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The Role of Solvent in the Partition of Procaine and *p*-Aminobenzoic Acid between Organic Solvent and Water

SATOSHI OKADA,* HIROSHI NAKAHARA, CHIKAKO YOMOTA, and KENSHU MOCHIDA[1)]

National Institute of Hygienic Sciences, Osaka Branch, 1-1-43, Hoenzaka, Higashi-ku, Osaka 540, Japan

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The role of the solvent in the partition of procaine (PC) and its hydrolysis product, *p*-aminobenzoic acid (PABA), into various organic solvents was investigated from the viewpoints of the solvent polarity, the proton-donating and/or -accepting properties, and the solvation of the solute. The partition coefficients of PC and PABA in a pentanol (PeOH)–cyclohexane (CyH) mixture/water system gradually increased with increase of the mixing ratio of PeOH, but the change with the mixing ratio was not linearly correlated with the change of solvent polarity. The electrophilicity and/or nucleophilicity of the solvents did not greatly influence the partition of PC, whereas the partition of PABA varied with different solvent systems as follows; nucleophilic solvents > amphiprotic solvents ≫ electrophilic solvents ≫ inert solvent.

Furthermore, although it was ascertained that an appropriate solvating agent is required for the partition of a polar solute, the specificity was not always high due to the amphiprotic properties of PC and PABA. The solvation number and the extraction constant were obtained for PeOH, ethyl acetate, and chloroform as solvating agents for PC and PABA, and the nature of the solute–solvent interaction is discussed.

Keywords——procaine; *p*-aminobenzoic acid; partition; solvent polarity; solvation; solute–solvent interaction

The widely used drug procaine (PC), a representative local anesthetic, is the diethylaminoethyl ester of *p*-aminobenzoic acid. It is not stable in aqueous solutions, being easily hydrolyzed by acid and/or alkali to *p*-aminobenzoic acid (PABA) and diethylaminoethanol.[2)] The partition behavior of PC and PABA in pentanol (PeOH)/water and in ethyl acetate (EA)/water system has been investigated, and it was clarified that only the neutral form of each solute can be partitioned into the organic phases.[3)] The intrinsic partition coefficients of PC in the two solvent systems and those of PABA in the PeOH/water system were linearly correlated with those in the octanol/water system. However, the log *P* values of PABA in the EA/water system and the octanol/water system showed slight deviation from a linear relationship.[3)]

It is suggested that a relatively strong solute–solvent interaction exists between PABA and EA, in contrast with the other partition systems. In order to clarify the nature of solute–solvent interaction, the role of solvent in the partition of PC and PABA was investigated from the viewpoints of the solvent polarity, the proton-donating and/or -accepting properties, and the ability to solvate the present solutes.

Experimental

Materials——Procaine hydrochloride was supplied by Fuso Pharmaceutical Industries Co., Ltd., and used without further purification. PABA was purchased from Tokyo Kasei Kogyo Co., Ltd., and purified twice from ethanol. The pH was adjusted by using the phosphate buffer or the citrate buffer, and the ionic strength was adjusted to 0.15 M in the final solutions by adding NaCl.

Various organic solvents were used to study the role of the solvent in the partition of PC and PABA—butanol, PeOH, and octanol as alcohols; EA, propyl acetate, butyl acetate, and amyl acetate as esters; diethyl ether, dibutyl ether as ethers; chloroform (CF) and dichloromethane as halogenated hydrocarbons; and cyclohexane (CyH) as an inert solvent. For an examination of the effect of solvent polarity and solvation of the solute, mixtures of a polar solvent with CyH were used in a wide mixing ratio. Prior to use, a solvent was washed with weak acid and/or weak alkali solutions several times, and finally with distilled water. By this procedure, the aqueous and organic phases were equilibrated with each other and the volume of each phase could be held constant.

Determination of the Apparent Partition Coefficients——Aqueous solutions of 4×10^{-5}—2×10^{-4} M PC and 3×10^{-5}—1.8×10^{-4} M PABA at a fixed pH in centrifugal tubes with stopper were shaken gently with an organic phase in a thermostated incubator at 25 ± 0.1 °C for about 3 h. After equilibration of the partition system, it was centrifuged and the mutual separation of both phases was completed. Then the concentrations of PC and PABA in the aqueous phase were determined spectroscopically with a Hitachi spectrophotometer, model 100-60, at each λ_{max}. The concentration of the solute in the organic phase (C_o) was calculated from the difference between the initial concentration in the aqueous phase (C_i) and the equilibrated concentration (C_w), $C_o=(C_i-C_w)(V_w/V_o)$, where V_w and V_o denote the volumes of aqueous and organic phases, respectively.[4] Thus, the apparent partition coefficient P' can be calculated as $P'=C_o/C_w$.

Both PC and PABA are weak electrolytes, and dissociate depending upon the hydrogen-ion concentration of the aqueous phase. As described in a previous paper,[3] since only the neutral form of each solute can be partitioned into the organic phase under the usual conditions, the apparent partition coefficients of PC and PABA are related to the intrinsic partition coefficients P° by Eqs. 1 and 2, respectively,

$$P'_{PC}=P^\circ_{PC}\left[1+\frac{(H^+)}{K_{a2}}+\frac{(H^+)^2}{K_{a1}K_{a2}}\right]^{-1} \quad (1)$$

$$P'_{AHB}=P^\circ_{AHB}\left[1+\frac{K_{a2}}{(H^+)}+\frac{(H^+)}{K_{a1}}\right]^{-1} \quad (2)$$

where K_{a1} and K_{a2} are the 1st and 2nd acid dissociation constants, respectively, and the subscripts "PC" and "AHB" designate the neutral species of each solute. If the apparent partition coefficient P' of each solute is determined, the intrinsic partition coefficient P° in the partition system can be calculated by means of Eqs. 1 or 2, using the known acid dissociation constants of the solutes.[5,6]

Results and Discussion

Effect of Solvent Polarity

Since it is well-known in the field of analytical chemistry that the extractability of a solute is greatly affected by the polarity of the organic phase, the effect with the present solutes was examined in PeOH–CyH/water system at a constant pH of 8.37 for PC and of 3.24 for PABA. The change of the apparent partition coefficient, P', is depicted in Fig. 1 as a function of the concentration of PeOH in a mole fraction scale, X_{PeOH}. As shown in Fig. 1, in a lower range of X_{PeOH}, the values of $\log P'$ for both solutes increase markedly with X_{PeOH}, and tend to

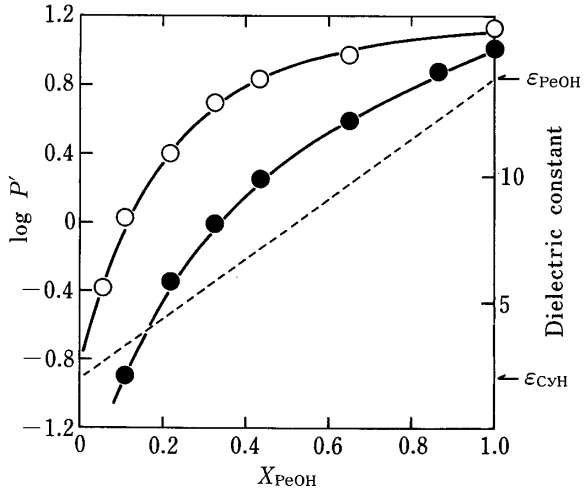

Fig. 1. Change of the Apparent Partition Coefficient, $\log P'$, with Solvent Composition of PeOH–CyH Mixture, X_{PeOH}

The partition experiments were done at fixed pH values of 8.37 for procaine and 3.24 for PABA. The dotted line indicates the change of dielectric constant of the solvent mixture, expected from the additivity rule, based on dielectric constants of PeOH and CyH of 13.9 and 2.02, respectively.[7]

—●—, PABA; —○—, procaine.

approach a constant in the high concentration range. This indicates that an increase of solvent polarity appears to enhance the partition of PC and PABA in the PeOH–CyH mixture. However, the correspondency seems to be fairly poor, because the change of the dielectric constant with the composition can be represented by the dotted line. In contrast with the expected linear change of dielectric constant with X_{PeOH}, the changes of both $\log P'_{PC}$ and $\log P'_{AHB}$ with X_{PeOH} are convex–upward. Especially in the lower region of X_{PeOH}, each $\log P'$ changes steeply with X_{PeOH}, and is not correlated with the change of dielectric constant, *i.e.*, that of solvent polarity. In this treatment, the dielectric constants of PeOH and CyH are 13.9 and 2.02, respectively,[7] and that of PeOH–CyH mixture was calculated by means of the additivity rule.

In real systems the organic phase is saturated with water and contains a small amount of polar solute (below 2×10^{-3} M), which may affect the dielectric constant. However, in nitrobenzene/carbon tetrachloride systems the values of the dielectric constant for the solvent mixtures differ only slightly from the values for the water-saturated solvent mixtures.[6] For example, at mole ratios of nitrobenzene/carbon tetrachloride of 1:0, 1:1, and 2:1, the dielectric constants were determined to be 34.8 (34.8), 16.52 (16.35), and 22.10 (22.05), respectively. The values in parentheses correspond to mixtures saturated with water.[6] This indicates that even a fair content of water in nitrobenzene (0.18 M)[8] hardly affects the dielectric constant of nitrobenzene. Thus, it is expected that the dissolved water and drug may only slightly affect the dielectric constant of the present solvent mixtures. Moreover, in methanol/carbon tetrachloride and ethanol/carbon tetrachloride systems, the deviation of the measured dielectric constant from the additivity rule is reported to be convex-downward,[9] contrary to the deviation for $\log P'$. Accordingly, the partition behavior of PC and PABA into PeOH–CyH mixture cannot be interpreted only in terms of the change of dielectric constant. Thus it seems that the partition of a polar solute should be considered from the viewpoint of the solute–solvent interaction.

Effect of Solvent Electrophilicity and/or Nucleophilicity

In the partition of the neutral forms of PC and PABA into the organic phase, the

TABLE I. Partition Coefficients of PC and PABA in Various Organic Solvent/Water Partition Systems at a Fixed pH of 8.37 for PC and of 3.24 for PABA

Organic solvents	Procaine[a]		PABA[a]		Dielectric constant[b]
	$\log P'_{PC}$	$\log P^{\circ}_{PC}$	$\log P'_{AHB}$	$\log P^{\circ}_{AHB}$	
Electrophilic solvents					
Chloroform	1.44	2.13	−1.01	−0.92	4.1
Dichlormethane	1.42	2.11	−0.89	−0.80	10.4
Nucleophilic solvents					
Ethyl acetate	1.12	1.81	1.20	1.29	6.4
Propyl acetate	0.91	1.60	0.97	1.06	6.3
Butyl acetate	0.73	1.42	1.08	1.17	5.0
Amyl acetate	1.07	1.76	0.72	0.81	4.75
Diethyl ether	1.11	1.80	0.45	0.54	4.34
Dibutyl ether	0.14	0.83	−0.52	−0.43	4.4
Amphiprotic solvents					
n-Butanol	1.13	1.82	0.90	0.99	17.8
n-Pentanol	1.30	1.99	0.81	0.90	13.9
n-Octanol	1.07	1.76	0.66	0.75	10.34
Inert solvent					
Cyclohexane	−0.82	−0.13	−3.34	−3.25	2.02

a) The intrinsic partition coefficients of PC and PABA were calculated by using Eqs. 1 and 2, respectively. *b*) Taken from ref. 7.

existence of the primary amino group and the carboxyl group attached to the aromatic ring acts as a negative factor. In Hansch analysis, the hydrophobic substituent constants in the octanol/water system are evaluated as $\pi_{NH_2} = -1.23$ and $\pi_{COOH} = -0.32$.[10] Thus, the partition coefficient of PABA in the octanol/water system is expected to be $\log P^{\circ}_{AHB} = 0.58$,[3] and the magnitude of the partition coefficient is not adequate for quantitative chemical analysis.

Now, if solvation at the primary amino group and the carboxyl group plays an important role in the partition of PC and PABA, the difference in solvating ability of various solvents for these solutes should be reflected in the partition behavior. The apparent partition coefficients of PC and PABA in various solvent systems, including electrophilic, nucleophilic, amphiprotic, and inert solvents, were determined at a fixed pH of 8.37 for PC and of 3.24 for PABA, as shown in Table I. Table I also shows the intrinsic partition coefficients, calculated by means of Eqs. 1 and 2.

In the case of PC, most of the solvents other than CyH are fairly effective in the partition, and in many solvent systems the values of $\log P^{\circ}_{PC}$ are over 1.5. The largest values are obtained in halogenated hydrocarbon/water systems. This indicates that although both electrophilic and nucleophilic solvents can effectively solvate the primary amino group of PC, the solvating ability of the former may be more effective and/or stronger. Apart from a slight superiority of electrophilic solvents to nucleophilic or amphiprotic solvents in the partition of PC, the difference in the solvent properties may be relatively unimportant for partition. This may be attributed to the amphiprotic character of the primary amino group of PC.

In the case of PABA, there seems to be a difference among solvent systems, and the magnitude of $\log P^{\circ}_{AHB}$ is roughly in the order: esters $\gtrsim$ alcohols $>$ ethers $\gg$ halogenated hydrocarbons $\gg$ inert solvent. The solvating ability of ethers for PABA seems to be less strong than that of esters, despite the similar nucleophilic character, and the difference between diethyl ether and dibutyl ether may arise from steric factors. It is surprising that the values of $\log P^{\circ}_{AHB}$ in the electrophilic solvent/water systems are remarkably small, compared with those in other solvent systems. This phenomenon cannot yet be explained, and further studies are required. As shown in Table I, both $\log P^{\circ}_{PC}$ and $\log P^{\circ}_{AHB}$ in the CyH/water system are extremely small, which also suggests the importance of solvation in the transfer of polar solutes to organic phases. Furthermore, it is clear that a solvent having a high dielectric constant is not necessarily a superior one for the extraction of the present solutes.

Role of Solvation in the Partition of PC and PABA

The partition coefficients of PC and PABA in the PeOH–CyH mixture/water system were greatly dependent on the concentration of PeOH, as shown in Fig. 1. Moreover, as shown in Table I, in the partition of polar solutes such as PABA the selection of the solvent seems to affect appreciably the extractability of the solute. Thus, the existence of appropriate solvating character appears to be required for the partition of polar solutes, as well as for the partition of ion-pairs having an unproportionate charge distribution.[11]

Now, let us consider the partition of PABA into the organic phase containing a solvating agent. If the neutral form of PABA, AHB, is solvated with n molecules of a solvating agent S in the organic phase, the partition equilibrium should be represented by Eq. 3,

$$(AHB)_w + n(S)_o \overset{K^{\circ}_{ex}}{\rightleftharpoons} (AHB \cdot S_n)_o \tag{3}$$

$$K^{\circ}_{ex} = \frac{[AHB \cdot S_n]_o}{[AHB]_w [S]^n_o} \tag{4}$$

where K°_{ex} designates the extraction constant, subscripts w and o are the aqueous and organic phases, respectively, and [ ] indicates the activity of each species. In practice, the activity of each species can conveniently be assumed to be the same as its analytical concentration in the

low concentration range. When the solute, AHB, in the organic phase exists only in the monomeric form, $[AHB \cdot S_n]_o$ in Eq. 4 is nearly equal to $[AHB]_o$ in the definition of the intrinsic partition coefficient, $P^\circ_{AHB}=[AHB]_o/[AHB]_w$. Thus Eq. 5 can be derived from Eq. 4.

$$K^\circ_{ex}=P^\circ_{AHB}\cdot[S]_o^{-n} \tag{5}$$

When the relation of Eq. 2 holds between P'_{AHB} and P°_{AHB}, Eq. 6 is given.

$$\log P'_{AHB}=\log(K^\circ_{ex}/A_{AHB})+n\log[S]_o \tag{6a}$$

$$A_{AHB}=1+\frac{K_{a2}}{(H^+)}+\frac{(H^+)}{K_{a1}} \tag{6b}$$

Thus, when the plot of $\log P'_{AHB}$ *vs.* $\log[S]_o$ gives a linear relation at a constant pH, both the solvation number, n, and the extraction constant, K°_{ex}, can be obtained from the slope and the intercept on the ordinate. However, in the case of PC, the constant A in Eq. 6 can be represented by Eq. 7.

$$A_{PC}=1+\frac{(H^+)}{K_{a2}}+\frac{(H^+)^2}{K_{a1}K_{a2}} \tag{7}$$

The constant A corresponds to the reciprocal of the existence ratio of the neutral species at a given pH.

Using PeOH as a solvating agent, the relations of $\log P'$ to $\log[S]_o$ for PC and PABA are plotted in Fig. 2. Since a good linear relation could be obtained in each partition system, n and K°_{ex} were determined by regression analysis, based on Eq. 6. Similar experiments were also done using other solvating agents of CF and EA, and it was ascertained that Eq. 6 is also valid in those solvent systems. These results are summarized in Table II.

The solvation numbers of PeOH, EA, and CF for PC are 1.2, 1.5, and 1.9, respectively, while those for PABA are 2.0, 3.7, and 2.7, respectively. The difference between PC and

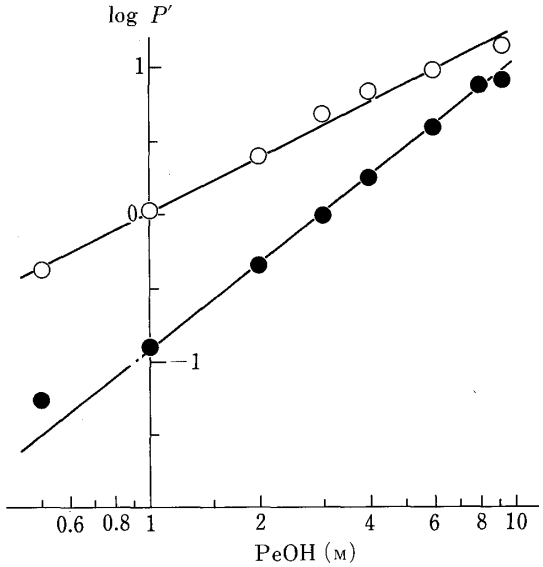

Fig. 2. Linear Correlation of the Apparent Partition Coefficient, $\log P'$, with the Logarithmic Concentration of PeOH as a Solvating Agent

The partition experiments were done at fixed pH values of 8.24 for procaine and 3.21 for PABA.
—●—, PABA; —○—, procaine.

TABLE II. The Solvation Number n and the Extraction Constant K°_{ex}

Solute	Solvating agent	n	K°_{ex}
Procaine	Pentanol	1.2	5.20
	Ethyl acetate	1.5	2.14
	Chloroform[a)]	1.9	0.31
PABA	Pentanol	2.0	1.42×10^{-1}
	Ethyl acetate	3.7	1.80×10^{-3}
	Chloroform	2.7	1.14×10^{-4}

a) The solvation of PC by chloroform differs from that in other solute–solvent systems, as described in ref. 13.

PABA may be attributable to the difference in polar groups, *i.e.* the ester group for PC and the free carboxyl for PABA. The hydrophobic character of the carboxyl and ester groups attached to the aromatic ring can be estimated to be $\pi_{COOH} = -0.32$ and $\pi_{COOCH_3} = -0.01$, respectively.[10] This suggests that the carboxyl group is fairly hydrophilic, while the ester group is almost neutral, comparable to the unsubstituted compound. The fact that the solvation number for PABA is greater than that for PC by about one or two molecules indicates that the carboxyl group of PABA requires more solvation than the ester group of PC.

The values of K°_{ex} are slightly larger than unity in the case of the partition of PC, using PeOH or EA as a solvating agent, whereas in all other partition systems they are below unity. In the partition of PABA using EA or CF as a solvating agent, the values are particularly low. This means that for the partition of such a polar solute as PABA in those solvent systems, a very high concentration of solvating agent is required due to the weak solute–solvent interaction. Thus the solute–solvent interaction in the present partition systems seems to be insufficiently strong to be regarded as hydrogen-bonding between the polar groups of the solute and solvent. As mentioned above, the difference in $\log P^{\circ}_{AHB}$ between EA and CF is significant, while the values of n and K°_{ex} of EA are not very much different from those of CF. On the other hand, the difference in $\log P^{\circ}_{AHB}$ between EA and PeOH is insignificant, but the values of n and K°_{ex} of EA are significantly different from those of PeOH. That is, the solvation number of PeOH for PABA is smaller than that of EA, but K°_{ex} of the former is much larger than that of the latter. This indicates that the solute–solvent interaction between PABA and PeOH might be stronger than that between PABA and EA, and PeOH is more effective than EA as a solvating agent for PABA.

In order to clarify the difference between organic solvents in the partition of polar solutes, it is necessary to know the solute–solvent interaction in detail. Although the solvation number and the extraction constant are useful, they alone cannot provide sufficient information to elucidate the interaction mechanism. Moreover, one great problem is the existence of water in the organic phases; it does not change the solvent polarity remarkably, as mentioned above, but it can interact with a polar solute, competing with the organic solvating agent. Undoubtedly this would influence the solute–solvent interaction in the organic phase, making interpretation complex and difficult.

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13) It was observed that the solvation of PC by CF is different from those of other solute–solvent pairs; the plot of $\log P'_{PC}$ *vs.* $\log [CF]$ is composed of two separate linear parts, which suggests two-step solvation of PC by CF. In Table II, the results of analysis of the solvation in the higher concentration range are shown. A study on this phenomenon is under way.