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Simultaneous Determination of Acid Dissociation Constants and True Partition Coefficients by Analysis of the Apparent Partition Coefficients. III. The Application of the Hammett Acid Function[1,2]

JIRO HANAMURA, KEIKO KOBAYASHI, KENJI KANO, and TANEKAZU KUBOTA*

Gifu College of Pharmacy, 6-1, Mitahora-higashi 5-chome, Gifu 502, Japan

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Equations for the simultaneous determination of acid dissociation constants (pK_a) and true partition coefficients (P) of a very weak base or acid have been derived by applying the Hammett acid functions H_0 and H_- and by analyzing the acid function dependence of the apparent partition coefficient (P_a). The equations show that P_a^{-1} is linearly related to h_0 ($h_0=10^{-H_0}$) and h_-^{-1} ($h_-=10^{-H_-}$) for a base and an acid, respectively. Some experimental systems for bases have been successfully measured using octanol as an organic solvent phase and suitably diluted H_2SO_4 solution as an aqueous phase, the H_0 value being used. The results obtained were reasonably consistent with the literature values. The application of this technique makes it possible to determine log P and pK_a simultaneously, the latter values being smaller than 0 for bases and larger than 14 for acids.

Keywords——dissociation constant; partition coefficient; octanol; structure–activity relationship; UV spectra; H_0 acid function; H_- acid function; theory of acid function dependence of apparent partition coefficient; pyridine *N*-oxide; quinoline *N*-oxide

In the foregoing papers[3,4] of this series we have reported a new technique for the simultaneous determination of the acid dissociation constants pK_a's and the true partition coefficient P of neutral species by analyzing the pH dependence of the apparent partition coefficient (P_a) of mono- or dibasic acids and mono- or diacidic bases. This procedure is simple and especially useful for the study of quantitative structure–activity relationships (QSAR). However, this method is only applicable to compounds, which undergo proton dissociation in aqueous solutions having usual pH scales. Accordingly, modification of the method is necessary for the case of very weak bases or acids, because these substances do not undergo proton dissociation in usual pH buffer solutions. The application of the Hammett acid function[5] should be very useful for the above substances. In this report details of the treatment are presented.

Experimental

Solvents——Pure octanol(1-) purchased from E. Merck Co. was carefully rectified (bp 193.5°C). This octanol showed no UV absorption due to impurities in the near UV region. Aqueous sulfuric acid solutions of various concentrations (weight percent) were prepared by suitably diluting conc. sulfuric acid (97%, purchased from Wako Junyaku Co.) with distilled water. The sulfuric acid concentration was determined by titration with standard NaOH solution of known concentration. The conversion from volume concentration (weight/volume) to weight percent concentration was done by referring to the published table.[6]

Samples——Guaranteed reagent-grade samples of 4-nitropyridine *N*-oxide (4NPO) and 4-nitroquinoline *N*-oxide (4NQO) were obtained from Aldrich Chemical Co. and Wako Junyaku Co., respectively. The former (mp 162°C) was recrystallized from acetone, while the latter (mp 155°C) was purified by repeated sublimation. Pure 4-cyanopyridine *N*-oxide (4CNPO) was kindly supplied by Dr. Yamakawa[7] of Shionogi Research Laboratories (recrystallized from acetone, mp 230°C).

Measurement of Apparent Partition Coefficient P_a——The details of the measurement of apparent partition coefficients were described in the foregoing papers.[3] All the partition and centrifugation processes were carried out at room temperature (~23°C). A temperature-regulated centrifuge was used for the latter operation. Sample concentration was determined by UV spectral measurement. Sulfuric acid concentrations were determined immediately after partitioning, the acid function H_0 values being evaluated from these. The relationship between H_0 and sulfuric acid concentration is given in Fig. 1 (see reference 8 for detailed values). These H_0 values tend toward a usual pH scale in highly diluted acid solutions with water. It should be noted that octanol could not be employed as an organic solvent phase in more than 50% conc. sulfuric acid because of its instability; the background absorption of the octanol increased gradually in such sulfuric acid solutions.

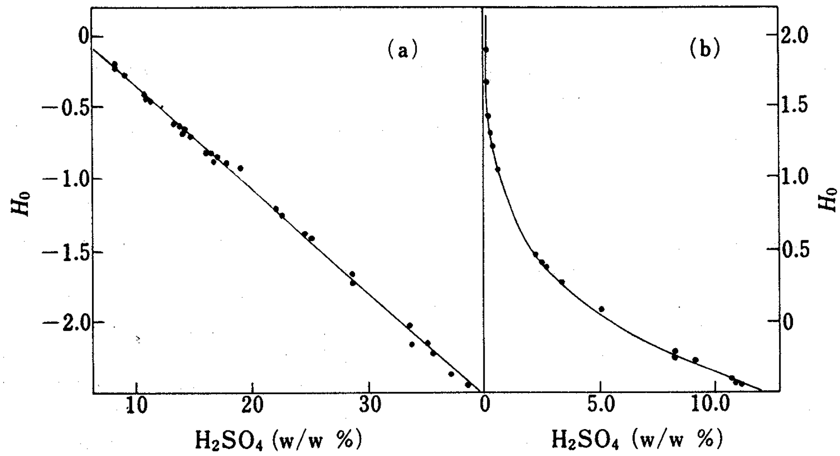

Fig. 1

The relationship between acid function H_0 values and sulfuric acid concentrations (weight percent) studied here. Refer to the left ordinate scale and the right one for figures (a) and (b), respectively. See ref. 8 in the text.

Results and Discussion

The H_0 Dependence of Apparent Partition Coefficients

First, let us consider very weak bases,[9,10] such as benzophenone, azobenzenes, 4NPO, 4NQO, *etc.*, the pK_a values of which are much less than 0, so that proton addition to the molecules does not occur in the usual pH region but occurs in very strong acids such as sulfuric acid. When we write a one-proton addition equilibrium of a weak base B as $BH^+ \rightleftharpoons B + H^+$, the equilibrium constant K_a^B is given by Eq. 1, where the activity coefficient f is explicitly taken into account.

$$K_a^B = \frac{f_B(B)\cdot f_{H^+}(H^+)}{f_{BH^+}(BH^+)} = \frac{(B)}{(BH^+)}\cdot\frac{f_B\cdot a_{H^+}}{f_{BH^+}} \qquad (1)$$

Here, $a_{H^+}=f_{H^+}(H^+)$ is the proton activity, f_{H^+} being the activity coefficient. According to the Hammett definition, $[f_B\cdot a_{H^+}/f_{BH^+}]=h_0$, the acid function H_0 is given by $H_0=-\log h_0$. The H_0 values derived originally by Hammett[5] have been reported for various kinds of acids by many authors,[8b] and those values have been refined.[8b] The H_0 values for a wide variety of H_2SO_4 concentrations were cited in Cookson's review[8b] and are used in the present study (see Fig. 1).[8a] In a solution at some H_0 the total concentration $(B)_W^0$ of the base is given by $(B)_W^0 = (B)_W + (BH^+)_W$. Thus we obtain $[(B)_W/(B)_W^0]=1-\alpha$, α being the dissociation degree written as $[(BH^+)_W/(B)_W^0]=[h_0/(K_a^B+h_0)]$. Combining this relation with the apparent partition coefficient $P_a=(B)_{org}/(B)_W^0$ and the true partition coefficient $P=(B)_{org}/(B)_W$, and also assuming that the neutral species B alone exists in the organic solvent phase,[11] we can easily derive Eq. 2.

$$P_a^{-1} = P^{-1} + (P\cdot K_a^B)^{-1}\cdot h_0 \qquad (2)$$

Here, note that Eq. 2 has the same form as was reported for the case of a one-proton addition equilibrium of a base in the usual pH region,[3] the only difference being that h_0 enters into Eq. 2 in place of proton concentration (H^+).

The H_- Dependence of Apparent Partition Coefficients

In the case of very weak acids such as 2,4-dinitroaniline, *N*-isopropylidene-*N'*-(4-nitrophenyl)hydrazine, thioacetamide, *etc.*[12] proton dissociation may occur in strongly basic solutions. The dissociation constant K_a^A is given by

$$K_a^A = \frac{f_{A^-}(A^-) \cdot f_{H^+}(H^+)}{f_{AH}(AH)} = \frac{(A^-)}{(AH)} \cdot \frac{f_{A^-} \cdot a_{H^+}}{f_{AH}} \qquad (3)$$

for the equilibrium $AH \rightleftarrows A^- + H^+$. The acid function H_- is now defined by $H_- = -\log h_-$, where $h_- = (f_{A^-} \cdot a_{H^+}/f_{AH})$.[13] Eq. 3 is now rewritten as $K_a^A = [(A^-) \cdot h_-/(AH)]$. Combining this equation with the relations $[(AH)/(AH)_W^0] = 1-\alpha$, where $\alpha = [(A^-)_W/(AH)_W^0] = K_a^A/[K_a^A + h_-]$ and $(AH)_W^0 = (AH)_W + (A^-)_W$, $P_a = (AH)_{org}/(AH)_W^0$, and $P = (AH)_{org}/(AH)_W$, and also applying the same treatment as for the derivation of Eq. 2, we easily obtain Eq. 4.

$$(P_a)^{-1} = P^{-1} + (K_a^A/P) \cdot h_-^{-1} \qquad (4)$$

Note here that Eq. 4 is also the same as that applied to the dissociation equibrium of AH in a usual pH region except that $(H^+)^{-1}$ is replaced by $(h_-)^{-1}$.

Comparison with Experimental Results

For the examination of Eq. 2, sulfuric acid solutions (hence H_0 values) were employed as an aqueous phase in the present experiments, as described in "Experimental," octanol being the organic solvent phase. The substances 4NPO, 4NQO, and 4CNPO have been used for the present partition experiments.[10] The results are listed in Table I, and Fig. 2 shows a linear plot based on Eq. 2 for 4NQO as an example. The log *P* values obtained here are compatible with those given in the literature[14] as can be seen in Table I.[15] Next, let us consider the basicity of 4NPO, 4NQO, and 4CNPO. The basicity of pyridine *N*-oxide (PNO) has been studied in detail, the reported pK_a values being 0.78—0.79.[10,16,17] The introduction of an electron-withdrawing substituent at the *para*-position with respect to the $\rangle N \rightarrow O$ group, therefore, brings about a decrease of the pK_a value of PNO due to the intramolecular charge transfer effect from the *N*-oxide group oxygen atom to the substituent, so the pK_a value should move to the negative scale. The pK_a value of 4NPO was previously reported to be −1.7 as determined by a UV method in dilute sulfuric acid solution.[17] Although this absolute value is a little larger[18] than that obtained here by using Eq. 2 (see Table I), the values are of the same order of magnitude. Now, from Table I it is clear that the pK_a values of 4NPO and 4NQO, derived by analysis based on Eq. 2, are significantly different (4NQO is a strong carcinogen). This is clearly because the NO_2 group in 4NQO cannot be coplanar with the quinoline *N*-oxide molecular

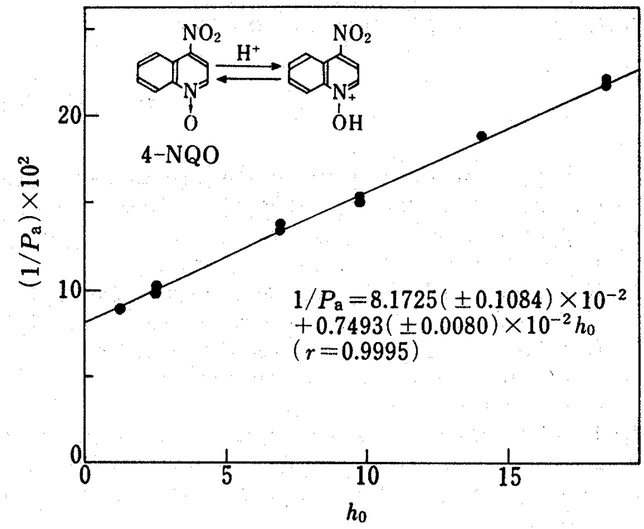

Fig. 2

Linear plot based on Eq. 2 in the text for 4-nitroquinoline *N*-oxide in octanol-sulfuric acid solution.

TABLE I. Dissociation Constants (pK_a) and log P Values of 4NPO, 4NQO, and 4CNPO obtained in the Present Work[a]

Compound	Present work[b]		Literature	
	log P	pK_a	log P	pK_a
4NPO	−0.69 (±0.01)	−1.25 (±0.02)	−0.57[c]	−1.7[e]
4NQO	1.09 (±0.01)	−1.03 (±0.02)	1.02[c]	—
4CNPO	−0.94 (±0.01)	−0.73 (±0.03)	(−0.98)[d]	—

a) The abbreviations 4NPO, 4NQO, and 4CNPO refer to 4-nitropyridine *N*-oxide, 4-nitroquinoline *N*-oxide, and 4-cyanopyridine *N*-oxide, respectively.
b) The system of octanol-sulfuric acid solution was used. The errors were calculated using the estimated standard error of the partial regression coefficients.[22]
c) Taken from ref. 14 in the text.
d) See note 15 in the text.
e) Taken from ref. 17 in the text.

plane due to the steric hindrance between the nitro group oxygen atoms and the proton at the *peri* position (5th position) of 4NQO.[20,21] Ultimately the basicity of the 4NQO *N*-oxide group oxygen atom is increased because of the decrease of the electron-withdrawing ability of the 4NQO NO_2 group owing to the steric hindrance. The CN group is a weaker electron-withdrawing substituent than the NO_2 group, as is readily apparent from the Hammett σ constants.[14] Thus it is not surprising that the pK_a value of 4CNPO is smaller than that of 4NPO in absolute value (see Table I).

An experimental check of Eq. 4 was made by using strongly basic solutions, such as conc. aqueous NaOH solutions, whose H− values are already known.[12] A survery of the literature indicated that compounds suitable for the present purpose are 2,4-dinitroaniline, thioacetamide, *etc.*, in which the NH_2 group dissociates ($R\text{–}NH_2 \rightleftarrows R\text{–}NH^- + H^+$) in strongly basic solutions.[12] The pK_a values of these compounds are known.[12] However, in spite of very careful experiments we could not determine reliable values of apparent partition coefficient P_a. The main reasons for this are as follows. The samples used, which have an NH_2 group in the molecules, are not stable in conc. NaOH solution, and the UV spectra are quite time-dependent in the course of partitioning between octanol and conc. (to 8 mol dm^{-3}) NaOH aqueous solution. Experimental verification of Eq. 4 must therefore await further study. Even so, Eq. 4 should be applicable to samples that are stable in strongly basic solutions during partitioning. The advantages of the present type of equations are dealt with in our foregoing papers,[2–4] where we have also discussed various problems in the present techniques.

References and Notes

1) This paper forms Part IV of "Studies on Structure–Activity Relationships." Part III: T. Kubota, K. Ezumi, M. Yamakawa, and B. Uno, *Chem. Pharm. Bull.*, **30**, 398 (1982).
2) Reported in part at the VIth International Symposium on Solute-Solute-Solvent Interaction, Osaka, Japan, July 6, 1982, Abstracts p. 84.
3) K. Ezumi and T. Kubota, *Chem. Pharm. Bull.*, **28**, 85 (1980).
4) T. Kubota and K. Ezumi, *Chem. Pharm. Bull.*, **28**, 3673 (1980).
5) L.P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., New York, 1940.
6) "CRC Handbook of Chemistry and Physics, 60th Edition," ed. by R.C. Weast and M.J. Astle, CRC Press, Florida, 1979.
7) To whom the authors wish to express their gratitude.
8) *a)* Data in Fig. 1 are cited from Table III of K.N. Bascombe and R.P. Bell, *J. Chem. Soc.*, **1959**, 1096; *b)* R.F. Cookson, *Chem. Rev.*, **74**, 5 (1974); *c)* L. Michaelis and S. Granick, *J. Am. Chem. Soc.*, **64**, 1861 (1942).
9) H. Hosoya, *Kōgaku Zōkan*, **26**, 137 (1966) (Review, in Japanese), Kagaku-Dojin Press, Kyoto.
10) E. Ochiai, "Aromatic Amine Oxidase," Elsevier Publishing Co., Amsterdam, 1967, p. 102.
11) For these reasons, care should be taken in applying Eq. 2 to actual problems. See ref. 10 and the section "Effect of Ionic Dissociation in the Organic Solvent Phase" in our first paper for a discussion of these

problems.[3] In conclusion, a linear P_a^{-1} *vs.* h_0 plot based on Eq. 2 should be obtained under the condition that the concentration of the dissociated ion molecule in the aqueous phase is not very large.

12) *a*) K. Bowden, *Chem. Rev.*, **66**, 119 (1966); *b*) J.T. Edward and I.C. Wang, *Can. J. Chem.*, **40**, 399 (1962); *c*) M.G. Harris and R. Stewart, *Can. J. Chem.*, **55**, 3800 (1977).

13) The activity coefficient due to ionic species appears in the numerator for h_-, but in the denominator for h_0. According to the Debye–Hückel theory on strong electrolytes the activity coefficient for an ionic species has a much more important physical significance than that for a neutral species, for which it seems to be near 1, so that H_- and H_0 may not be defined by the same scale. Also, refer to A. Albert and E.P. Sergeant, "The Determination of Ionization Constants," Chapman and Hall, London, 1971.

14) C. Hansch and A.J. Leo, "Substituent Constants for Correlation Analysis in Chemistry and Biology," John Wiley & Sons, New York, 1979.

15) The log P value of 4CNPO is calculated as $[\log P(\text{4NPO}) - \pi(\text{NO}_2) + \pi(\text{CN})] = [-0.69 - (-0.28) + (-0.57)] = -0.98$, which is in quite good agreement with the observed value, -0.94.

16) *a*) T. Kubota, *Yakugaku Zasshi*, **75**, 1546 (1955); *b*) T. Kubota and H. Miyazaki, *Nippon Kagaku Zasshi*, **79**, 924 (1958); *c*) T. Kubota, *Bunkō Kenkyū*, **10**, 83 (1962).

17) *a*) H.H Jaffé and G.O. Doak, *J. Am. Chem. Soc.*, **77**, 4441 (1955); *b*) L.P. Hammett and A.J. Deyrup, *J. Am. Chem. Soc.*, **54**, 2721 (1932); *c*) L.P. Hammett and M.A. Paul, *J. Am. Chem. Soc.*, **56**, 827 (1934).

18) In the original paper, details of the experimental analyses were not given. Since revised H_0 values of sulfuric acid solutions were subsequently reported,[8] one of the reasons for the small difference may be these H_0 values. Note that the pK_a value of 2,6-dimethyl-4NPO was reported to be -1.02.[19]

19) C.D. Johnson, A.R. Katritzky, and N. Shakir, *J. Chem. Soc.* (*B*), **1967**, 1235.

20) The twist angle of the NO_2 group seems to be less than 49°, the value observed for 1,5-dinitronaphthalene.[21]

21) J. Trotter, *Acta Cryst.*, **13**, 95 (1960).

22) *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 & Sons, New York, 1968.