Chem. Pharm. Bull.
32(8)3287—3290(1984)

Partition Behavior of Procaine and *p*-Aminobenzoic Acid in Pentanol/Water and Ethyl Acetate/Water Systems

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(Received December 8, 1983)

The partition behavior of procaine and its hydrolyzed product, *p*-aminobenzoic acid (PABA), in pentanol/water and ethyl acetate (EA)/water systems were investigated. From the pH-dependent partition behavior, it was evident that only the neutral form of each solute can be partitioned into the organic phase. The intrinsic partition coefficient (P°) in each system was also determined. log P° in each solvent system was linearly correlated with that in the octanol/water system, except for that of PABA in the EA/water system. This indicates that the primary solvation forces in these partition systems are fundamentally similar to each other.

Keywords——procaine; *p*-aminobenzoic acid; partition; pentanol/water; ethyl acetate/water; solute–solvent interaction

It is well-known that procaine (PC) can be hydrolyzed to *p*-aminobenzoic acid (PABA) and diethylaminoethanol.[2)] For quality control of pharmaceutical preparations of unstable drugs, not only should the degradation products be known, but also their amounts should be examined. In order to develope a solvent extraction method to detect the degradation products of PC, the partition behavior of PC and PABA in pentanol (PeOH)/water and ethyl acetate (EA)/water systems were examined.

Experimental

Materials——PC was provided by Fuso Pharmaceutical Industry, and PABA was supplied by Tokyo Kasei Kogyo. PeOH and EA were purchased from Wako Pure Chemical Industry. All other chemicals were of analytical reagent grade.

Determination of Partition Coefficients——Partition experiments were performed at 25 ± 0.1 °C, according to the usual method. The concentrations of PC and PABA were determined by spectroscopy with a Hitachi spectrophotometer, model 100-60, at $\lambda_{\max}$.

Results and Discussion

Partition Equilibria of PC and PABA

Both PC and PABA are weak electrolytes; PC is a diacidic base, while PABA is an amphoteric molecule. If only the neutral form of each solute can be partitioned into the organic phase, the partition equilibria can be expressed as follows. Firstly, in the case of PC, an apparent partition coefficient, P'_{PC}, is defined by Eq. 1, where PC, PCH^+, and PCH_2^{2+} denote neutral,

$$P'_{PC}=\frac{[PC]_o}{[PC]_w+[PCH^+]_w+[PCH_2^{2+}]_w} \tag{1}$$

mono- and diprotonated forms of procaine, and subscripts w and o represent water and organic phases, respectively. From Eq. 1, the acid dissociation constant, and the definition of the intrinsic partition coefficient, $P^{\circ}_{\mathrm{PC}}=[\mathrm{PC}]_{\mathrm{o}}/[\mathrm{PC}]_{\mathrm{w}}$, Eq. 2 can be derived.

$$P'_{\mathrm{PC}}=P^{\circ}_{\mathrm{PC}}\left\{1+\frac{(\mathrm{H}^{+})}{K_{\mathrm{a2}}}+\frac{(\mathrm{H}^{+})^{2}}{K_{\mathrm{a1}}K_{\mathrm{a2}}}\right\}^{-1} \qquad (2)$$

When $\mathrm{pH} \gg \mathrm{p}K_{\mathrm{a1}}$, Eq. 2 can be simplified to Eq. 3. In practice, this is a partition equation for a

$$P'_{\mathrm{PC}}=P^{\circ}_{\mathrm{PC}}\left\{1+\frac{(\mathrm{H}^{+})}{K_{\mathrm{a2}}}\right\}^{-1} \qquad (3)$$

monoacidic base, when only a neutral species can be transferred to the organic phase. When $\mathrm{pH} \gg \mathrm{p}K_{\mathrm{a2}}$, Eq. 3 can be simplified to Eq. 4, and when $\mathrm{pH} \ll \mathrm{p}K_{\mathrm{a2}}$, to Eq. 5. Thus, when $\mathrm{pH} \gg \mathrm{p}K_{\mathrm{a2}}$, P'_{PC} is equal to P°_{PC}, and when $\mathrm{pH} \ll \mathrm{p}K_{\mathrm{a2}}$, $\log P'_{\mathrm{PC}}$

$$\log P'_{\mathrm{PC}}=\log P^{\circ}_{\mathrm{PC}} \qquad (4)$$

$$\log P'_{\mathrm{PC}}=\mathrm{pH}-\mathrm{p}K_{\mathrm{a2}}+\log P^{\circ}_{\mathrm{PC}} \qquad (5)$$

increases with pH with a slope of +1.

Secondly, the partition mechanism of PABA was studied, and we investigated the applicability of the partition equation (Eq. 6) derived by Terada,[3)]

$$P'_{\mathrm{AHB}}=P^{\circ}_{\mathrm{AHB}}\left\{1+\frac{K_{\mathrm{a2}}}{(\mathrm{H}^{+})}+\frac{(\mathrm{H}^{+})}{K_{\mathrm{a1}}}\right\}^{-1} \qquad (6)$$

where the partition of only the neutral form is assumed, neglecting the participation of a zwitterionic form, and the subscript AHB denotes the neutral form of PABA. Equation 6 implies that when $\mathrm{pH} \ll \mathrm{p}K_{\mathrm{a1}}$, $\log P'_{\mathrm{AHB}}$ increases with pH with a slope of +1, and when $\mathrm{pH} \gg \mathrm{p}K_{\mathrm{a2}}$, it decreases with pH with a slope of −1.[3)]

pH Dependence of the Partition of PC and PABA

The pH dependence of the partition of PC and PABA between EA and water is shown in Fig. 1. Each point indicates the experimental value of $\log P'$ at the indicated pH and the solid lines show the calculated relations of $\log P'$ *vs.* pH predicted by Eqs. 3 and 6 for PC and PABA, respectively. It is clear that the experimental points are in good agreement with the calculated lines in both cases.

In the case of PC, $\log P'_{\mathrm{PC}}$ increases with pH in the low pH region with a slope of +1, while in the higher pH region it gradually approaches a constant value. Since this pH-profile of $\log P'_{\mathrm{PC}}$ is predicted by Eq. 3, at least in this pH range, the partition equilibrium of PC

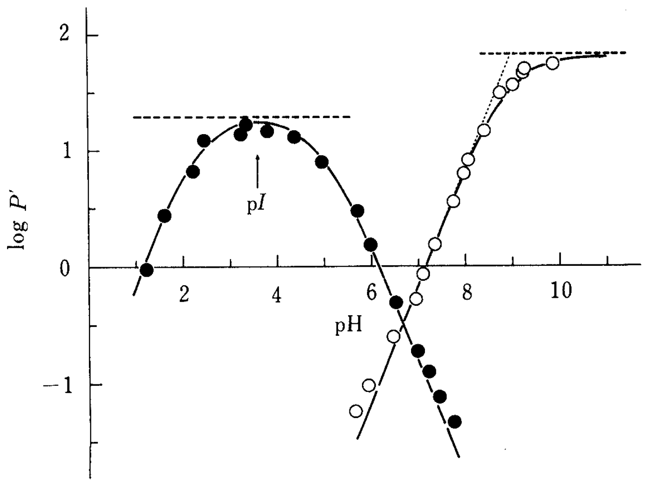

Fig. 1. pH-Dependences of Apparent Partition Coefficients, $\log P'$, of Procaine (○) and PABA (●) in the Ethyl Acetate/Water System

Solid line: calculated by using Eq. 3 for PC and Eq. 6 for PABA. Dashed line: the intrinsic partition coefficient, $\log P^{\circ}$. Dotted line: calculated by using Eq. 4. p*I* indicates the isoelectric point of PABA.

Table I. Intrinsic Partition Coefficients, $\log P^{\circ}$, of Procaine and PABA in Pentanol (PeOH)/Water and Ethyl Acetate (EA)/Water Systems

Partition systems		PeOH/water	EA/water	Octanol/water
Procaine	$\log P^{\circ}_{\mathrm{PC,expl}}$	1.99	1.81	$1.87^{a)}$
	$\log P^{\circ}_{\mathrm{PC,calc}}$	$1.78^{c)}$	$1.79^{c)}$	$1.86^{b)}$
PABA	$\log P^{\circ}_{\mathrm{AHB,expl}}$	0.90	1.29	$0.68^{a)}$
	$\log P^{\circ}_{\mathrm{AHB,calc}}$	$0.82^{c)}$	$0.69^{c)}$	$0.58^{b)}$

a) Taken from Ref. 4.
b) Calculated by using the hydrophobic substituent constants.[4]
c) Calculated by using Eq. 7, with reference to the octanol/water system.

seems to be well described by Eq. 3. Due to possible alkaline hydrolysis of PC at a higher pH region, P°_{PC} cannot be determined directly from Eq. 4. By using the acid dissociation constant ($\mathrm{p}K_{\mathrm{a2}}=8.96$) and the value of pH at the intercept with the abscissa, $\log P^{\circ}_{\mathrm{PC}}$ can be determined by the use of Eq. 5 (dashed line in Fig. 1). With this value of $\log P^{\circ}_{\mathrm{PC}}$ and $\mathrm{p}K_{\mathrm{a2}}$, $\log P'_{\mathrm{PC}}$ at various pH values can be calculated by using Eq. 3, as shown in Fig. 1 by the solid line. Despite a slight deviation from the calculated line at the lower pH region, Eq. 3 is essentially valid above pH 6.5. Below pH 6.5, the partition of ion-pairs with buffer components appears not to be negligible.

On the other hand, in the case of PABA, the plot of $\log P'_{\mathrm{AHB}}$ *vs.* pH seems to be parabolic: in the lower pH region it increases with pH with a slope of +1, and in the higher pH region it decreases with pH with a slope of −1, as expected from Eq. 6. The intrinsic partition coefficient of PABA was determined as in the case of PC as indicated in Fig. 1. In Fig. 1 p*I* indicates the isoelectric point of PABA, at which the pH-profile of $\log P'_{\mathrm{AHB}}$ shows a maximum, as reported by Terada.[3] Similar experiments were performed in another partition system of PeOH/water, and $\log P^{\circ}$ values for both solutes were also determined in the same way.

log P°_{PC} and log P°_{AHB} in PeOH/Water and EA/Water Systems

The intrinsic partition coefficients of PC and PABA in the two partition systems of PeOH/water and EA/water are summarized in Table I. For reference, $\log P^{\circ}$s for PC and PABA in the octanol/water system are also listed.[4] Based on linear free energy relationships, the partition coefficient of a solute in octanol/water can be calculated by using the hydrophobic substituent constant, unless a strong intra- and/or intermolecular interaction is present.[4] $\log P^{\circ}_{\mathrm{PC}}$ and $\log P^{\circ}_{\mathrm{AHB}}$ in octanol/water system were estimated to be 1.86 and 0.58, respectively, in good agreement with the reported values. Thus, in the octanol/water system no specific interaction seems to occur.

Since the hydrophobic substituent constant in the present partition systems has not been obtained, $\log P^{\circ}$s for PC and PABA cannot be estimated by the above method for the octanol/water system. Leo[5] states that a linear relationship between $\log P'$s will exist if the primary solvation forces in the two solvent systems are similar to one another, where a and b are constants,

$$\log P_2 = \mathrm{a}\ \log P_1 + \mathrm{b} \tag{7}$$

and subscripts 1 and 2 denote the respective solvent systems.Choosing octanol/water as a standard solvent system (P_1), $\log P^{\circ}$s for PC and PABA in the two partition systems were estimated by using the constants given by Leo.[5] Since $\log P^{\circ}_{\mathrm{PC,calc}}$ agreed well with the experimental values in both partition systems, the partition of PC in these solvent systems is linearly related to that in the octanol/water system and the primary solvation forces between

PC and these solvents seem to be similar to each other.

The partition of PABA in PeOH/water is also linearly correlated to that in octanol/water as expected, while that in the EA/water system cannot be simply predicted by Eq. 7. In the EA/water system, $\log P^{\circ}_{\mathrm{AHB,expl}}$ is about twice the calculated value. Since no abnormality in the concentration dependence of $\log P'_{\mathrm{AHB}}$ in EA/water system was observed, a change of the dissolution state of PABA in both phases such as a polymerization can be ruled out. Thus, there may be a slightly different solute–solvent interaction between PABA and EA, in contrast to the case of PABA and alcoholic solvents. Although the detailed interaction mechanism is not clear, presumably the nucleophilicity or the basicity of EA might enhance the affinity for the amphoteric solute of PABA.

Acknowledgement The authors are grateful to Dr. S. Kanoh, the director of this institute, for his kind advice and encouragement.

References and Notes

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