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Group Contribution Data Obtained by Ion-Pair Extraction of Prostaglandin B₂ with Aliphatic Amines

To the Editor:

The concept of ion-pairs (1) is significant in various areas including chemistry, organic synthesis (2), solvolytic reactions (3), partition chromatography (4–6), pharmaceutical analysis (7–10), and facilitated absorption of quaternary ammonium compounds in the GI lumen (11). Also, systematic studies on the ion-pair extraction successfully yielded functional group contributions to the thermodynamic parameters involved in transferring a given molecule from one phase to another (12, 13). Although our original study (14) was initiated to explore the practical utility of ion-pair formation of prostaglandins in all of these aspects, the present communication is specifically concerned with the free energy of transferring a methylene group from water to chloroform as determined by the ion-pair extraction process.

The importance of the lipophilicity of a drug molecule in the structure–activity relationship has been recognized and, consequently, great effort has been directed toward developing some means by which the partition coefficient of a drug molecule between oil (or lipophilic biological membrane) and aqueous systems can be predicted accurately (13, 15–17). Presently, the most popular way to achieve this goal appears to be the group contribution approach, in which the partition coefficient of a molecule is considered as the sum of the contribution of each functional group present in the molecule. Experimentally, an accurate measurement of the partition coefficient becomes very difficult as the value becomes either too large or too small.

Since the apparent partition coefficient of acidic or basic compounds depends not only upon the pH of the aqueous layer but also upon the type and the concentration of the counterions, by adjusting these factors it is possible to control the partition coefficient so that it is experimentally measurable. For a given charged solute, if the structural changes in a series of counterions are far removed from the electrostatically interacting charged portions of the molecules, it can be assumed that the change in the partition coefficient observed is independent of the electrostatic interaction and that the change solely reflects the structural modification of the counterions.

We determined the ion-pair extraction coefficient (K) of prostaglandin B₂ (I) with 10 simple aliphatic amines ranging from n-propyl- to n-dodecylamine at 25°:

$$K = \frac{\{A^-BH^+\}}{[A^-]\{BH^+\}} \tag{Eq. 1}$$

The partition system consisted of chloroform presaturated with water and tromethamine buffer (pH 8 and ionic strength 0.1 M) also presaturated with chloroform. Throughout the paper, prostaglandin B₂, amines, and ion-pair are represented as AH, B, and A^-BH^+, respectively. The braces { } and brackets [ ] indicate the molar concentrations of a given species in chloroform and aqueous buffer, respectively. Since, in most cases, the system is very dilute with respect to prostaglandin B₂ and amines, the molar concentrations are assumed to be equal to thermodynamic activities.

Simple algebraic manipulation of Eq. 1 yields:

$$P_{obs} = K[BH^+] + P_{app} \tag{Eq. 2}$$

where:

$$P_{obs} = \frac{\{A^-BH^+\} + \{AH\}}{[A^-] + [AH]} \tag{Eq. 3}$$

and:

$$P_{app} = \frac{\{AH\}}{[AH] + [A^-]} \tag{Eq. 4}$$

In deriving Eq. 2, it was assumed that ion-pairs do not exist in aqueous solutions, not only because the dielectric constant of water is extremely high but also because the concentrations of prostaglandin B₂ and amines in aqueous solutions are low. The dissociation constant of ion-pairs in an organic solvent of low dielectric constant is usually in the order of from 10^{-4} to 10^{-7} M (12, 18). Therefore, in our study, it was assumed that no significant dissociation takes place in chloroform. Moreover, at pH 8 the concentrations of neutral species of our acid and bases (*i.e.*, AH and B) are in the order of 0.1% of the total concentrations, because the pKa values of prostaglandin B₂ and
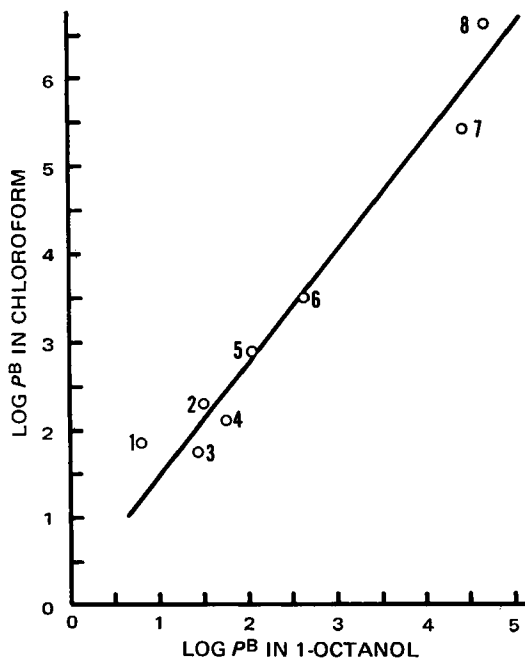

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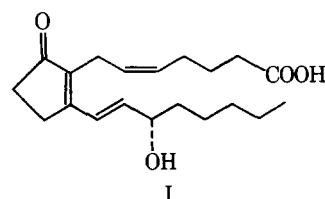
Figure 1—*Relationship between the intrinsic partition coefficient* (P^B) *of aliphatic amines between chloroform and water and that of the same amines between 1-octanol and water at 25°. The straight line is* $\log P^B_{CHCl_3} = 1.276 \log P^B_{oct} + 0.171$. *Amines are: 1, n-butyl; 2, n-hexyl; 3, triethyl; 4, dipropyl; 5, n-heptyl; 6, dibutyl; 7, tributyl; and 8, dihexyl.*

amines employed are approximately 5 (14) and 11, respectively. Equation 2 predicts a linear relationship between P_{obs} and $[BH^+]$.

The values of P_{obs} were determined by spectrophotometrically measuring the total concentrations of prostaglandin B_2 ($\epsilon_{280} \sim 2.50 \times 10^4$ in water) both in chloroform and aqueous layers at distribution equilibrium for at least five different amine concentrations. To reduce the experimental errors in determining P_{obs}, from 1:1 to 1:20 phase volume ratios were employed. We found it very difficult to measure $[BH^+]$ accurately at equilibrium, because in most cases $[BH^+]$ was extremely low. Therefore, we calculated $[BH^+]$ from the independently determined apparent partition coefficients of amines at pH 8, the initial concentrations of amines, $[A^-]$ measured at equilibrium in the presence of amines, P_{obs}, and the known phase volume ratio. The entire calculation procedure was described elsewhere (14).

The apparent partition coefficients of amines at pH 8 were obtained by dividing {B} at equilibrium by the amine concentrations in the aqueous layer. The former was determined by nonaqueous titration (19), and the latter was obtained by subtracting {B} from the known initial concentration of the amine in chloroform. Experimental data, when plotted following Eq. 2, invariably showed negative deviations from the linearity as $[BH^+]$ increased, presumably due to the premicellar aggregation of the amines in the aqueous layers. Therefore, the initial slopes were measured as the values of K.

Determination of the apparent partition coefficient of amines at pH 8 not only allowed us to calculate $[BH^+]$ in Eq. 2 at distribution equilibrium but
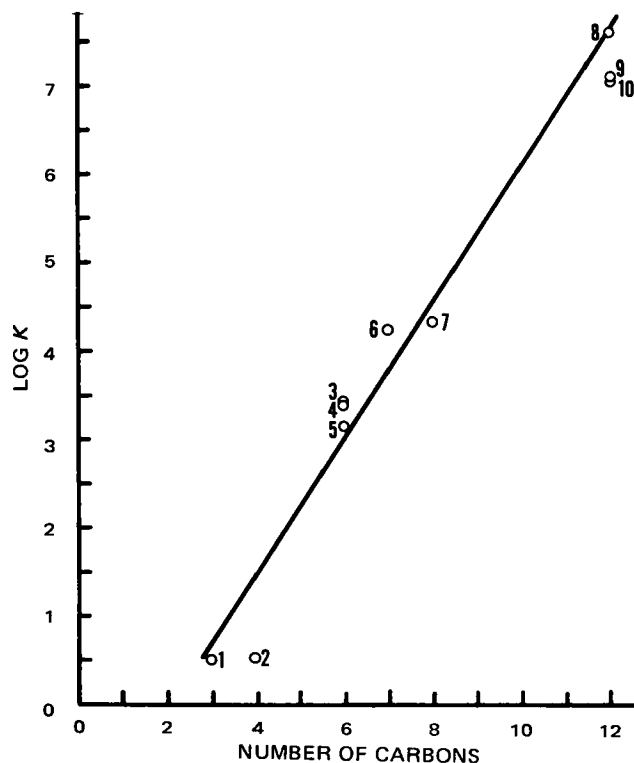

Figure 2—*Logarithm of the ion-pair extraction coefficient* (K) *for prostaglandin* B_2*–amine pairs at 25° as a function of carbon numbers in the amines. The slope of the straight line is 0.77. Amines are: 1, n-propyl; 2, n-butyl; 3, triethyl; 4, dipropyl; 5, n-hexyl; 6, n-heptyl; 7, dibutyl; 8, n-dodecyl; 9, dihexyl; and 10, tributyl.*

also resulted in an independent measurement of methylene group contribution. The intrinsic partition coefficients of the amines were calculated from the apparent partition coefficients determined at pH 8 and the known pKa values of amines (20). The pKa values at ionic strength 0.1 M were obtained from the so-called thermodynamic pKa values (pKa°) by correcting the activity coefficient of ionic species using the Davies equation (21); pKa at ionic strength 0.1 M = pKa° + 0.224. The pKa° values of dihexyl- and n-dodecylamine, which were not available in the literature, were assumed to be the same as those of dibutyl- and n-heptylamine, respectively.

The intrinsic partition coefficients of amines thus obtained were linearly correlated with those for the 1-octanol–water system (15), as shown in Fig. 1. The slope, intercept, and correlation coefficient are 1.27, 0.20, and 0.978, respectively, in excellent agreement with the relationship reported in the literature (22). Since the methylene group contribution to the partition coefficient for a 1-octanol–water system is 0.50 in terms of a logarithmic scale (*i.e.*, $\pi_{CH_2} = 0.50$) (15), the linear relationship shown in Fig. 1 strongly implies that the methylene group contribution to the partition coefficient for a chloroform–water system is $0.5 \times 1.27 = 0.64$. This value is again in good agreement with the value reported in the literature (13, 23, 24).

When the logarithms of the intrinsic partition coefficients of amines we obtained were plotted

against the carbon number, a slope of 0.64 was obtained for a series of primary amines. As expected, secondary and tertiary amines showed somewhat smaller partition coefficients than the primary amines of the same carbon number.

On the other hand, when the logarithms of K were plotted against the carbon number of the amines (Fig. 2), a slope of 0.77 was obtained. The discrepancy between the methylene group contribution obtained by the ion-pair extraction studies (0.77) and that obtained by the measurement of the intrinsic partition coefficient (0.64) was small but significant and deserves further comment. The ion-pair extraction coefficient defined by Eq. 1 is an overall equilibrium constant for the formation as well as the partition of ion-pairs:

$$K_1 = \frac{[A^-BH^+]}{[A^-][BH^+]} \qquad \text{(Eq. 5)}$$

$$K_2 = \frac{\{A^-BH^+\}}{[A^-BH^+]} \qquad \text{(Eq. 6)}$$

$$K = K_1 K_2 \quad \text{or} \quad \log K = \log K_1 + \log K_2 \qquad \text{(Eq. 7)}$$

According to Eq. 5, ion-pairs are formed in the aqueous phase. In reality, this formation most likely would occur at the interface. No matter where the ion-pairs are formed, the separation of K into K_1 and K_2 shown in Eq. 7 should be valid. As shown in Eq. 7, for the slope shown in Fig. 2 truly to represent K_2 and ultimately the methylene group contribution, K_1 must be constant regardless of the amine employed. The fact that we obtained 0.77 for the methylene group contribution from ion-pair extraction studies and 0.64 from ordinary partition experiments strongly suggests that, at least in certain cases, the group contribution data obtained from ion-pair studies may not be generally the same as obtained in straightforward partition experiments.

In the present study, as the amine becomes more hydrophobic, the equilibrium constant for the ion-pair formation (K_1) appears to increase at a rate of $\Delta \log K_1/CH_2 = 0.13$. It is to be seen in the future whether such a deviation, intrinsically derived from the assumption that the change in the ion-pair extraction coefficient (ΔK) is independent of the electrostatic interaction, is universal or not.

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