of electron transport in mitochondria [9], the non-specific inhibition of mitosis in plant cells [10], the inhibition of photosynthesis in green algae [11] and the inhibition of enzymes *in vitro* and *in vivo* [12–15]. These effects of chemicals on biological systems can be grouped under the general heading of "physical toxicity" [16].

Because physical toxicity is determined by the partition of compounds into a hydrophobic biophase, and because partition of neutral molecules between biophase/water is primarily a function of the volume of the molecule, it is often useful to correlate physical toxicity with molecular volume. The first molecular volume parameter used in these correlations was the *parachor* [17]. The parachor was used as a tool for structural analysis [18] but it does not have the dimensions of a molecular volume and for this reason the characteristic volume (V_x), which is the molecular volume of the compound at the absolute zero [19, 20], is more satisfactory. The V_x can be calculated from densities and isothermal compressibilities, or obtained by division of the parachor (in c.g.s. units) by the factor 2.835×10^6 to give V_x in S.I. units ($m^3\,mole^{-1}$) [21]. The parachor and the characteristic volume (V_x) can be estimated for a molecule by addition of the individual atomic parachors [22–25] or atomic characteristic volumes [20]. It was recognised that the partition of compounds between an organic phase and water is dependent on the molecular volume (V_x) of the compound, and that

$$\log_{10}\left(\frac{\text{concn in organic phase}}{\text{concn in water}}\right) = kV_x,$$

where k is a constant which for many organic liquids appears to be about 36,000. For groups which show interactions with one or both phases it is necessary to correct the above expression to give

$$\log_{10}\left(\frac{\text{concn in organic phase}}{\text{concn in water}}\right) = kV_x - E_B$$

where E_B is the interaction term [26–29]. Figure 1 shows the use of this relationship to predict the partition coefficients (X) of hydrocortisone and prednisolone derivatives between diethyl ether and water [20]. The straight line is the predicted partition coefficient and the points show the measured values. For further details of the calculation of partition coefficients from V_x and E_B, see Refs. 20 and 26.

† Author to whom all correspondence should be addressed.
§ J. Ferguson, in "Mécanisme de la Narcose", Symposium No. 26 *Centre National de la Recherche Scientifique, Paris* pp. 25–39 (1951).

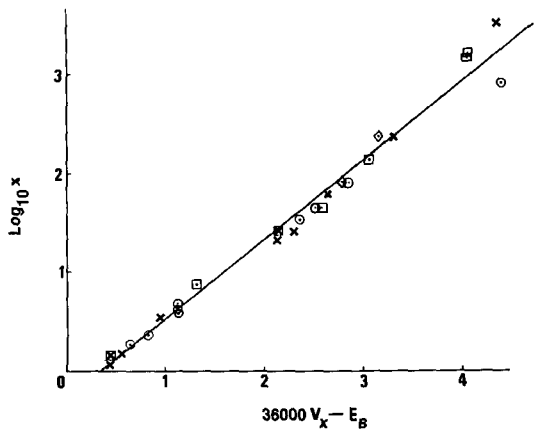

Fig. 1. Relationship between characteristic molecular volumes (V_x), interaction terms (E_B), and the measured partition coefficients (X) between diethyl ether and water for 29 hydrocortisone and prednisolone derivatives. The figure is modified from Ref. 20, and the partition coefficients are the values measured by Flynn [30] at 23°. The interaction terms used are $E_B = +1.9$ for each group containing oxygen, and $E_B = -0.15$ for halogen atoms. Individual steroids are identified in Refs. 20 and 26. The classes of steroids are: (⊙) compounds containing one fluorine atom per molecule, (⊡) compounds containing two fluorine atoms per molecule, (◇) compounds containing three fluorine atoms or two fluorine atoms and one chlorine atom per molecule, (⊠) compounds with > C = 0 in place of > CHOH at C_{11}, and (×) compounds with an 11-hydroxyl group and no halogen atoms.

The characteristic volumes (V_x) used in Fig. 1 were calculated by the addition of the atomic values for all the atoms in the molecule and the subtraction of 6.56×10^{-6} m^3 mol^{-1} for every bond regardless of whether it is a single, double or triple bond [20, 29]. Atomic factors for elements of biochemical importance, in m^3 mole^{-1}, are hydrogen (8.71×10^{-6}); carbon (1.635×10^{-5}); nitrogen (1.439×10^{-5}); oxygen (1.243×10^{-5}); fluorine (1.048×10^{-5}) phosphorus (2.487×10^{-5}); sulphur (2.291×10^{-5}); chlorine (2.095×10^{-5}); bromine (2.621×10^{-5}); and iodine (3.453×10^{-5}). As an example, the characteristic molecular volume for ethanol (C_2H_6O, with 9 bonds) in m^3 mole^{-1} is the sum of ($2 \times 1.635 \times 10^{-5}$) + ($6 \times 8.71 \times 10^{-6}$) + ($1.243 \times 10^{-5}$) − ($8 \times 6.56 \times 10^{-6}$), which is equal to 4.492×10^{-5} m^3 mole^{-1}.

The interaction term E_B deserves more attention since it can be used to estimate the number of hydroxyl groups from water which are associated with a solute in aqueous solution. A relationship can be derived, based on characteristic molar volume V_x and interaction terms E_B, which relates these to the solubility of amino acids in mixtures of ethanol and water [31]. From a plot of log (solubility) + V_x(0.65 [H_2O] + 0.18 [C_2H_5OH] + 2.7 [amino acid]) versus the $\log_{10}$ (total hydroxyl content of solvent), straight lines were obtained for amino acids with non-polar side-chains. In all cases the slope of the straight line was 12, and this value suggests that each of the amino acid molecules interacts with 12 hydroxyl groups in the solvent.

The ratio of the solubility of a molecule in ethanol to its solubility in water has been used to define the "hydrophobicity" of the molecule [32]. The ratios for these solubilities can be estimated using the V_x values for the non-polar side-chains of amino acids [32]. The "hydrophobicities" estimated in this manner are close to the values measured from the solubilities of the amino acids in ethanol and in water (Table 1). This treatment has not yet been extended to amino acids with polar side-chains which will strongly interact with hydroxyl groups of water or ethanol.

The equation, $\log_{10}X = kV_x - E_B$, used above to predict partition coefficients (X) from V_x and E_B, can also be used to predict biological activity where that depends on a compound achieving a certain concentration (C_B) in the non-aqueous biophase [17, 33, 34]. Physical toxicity, anesthesia and narcosis, and membrane enzyme inhibition are all examples of such biological activity. It has been shown that, for many biological organisms or cell suspensions, the above equation can be modified and rearranged to give $C_t = A + B \times 10^{-(kV_x - E_B)}$ where C_t is the toxic concentration of the compound required in the aqueous phase to produce a given effect, k, V_x and E_B are as defined earlier, and A and B are constants for the particular system. When this relationship holds, a plot of $-\log C_t$ against $kV_x - E_B$ for all compounds will give a curve like that of Fig. 2 consisting of two regions. For lower V_x compounds, a line of slope 1 is obtained, corresponding to increasing partition coefficients as V_x increases. The intercept of this line on the y axis gives the constant B. The second region is a horizontal line at higher V_x values, for which the value $-\log C_t$ equals $-\log A$. Curves of this type have been obtained for the inhibition by anesthetics of hypotonic hemolysis in erythrocytes [19], the inhibition of membrane-bound mouse brain synaptosomal acyl CoA:lysophosphatidylcholine acyltransferase [19], the mouse spleen cell cytotoxicity of alcohols and halogenated hydrocarbons and the inhibition of mouse spleen lymphocyte 5′-nucleotidase by halo-

Table 1. Characteristic molar volumes and hydrophobicities of amino acids

Amino acid	Formula (bonds)	V_x (m^3 mole^{-1})	$1.88 \times 10^8(V_x - 5.65 \times 10^{-5})$	Hydrophobicity (J mole^{-1})
Glycine	$C_2H_5NO_2$(9)	5.65×10^{-5}	0	0
Alanine	$C_3H_7NO_2$(12)	7.06×10^{-5}	2.65×10^3	2.09×10^3
Valine	$C_5H_{11}NO_2$(18)	9.87×10^{-5}	7.93×10^3	6.28×10^3
Leucine	$C_6H_{13}NO_2$(21)	1.13×10^{-4}	1.06×10^4	7.53×10^3
nor-Leucine	$C_6H_{13}NO_2$(21)	1.13×10^{-4}	1.06×10^4	1.09×10^4
Phenylalanine	$C_9H_{11}NO_2$(23)	1.31×10^{-4}	1.40×10^4	1.05×10^4

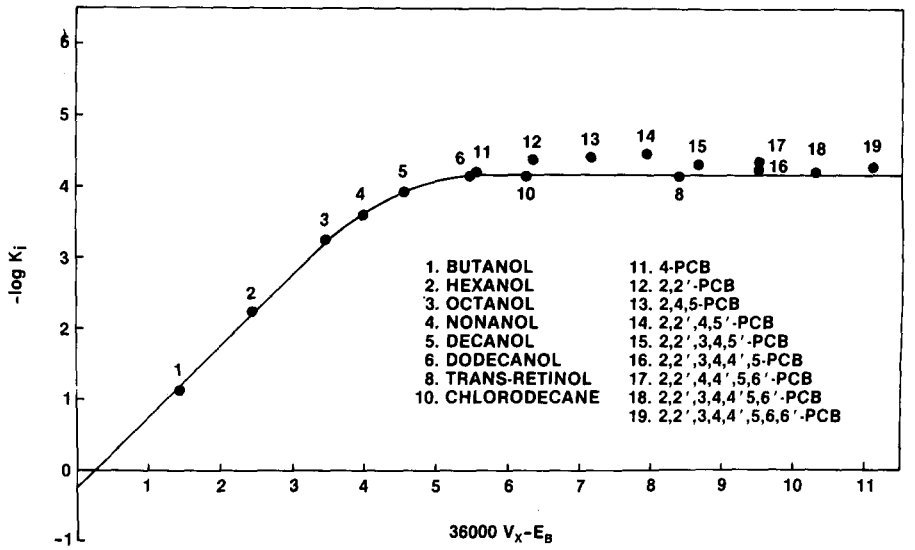

Fig. 2. Characteristic molecular volumes (V_x), interaction terms (E_B) and the inhibition of membrane bound mouse lymphocyte 5'-nucleotidase for some lipophilic compounds including polychlorinated biphenyls (PCBs). The interaction terms used were +1.2 for hydroxyl groups and − 0.35 for chlorines. The K_i is the concentration of the compound needed to inhibit the enzyme by 50%. The figure is adapted from that in Ref. 15.

genated hydrocarbons and alcohols [15]. Figure 2 shows the curve for the latter example. All compounds tested fit on this curve and therefore show no specific inhibition of the membrane enzyme, only a non-specific inhibition. A chemically-specific inhibitor would produce a point lying above the physical toxicity curve.

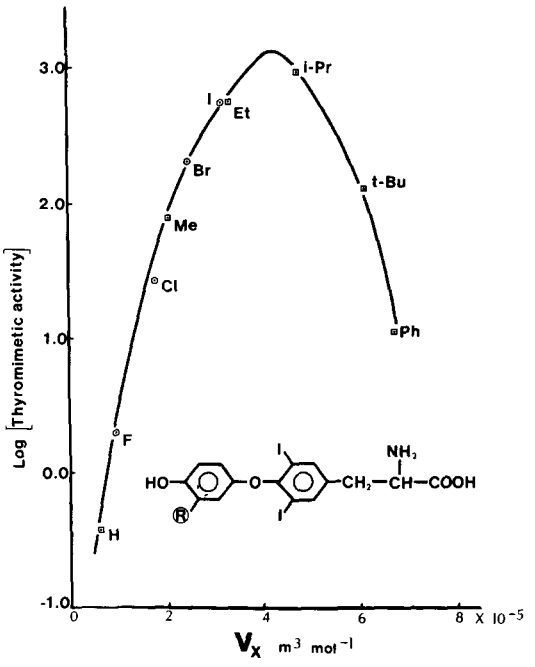

Fig. 3. Thyromimetic activity of the 3'-substituted thyroxine derivatives [35, 36] as a function of the characteristic molecular volume (V_x) of the 3'-substituent atom or group (R). The R groups are Me: methyl; Et: ethyl; i-Pr: isopropyl; t-Bu: *t*-butyl; and Ph: phenyl. The figure is modified from Ref. 37.

Examination of the constants A and B for the system will yield some useful information. It can be shown that constant B, given by the intercept on the y axis ($-\log B$), is the concentration in the lipid biophase necessary for the biological effect, for any compound on the line [19]. From Fig. 2 then we can see that a 1–2 M concentration of the inhibitors is required in the lipid biophase of the mouse spleen cells to give 50% inhibition of 5'-nucleotidase. This high concentration in the lipid biophase will be given when many compounds are present at 10^{-4} M in the aqueous phase. It can also be shown that the value of A/B is equal to the ratio of non-aqueous phase volume to aqueous phase volume. This is in accord with the familiar observation that organisms or systems containing much lipid require higher doses of lipophilic drugs than systems with less lipid. From the value of A/B we can estimate the volume of lipid biophase in a system, and these values are in accord with other estimates [15].

There have been few studies on the significance of the molecular volume in ligand–receptor binding. Such binding is usually of high affinity and of high stereochemical specificity. For some hormone–receptor binding, however, the molecular volume of the hormone appears to be critical. For example, the molecular volumes of 3'-substituted thyroxine analogs correlate much better with the thyromimetic potencies of the compounds than do the partition coefficients [37]. Figure 3 show that 3'-isopropyl thyroxine appears to be optimal in molecular volume, followed by the 3'-iodo- and 3'-ethyl derivatives. It is likely that the influence of molecular volume in this example is quite different from that in the previous examples. Here the optimum size for the hormone may be due to a requirement for filling a cavity on the receptor surface.

In the past a number of parameters have been used to predict partition coefficients, solubilities and

hydrophobicities of biologically active compounds. For example, surface area has been used [38–41] and for a homologous series this will be simply related to the molecular volume. However, the surface area is less easy to define and calculate, whereas the characteristic molecular volume can be readily estimated from a summation of the volumes of individual atoms, and a single value for all bonds. In a study of the correlation between 1-octanol/water partition coefficients and molecular volumes or surface areas, it was found that molecular volumes correlated better than any of the estimates of surface area [42]. There is no limit to the size of compounds for which molecular volumes can be estimated and the estimation is rapid and precise even for complex biological molecules. Further use of molecular volumes should be encouraged in the study of chemical–biological interactions.

Acknowledgements—The authors thank Dr. P. Ahmad for his collaboration and N.S.E.R.C. and M.R.C. Canada for financial support.

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