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## Simultaneous Determination of Acid Dissociation Constants and True Partition Coefficients by Analyses of the Apparent Partition Coefficients. I

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Since the partition coefficient  $P$  and the dissociation constant  $pK_a$  are important in studies of structure-activity relationships, the pH dependence of the apparent partition coefficient  $P_a$  was quantitatively studied for the cases of acids and bases. Linear relations for mono-acids and mono-bases, but parabolic relations for the dissociation of two functional groups in the pH region studied were derived between  $P_a$  and proton concentration. The equations, however, are different for acids and bases. These equations make it possible to determine  $pK_a$  and the  $\log P$  simultaneously. Some experimental systems have been measured and compared with the results obtained by other techniques; the agreement was good. A consideration of the dissociation in the organic phase showed that the correction factor due to ionic dissociation in the organic phase for an octanol-buffer system is negligible in the pH region near the  $pK_a$  value, but is not negligible in the general equation connecting the  $P_a$  and  $P$  values.

**Keywords**—dissociation constant; partition coefficient; structure-activity relationship; octanol; dissociation in organic phase; UV spectra; correlation coefficient in least-squares method; theory of pH dependence of apparent partition coefficient

In the study of quantitative structure-activity relationships (QSAR), it is well known that the partition coefficient ( $P$ ) is an important parameter for describing the transport process and the hydrophobic bonding of various drugs in biological systems.<sup>2)</sup> The  $P$  values obtained using the octanol-water system were recommended by the Hansch group for QSAR studies.<sup>3)</sup> Various methods for the measurement of  $P$  values have been reported.<sup>4)</sup> However, it should be noted that if the drug in question is an acid or a base, it will be in equilibrium with the ions in the water and also in the organic phase. The apparent partition coefficient ( $P_a$ ) is therefore pH-dependent.<sup>5-7)</sup> In order to obtain the true partition coefficient,  $P$ , which is much more important than the  $P_a$  values from the viewpoints of physicochemical and QSAR studies, the  $pK_a$  values of acids or bases should be determined.<sup>8)</sup> In this report we present a new method for obtaining the  $pK_a$  and the  $P$  values simultaneously by analyzing the pH dependence of the  $P_a$  values.

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### Experimental

**Solvents and Buffer Solutions**—Pure octanol purchased from E. Merck Co. was carefully rectified (bp 193.5°). This octanol shows no UV absorption in the near UV region due to impurities. Heptane used was spectrograde in purity, obtained from Wako Junyaku Co. The following buffer systems were employed: HCl-KCl (1.9—2.55), CH<sub>3</sub>COONa-HCl-NaCl (2.65—5.60), CH<sub>3</sub>COONa-CH<sub>3</sub>COOH-NaCl (4.47—5.50), Na<sub>2</sub>HPO<sub>4</sub>-KH<sub>2</sub>PO<sub>4</sub>-NaCl (4.99—6.17). The values in parentheses are the pH regions of application. The ionic strength of buffer solutions was adjusted to 0.5 by adding NaCl, except for the HCl-KCl system, which was adjusted with KCl.

**Samples**—Guaranteed reagent grade samples of phenoxyacetic acid (POAA), *p*-methylbenzoic acid (PMBA), *p*-methyl aniline (PMA), and *p*-methoxy aniline (PMOA) obtained commercially were purified as follows; PMA (mp 44—45°) was used without purification. Repeated sublimation was used to purify POAA (mp 99—100°) and PMOA (mp 59—60°). PMBA was recrystallized from aqueous alcohol (mp 186°). A pure sample of 5-methyl isoxazole-3-carboxylic acid (MICA; mp 178°) was kindly supplied by Mr. Honma of our laboratory.

**Measurement of Apparent Partition Coefficient  $P_a$** —The measurement of  $P_a$  was carried out by the method recommended by Hansch and Purcell *et al.*<sup>4)</sup> All the experiments were carried out in an air-conditioned room at ~23°.

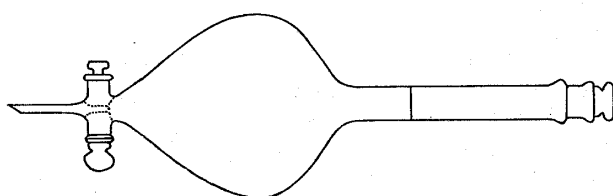


Fig. 1. The Flask used for Measuring  $P_a$  by the Shaken Flask Method

This has a constant volume and is especially convenient for samples with  $P_a < 1$ . The water phase is easily separated, using a good stopcock.

Before partitioning the organic solvent and buffer solution were shaken well in order to saturate both solvents with each other, then centrifuged after standing. An accurately weighed sample was dissolved completely in the phase in which it was most-soluble. A calculated amount of another solvents was added, the two phases being mixed well by inverting the bottles by hand for about two minutes. However, when the volume ratio of water and organic solvents was large, the mixing time was increased to about 5 to 10 minutes. After standing, the phases were centrifuged for at least 15 min at 4000 rpm and at ~23° with a

temperature-regulated centrifuge. For these experimental procedures the container shown in Fig. 1 is very convenient, especially in the case of samples with  $P_a < 1$ .

The concentration was determined by the UV method using the solvent phase in which the sample has been dissolved. The concentration in the other phase was obtained by subtracting the above concentration from that of the standard, the spectrum of which was recorded as soon as possible after dissolving the sample in the same solvent. The sample concentrations were of the order of  $10^{-4}$  to  $10^{-5}$  mol dm<sup>-3</sup>. The  $P_a$  value was calculated as follows:  $P_a = \frac{[C_{org}/V_{org}]/[C_w/V_w]}{[D_{org}/V_{org}]/\{(D_{org}^0 - D_{org})/V_w\}} = \frac{[(D_w^0 - D_w)/V_{org}]/[D_w/V_w]}{[D_{org}/V_{org}]/\{(D_{org}^0 - D_{org})/V_w\}}$ .<sup>9)</sup> Here  $C_x$  and  $V_x$  mean the concentration and the volume in the X-phase (organic or water phase), respectively, and  $D_x^0$  and  $D_x$  represent the optical density in the X-phase before and after partitioning, respectively. The cell length used throughout was 1 cm with this equation. The sample concentration and the volume ratio of  $V_{org}$  and  $V_w$  were chosen so that the difference between  $D_x$  and  $D_x^0$  was sufficient for accurate measurement. It should be noted that for the case of  $P_a \approx 1$  the  $P_a$  values obtained separately from the organic phase and the water phase agreed well. However, the larger the volume ratio of water and organic phases, the smaller the sample concentration in the larger volume phase after partitioning (*vide supra* for sampling). This leads to an additional error in determining the concentration in the larger volume phase, since the first (standard) sample concentration is of the order of  $10^{-4}$ — $10^{-5}$  mol dm<sup>-3</sup>.<sup>10)</sup> In these cases, concentration measurements of the smaller volume phase seem to be sufficient to obtain reliable  $P_a$  values, as reported in the literature,<sup>4,9)</sup> but partitionings at different concentrations should be repeated to ensure the reproducibility of  $P_a$  values.

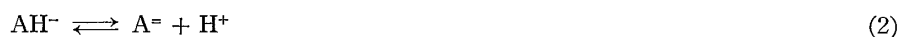
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10) Self-association or ion-pair formation of the sample in the organic phase was neglected. Here we employed the UV spectral method for the measurement of concentration, which would be of the order of  $10^{-4}$  mol dm<sup>-3</sup>, so that the error due to the above phenomena may reasonably be neglected. In addition it should be noted that a value such as  $P_{12} = (AH^{\ominus})_{org}/(AH^{\ominus})_w$  will be smaller than  $P_1 = (AH^{\ominus})_{org}/(AH^{\ominus})_w$ , since the contribution from the more charged species would become even smaller in the organic phase.

## Results and Discussion

### The pH Dependence of Apparent Partition Coefficients

Let us consider the dissociation equilibria of a diacid  $AH_2$  in aqueous buffer solution. Equations 1 and 2 can be written for these equilibria.<sup>11)</sup> From Eqs. 1 and 2,  $K_{a1}^A = (AH^-)_w \times (H^+) / (AH_2)_w$  and  $K_{a2}^A = (A^-)_w (H^+) / (AH^-)_w$  are defined, so the  $pK_{a1}^A$  and  $pK_{a2}^A$  values are given by Eqs. 3 and 4, respectively, under the assumption that the activity coefficient  $f=1$  for all the species.<sup>12)</sup>



$$pK_{a1}^A = pH + \log [(AH_2)_w / (AH^-)_w] \quad (3)$$

$$pK_{a2}^A = pH + \log [(AH^-)_w / (A^-)_w] \quad (4)$$

At some pH the total concentration of diacid  $AH_2$  becomes  $(AH_2)_w^0 = (AH_2)_w + (AH^-)_w + (A^-)_w$ , and then the relation of Eq. 5 holds.

$$\frac{(AH_2)_w}{(AH_2)_w^0} = 1 - \frac{(AH^-)_w}{(AH_2)_w^0} - \frac{(A^-)_w}{(AH_2)_w^0} = 1 - \alpha_1 - \alpha_2 \quad (5)$$

Here,  $\alpha_1$  and  $\alpha_2$  are, respectively, the degrees of ionization to the  $AH^-$  and  $A^-$  species. The relation  $(AH_2)_w^0 = (AH_2)_w / (1 - \alpha_1 - \alpha_2)$  can now be derived from Eq. 5. The apparent partition coefficient is given by  $P_a = (AH_2)_{org} / (AH_2)_w^0$ , giving Eq. 6, where  $P = (AH_2)_{org} / (AH_2)_w$  is the true partition coefficient. Here it was assumed that the  $AH_2$  species alone (*i.e.*, unionized) exists in the organic solvent phase (*vide infra*).

$$P_a = P(1 - \alpha_1 - \alpha_2) \quad (6)$$

Using  $K_{a1}^A$ ,  $K_{a2}^A$ , and the proton concentration  $(H^+)$ ,  $\alpha_1$  is easily obtained as follows.

$$\alpha_1 = \left[ 1 + \frac{(H^+)}{K_{a1}^A} + \frac{K_{a2}^A}{(H^+)} \right]^{-1} \quad (7)$$

On the other hand, in view of the relation  $(A^-)_w = K_{a1}^A K_{a2}^A (AH_2)_w / (H^+)^2$ , Eq. 8 should hold for  $\alpha_2$ .

$$\alpha_2 = \left[ 1 + \frac{(H^+)^2}{K_{a1}^A K_{a2}^A} + \frac{(H^+)}{K_{a2}^A} \right]^{-1} \quad (8)$$

11) Strictly speaking, equation 1 should be written as  $AH_2 + H_2O \rightleftharpoons AH^- + H_3O^+$ . In an aqueous medium the water concentration is considered as constant, so the equations given in the text were used for convenience.

12) For thermodynamic  $pK_a$  values the equilibrium concentrations (see Eqs. 3, 4, 13, and 14) of solutes should be expressed in an activity scale; that is, correction for the ionic strength of the buffer solution should be made.<sup>13,14)</sup> Unfortunately it is not clear whether many  $pK_a$  values in the literature are corrected or not;  $pK_a$  values which have been employed in QSAR studies appear not to be corrected for ionic strength, so no correction was carried out here. If correction is necessary, the following equations are recommended to obtain the  $\log f$  value,  $f$  being the activity coefficient.<sup>13,15,16)</sup>  $\log f = -Az^2\sqrt{\mu} / (1 + \sqrt{\mu})$ , or  $\log f = -A[\{\sqrt{\mu} / (1 + \sqrt{\mu})\} - 0.3\mu]$ . The latter is suitable for the case of the formal charge  $Z=1$  (monocation or monoanion),  $f=1$  being postulated for the neutral species. The Lewis ionic strength  $\mu$  is given by  $1/2 \sum_i C_i Z_i^2$ , and  $A$  is a constant dependent on the temperature and the dielectric constant of the solution. Usually  $A$  is 0.505—0.509 at 20—25°. The  $\mu$  value of buffer solutions used here was 0.5. The proton concentration was determined with a glass electrode pH meter, so the proton concentration in this study is expressed on the activity scale  $(H^+)$ .

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We now obtain Eq. 9 by introducing Eqs. 7 and 8 into Eq. 6.

$$\frac{1}{P_a} = \frac{1}{P} + \frac{K_{a1}^A}{P} \left[ \frac{1}{(H^+)} \right] + \frac{K_{a1}^A K_{a2}^A}{P} \left[ \frac{1}{(H^+)} \right]^2 \quad (9)$$

Eq. 9 means that  $1/P_a$  and  $1/(H^+)$  are in a parabolic relation, and  $P$ ,  $K_{a1}^A$ , and  $K_{a2}^A$  can be determined by least-squares treatment of Eq. 9. See "Appendix" for details.<sup>17)</sup> The differentiation of Eq. 9 with respect to  $(H^+)^{-1}$  gives the relation  $[1/(H^+)]_{\min}^h = [-K_{a1}^A/P]/[2K_{a1}^A K_{a2}^A/P]$  at the minimum point of the parabolic curve of Eq. 9, so  $K_{a2}^A = -(H^+)_{\min}^h/2$ . In other words,  $pK_{a2}^A$  can also be estimated by using the hypothetical proton concentration  $(H^+)_{\min}^h$  at the minimum point of the least-squares fitted curve of Eq. 9 (*i.e.*  $P_a^{-1}$  vs.  $(H^+)^{-1}$ ). It should be pointed out that this minimum point appears in the negative scale of  $(H^+)^{-1}$ , since  $K_{a2}^A$  is positive. When Eq. 2 is disregarded in the pH region studied, that is,  $(A^-)_w$  is put equal to zero, then  $K_{a2}^A$  becomes zero, so that Eq. 9 can be replaced by Eq. 10, which shows that the values of  $(H^+)/P_a$  and  $(H^+)$  are in a linear relation.<sup>18)</sup>

$$(H^+)/P_a = K_{a1}^A/P + [1/P](H^+) \quad (10)$$

Eq. 10 can be rewritten as  $P_a = P - [K_{a1}^A P_a/(H^+)]$ , showing a linear relation between  $P_a$  and  $P_a/(H^+)$ . This would be useful as a check on Eq. 10.

Next, we will consider the proton addition equilibria to bases, written formally as follows:



Since  $K_{a1}^B = (B)_w(H^+)/ (BH^+)_w$  and  $K_{a2}^B = (BH^+)_w(H^+)/ (BH_2^{++})_w$ ,  $pK_{a1}^B$  and  $pK_{a2}^B$  are given by Eqs. 13 and 14, respectively.

$$pK_{a1}^B = pH + \log [(BH^+)_w / (B)_w] \quad (13)$$

$$pK_{a2}^B = pH + \log [(BH_2^{++})_w / (BH^+)_w] \quad (14)$$

Note that on comparing the second term in the right-hand side of Eqs. 13 and 14 with that of Eqs. 3 and 4, more charged species in the equilibria appear in the numerator for bases but in the denominator for acids. Keeping this in mind, the treatment used for the derivation of Eq. 9 leads to Eq. 15 for the dissociation equilibria of protonated bases;  $1/P_a$  has a parabolic relation with respect to the proton concentration in buffer solutions.<sup>17)</sup>

$$\frac{1}{P_a} = \frac{1}{P} + \frac{(H^+)}{P \cdot K_{a1}^B} + \frac{(H^+)^2}{P \cdot K_{a1}^B \cdot K_{a2}^B} \quad (15)$$

The proton concentration  $(H^+)_{\min}^h$  at the minimum point of this parabolic curve is given by  $(H^+)_{\min}^h = [-1/P \cdot K_{a1}^B] [P \cdot K_{a1}^B \cdot K_{a2}^B / 2] = -K_{a2}^B/2$ , so  $K_{a2}^B = -2(H^+)_{\min}^h$ . If the  $(BH_2^{++})_w$  concentration is ignored in the pH region studied,  $1/K_{a2}^B$  becomes zero. Eq. 15 is then simplified to Eq. 16, where  $1/P_a$  and  $(H^+)$  are in a linear relation.<sup>18)</sup>

$$1/P_a = 1/P + [1/P \cdot K_{a1}^B](H^+) \quad (16)$$

Note that Eq. 16 can be rewritten as  $P_a = P - [1/K_{a1}^B][P_a \cdot (H^+)]$ , showing the linear relation of  $P_a$  to  $P_a(H^+)$ .

### Effect of Ionic Dissociation in the Organic Solvent Phase

In the derivation of Eqs. 9 and 15 we have assumed no contribution from ionic dissociation in the organic phase. However, if ionic dissociation occurs in the organic phase, the true

17) The application of Eqs. 9 and 15 should be particularly useful for the case where  $K_{a1}$  and  $K_{a2}$  are similar in magnitude. If the separation between the values is large, these equations may not be applicable, at least, for the octanol-water system, since the ionic dissociation effect in the organic solvent phase ceases to be negligible. This is discussed later in the text.

18) Equations 10 and 16 are quite similar to the Benesi-Hildebrand equation and its modified forms for charge transfer complex formation, H.A. Benesi and J.H. Hildebrand, *J. Am. Chem. Soc.*, **71**, 2703 (1949); R.S. Mulliken and W.B. Person, "Molecular Complexes," Wiley-Interscience, 1969.

partition coefficient  $P_i$  due to the ionic species should be considered; this is expressed by  $P_i = (\text{AH}^-)_{\text{org}}/(\text{AH}^-)_{\text{w}}$  for the case of Eq. 1. If  $\Delta \log P$  is given by  $\Delta \log P = \log P_i - \log P$ , then  $\log [(\text{AH}^-)_{\text{org}}/(\text{AH}_2)_{\text{org}}]$  is equal to  $\Delta \log P - \log [(\text{AH}_2)_{\text{w}}/(\text{AH}^-)_{\text{w}}]$ . Eqs. 17 and 18 are easily obtained,<sup>6)</sup> the latter being for the case of Eq. 11.

$$\log [(\text{AH}^-)_{\text{org}}/(\text{AH}_2)_{\text{org}}] = (\log P_i - \log P) - (\text{p}K_{\text{a1}}^{\text{A}} - \text{pH}) \quad (17)$$

$$\log [(\text{BH}^+)_{\text{org}}/(\text{B})_{\text{org}}] = (\log P_i - \log P) - (\text{pH} - \text{p}K_{\text{a1}}^{\text{B}}) \quad (18)$$

In usual cases,  $\log P_i$  is much smaller than  $\log P$ . The values of  $\Delta \log P$  were reported to be of the order of  $-3$  to  $-4$  for usual acids or bases in an octanol-water system.<sup>6,9a,10)</sup> Therefore, in the pH region near  $\text{p}K_{\text{a1}}^{\text{A}}$  or  $\text{p}K_{\text{a1}}^{\text{B}}$ , the concentration of the ion species in water (buffer solution)-saturated octanol is only of the order of  $10^{-3}$ – $10^{-4}$  of the concentration of neutral species. The assumption that ion species in the organic phase can be neglected thus seems reasonable in the above pH region and also at lower (for Eq. 17) or higher (for Eq. 18) pHs (*vide infra*). It follows from this viewpoint that the use of such solvents as heptane is preferable for  $\text{p}K_{\text{a}}$  determination, since ion dissociation is more difficult in solvents like heptane.

The relation between  $\log P_{\text{a}}$  and  $\log P$  can now be derived as follows. When ionic dissociation in the organic phase is taken into consideration,  $P_{\text{a}}$  can be expressed as  $P_{\text{a}} = [(\text{AH}_2)_{\text{org}} + (\text{AH}^-)_{\text{org}}]/[(\text{AH}_2)_{\text{w}} + (\text{AH}^-)_{\text{w}}]$  for the equilibrium of Eq. 1. Based on this relation, the equation  $P_{\text{a}} = P[(\text{H}^+)/\{K_{\text{a1}}^{\text{A}} + (\text{H}^+)\}] + P_i[K_{\text{a1}}^{\text{A}}/\{K_{\text{a1}}^{\text{A}} + (\text{H}^+)\}]$  is obtained, which then leads to Eq. 19.

$$P_{\text{a}} = \left[ \frac{(\text{H}^+)}{K_{\text{a1}}^{\text{A}} + (\text{H}^+)} \right] \left[ 1 + \frac{(\text{AH}^-)_{\text{org}}}{(\text{AH}_2)_{\text{org}}} \right] P \quad (19)$$

Here we see that in a series of similar compounds, whose  $\text{p}K_{\text{a}}$  and  $\Delta \log P$  values are almost equal, or whose  $(\text{AH}^-)_{\text{org}}$  values are essentially zero, the coefficient of  $P$  in Eq. 19 comes out constant at a given pH. Thus  $\log P_{\text{a}}$  and  $\log P$  are in a linear relation to each other with slope 1.<sup>19)</sup> Note that in the case of the base dissociation given by Eq. 11, Eq. 20 should be used instead of Eq. 19.

$$P_{\text{a}} = \left[ \frac{K_{\text{a1}}^{\text{B}}}{K_{\text{a1}}^{\text{B}} + (\text{H}^+)} \right] \left[ 1 + \frac{(\text{BH}^+)_{\text{org}}}{(\text{B})_{\text{org}}} \right] P \quad (20)$$

### Comparison with Experimental Results

Examples of the application of Eq. 10 are listed in Table I for POAA, PMBA, and MICA. To test Eq. 16, PMOA and PMA were used, the data being collected in Table I.<sup>12,20–23)</sup> The system of octanol buffer solution was chosen for all samples, since the  $\log P$  values obtained from this system are very useful for QSAR studies. In addition, an experiment in heptane-buffer solution was also carried out for PMOA. As examples, Figs. 2 and 3 show the applicability of Eqs. 10 and 16 for POAA and PMOA, respectively. In the case of the parabolic relation given by Eqs. 9 and 15, studies are now proceeding on some representative samples, and the results will be reported shortly. From the data given above, it is evident that the theoretical equations are consistent with the experimental results, and that the  $\text{p}K_{\text{a}}$  values obtained agree with the literature values determined by other techniques.

The advantages of this technique are as follows. (i) The method is very simple. (ii) The true  $P$  value and the  $\text{p}K_{\text{a}}$  value can be determined simultaneously, which is very useful

- 19) It has been found that this relation holds in some cephalosporin series compounds with a COOH group at the 4-position, the  $\text{p}K_{\text{a}}$  values of which are of the order of 2.5. To be published by us.
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TABLE I. Comparison of  $\log P$  and  $pK_a$  Values obtained in the Present Work with Literature Values

Compound <sup>a)</sup>	Present work <sup>b)</sup>		Literature	
	$\log P$	$pK_a$	$\log P$	$pK_a$
MICA	0.68(±0.01)	2.20(±0.02)	0.57 <sup>d)</sup>	2.22 <sup>e)</sup>
POAA	1.35(±0.04)	2.91(±0.01)	1.34, <sup>g)</sup> 1.26 <sup>f)</sup>	3.00, <sup>e)</sup> 3.17 <sup>f)</sup>
PMBA	2.36(±0.02)	4.32(±0.04)	2.27 <sup>f)</sup>	4.37 <sup>i)</sup>
PMA	1.56(±0.02)	5.26(±0.04)	1.39 <sup>f)</sup>	5.08 <sup>i)</sup>
PMA	0.49(±0.03) <sup>c)</sup>	5.11(±0.04) <sup>c)</sup>		5.08 <sup>i)</sup>
PMOA	0.80(±0.02)	5.38(±0.04)	0.95 <sup>g, h)</sup>	5.26 <sup>i)</sup>

a) See the text for abbreviations.

b) The system of octanol-buffer solution was used throughout unless otherwise indicated. The errors were calculated using the estimated standard error of the partial regression coefficients.<sup>24)</sup>

c) These values were obtained with an *n*-heptane-buffer system.

d) Taken from our previous data (to be published).

e) Our experimental data obtained by UV (2.22) or potentiometric titration (3.00).

f, g, h, and i) See refs. 20, 21, 22, and 23 in the text, respectively.

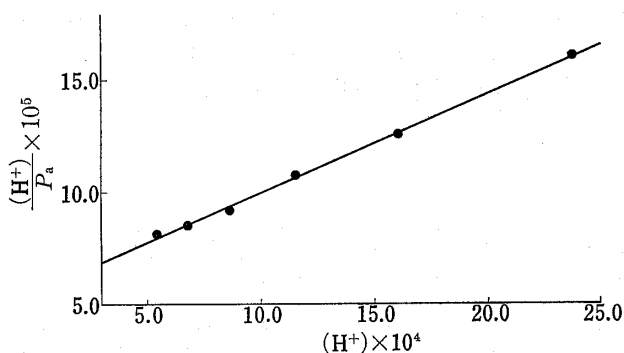


Fig. 2. The Linear Relation of Eq. 10 in the Text for Phenoxyacetic Acid in Octanol-buffer Solution

$$\frac{(H^+)}{P_a} = 4.463 \times 10^{-2}(H^+) + 5.531 \times 10^{-5}$$

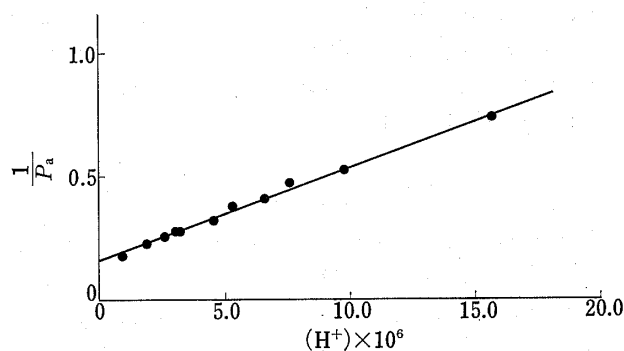


Fig. 3. The Linear Relation of Eq. 16 in the Text for *p*-Methoxyaniline in Octanol-buffer Solution

$$\frac{1}{P_a} = 3.770 \times 10^{-4}(H^+) + 0.1576$$

for QSAR studies. (iii) In addition, in cases where the application of the spectrophotometric or potentiometric titration method is difficult or not sufficiently accurate to determine the  $pK_a$  value, the present method can still be employed by selecting the most suitable technique, such as UV, IR, gas- or liquid-chromatographic methods, *etc.*, for concentration determination in the organic phase or aqueous buffer solutions.<sup>24)</sup> In addition these techniques make it possible to use a small quantity ( $10^{-4}$  mol  $dm^{-3}$  or less) of samples. However, there is one difficulty in the partition coefficient method reported here. This is that the theoretical equations pertinent to acids and bases are different, as mentioned previously. If acid and base groups in a molecule show  $pK_a$  values of the same order of magnitude, then analyses of the pH dependence of the apparent partition coefficient become quite tedious.<sup>5)</sup>

Quite recently a short paper on the determination of  $pK_a$  by analyses of the pH dependence of the  $P_a$  values was independently published by Unger *et al.*<sup>25)</sup> They used

24) In the case of substances such as POAA, the change in UV upon ion dissociation is small, so the UV method may not give accurate  $pK_a$  values. For compounds whose  $pK_a$  values are near the ends of the pH scale ( $pK_a \leq 2.0$ , or  $pK_a > 11$ ), the usual potentiometric titration may not be efficient because of the small change of pH scale during the titration.

25) S.H. Unger, J.R. Cook, and J.S. Hollenberg, *J. Pharm. Sci.*, **67**, 1364 (1978). See also P. Seiler, *Eur. J. Med. Chem.*, **9**, 663 (1974).

different equations and different techniques, such as high pressure liquid chromatography. However there was no discussion on the theoretical treatment of the cases of di-acids and di-bases, or on the effect of ion dissociation in the organic phase. In the case of octanol, which is important in the field of QSAR studies, the  $\Delta \log P$  is of the order of  $-3$  to  $-4$ . The ion species in the organic phase, therefore, may amount to 50% at pH values where  $(\text{p}K_{\text{a1}}^{\text{A}} - \text{pH})$  in Eq. 17 and  $(\text{pH} - \text{p}K_{\text{a1}}^{\text{B}})$  in Eq. 18 amount to  $-3$  to  $-4$ . The deviation from linearity of Eqs. 10 and 16 may be very large under the above conditions.

### Appendix

Eqs. 9 and 15 in the text have the form of Eq. A-1.

$$y = a_0 + a_1x + a_2x^2 \quad (\text{A-1})$$

In general the correlation coefficient  $r_{12} = \frac{\sum_i^n (x_i - \bar{x})(x_i^2 - \bar{x}^2)}{[\sum_i^n (x_i - \bar{x})^2]^{1/2} \cdot [\sum_i^n (x_i^2 - \bar{x}^2)]^{1/2}}$  between  $x$  and  $x^2$  is very high and near the maximum value, 1. In such a case direct application of the least-squares method may sometimes be inappropriate for obtaining the  $a_0$ ,  $a_1$  and  $a_2$ .<sup>26)</sup> Very recently, Bradley and Srivastava<sup>27)</sup> reported a general treatment to overcome this difficulty, *i.e.*, to reduce the  $r_{pq}$  value between the  $x^p$  and  $x^q$  terms. Applying their method to the present case, we transform Eq. A-1 to Eq. A-2, so that the correlation coefficient between  $(x - \bar{x} - \lambda)/\sqrt{m_2}$  and  $[(x - \bar{x} - \lambda)/\sqrt{m_2}]^2$  becomes zero; in other words, the values of  $b_0$ ,  $b_1$  and  $b_2$  can be accurately and simply obtained by the least-squares method.

$$y = b_0 + b_1 \left[ \frac{x - \bar{x} - \lambda}{\sqrt{m_2}} \right] + b_2 \left[ \frac{x - \bar{x} - \lambda}{\sqrt{m_2}} \right]^2 \quad (\text{A-2})$$

This transformation means that the origin of the input data  $(x_1, x_2, \dots, x_n)$  of the predictor variable  $x$  is shifted from  $\bar{x} = \sum_i^n x_i/n$  of Eq. A-1 to  $-\lambda/\sqrt{m_2}$  of Eq. A-2,  $\lambda$  and  $m_2$  being given by Eq. A-3.

$$\left. \begin{aligned} m_2 &= \frac{\sum_i^n (x_i - \bar{x})^2}{n} \\ \lambda &= 1/2 \left[ \frac{\sum_i^n (x_i - \bar{x})^3}{\sum_i^n (x_i - \bar{x})^2} \right] \end{aligned} \right\} \quad (\text{A-3})$$

Finally, the relations between  $a_0, a_1, a_2$  in Eq. A-1 and  $b_0, b_1, b_2$  in Eq. A-2 give rise to Eq. A-4.

$$\left. \begin{aligned} a_0 &= b_0 - \frac{b_1}{\sqrt{m_2}}(\bar{x} + \lambda) + \frac{b_2}{m_2}(\bar{x} + \lambda)^2 \\ a_1 &= \frac{b_1}{\sqrt{m_2}} - \frac{2b_2}{m_2}(\bar{x} + \lambda) \\ a_2 &= \frac{b_2}{m_2} \end{aligned} \right\} \quad (\text{A-4})$$

Using  $a_0$ ,  $a_1$ , and  $a_2$  obtained from Eq. A-4, the necessary physical constants are derived as described in the text. It should be noted that in the case of Eq. A-1 or A-2, three physical constants can be obtained from one parabolic relation. This, however, means that there is a decrease in the number of degrees of freedom, so that the number of experimental data should be larger than in the case of a linear relation such as Eq. 10 or 16 in the text, if accurate constants are to be obtained.

26) a) T. Okuno, H. Kume, T. Haga and T. Yoshizawa, "Multivariate Analysis," Nikkagiren Press, Tokyo, 1978 (in Japanese), Chapter II; b) N.R. Draper and H. Smith, "Applied Regression Analysis," John Wiley and Sons, New York, 1968.

27) R.A. Bradley and S.S. Srivastava, *The American Statistician*, 33, 11 (1979).