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# Ion-Pair Partition Mechanism of Methyl Orange with Aminoalkanols and Alkylamines

Yuko YOSHIKAWA and Hiroshi TERADA*

*Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi-1, Tokushima 770, Japan.*

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**Ion-pair partition of methyl orange anion with aminoalkanol and alkylamine cations between *n*-octanol and buffer solution was studied under various conditions. Transfer of methyl orange from the aqueous to the octanol phase increased with increase in the concentration of amines, and with increase in their alkyl chain length. The ion-pair partition consists of two steps: formation of the ion-pair complex in the aqueous phase and its transfer into the octanol phase. Although the ion-pair formation constant was almost independent of alkyl chain length of the amine and temperature, the ion-pair partition coefficient increased with increase in the chain length, both of these parameters being greater with alkylamines than with aminoalkanols. From the values of thermodynamic parameters, the mechanism of ion-pair partition of methyl orange with amine cation was discussed.**

**Keywords** ion-pair partition; methyl orange; aminoalkanol; alkylamine; hydrophobicity; *n*-octanol

For understanding of action mechanisms of bioactive compounds their transfer mechanism into the hydrophobic region is of primary importance, because their action sites are generally hydrophobic such as biomembranes and proteins. In fact, induction of biological activities of bioactive compounds is well known to be dependent on their hydrophobic property represented by oil-water partition coefficient ($P$), *n*-octanol being commonly used as an oil, and the partition mechanism has been extensively studied.[1,2] Thus, the logarithmic form of partition coefficient between octanol and water ($\log P_{\mathrm{oct}}$) of bioactive compounds is practically used as their hydrophobic parameter,[1] although $P_{\mathrm{oct}}$ does not solely represent the hydrophobicity of bioactive compounds.[2] However, studies on the partition process as well as determination and characterization of $P_{\mathrm{oct}}$ are performed mainly on the unionizable compounds[1] and partition process of the ionic compounds does not seem to be well known.

As the amount of transfer of an ionic compound into hydrophobic region such as *n*-octanol is regarded as very low due to the high polarity of the compound,[3] transfer is mainly achieved by formation of an ion-pair complex with inorganic and organic counter ions.[3] Thus, the ion-pair partition mechanism is important for understanding of partition mechanism of bioactive ions. The dye methyl orange can be regarded as a suitable model compound of the anionic form of bioactive compounds, because it has a strong acidic sulfonic acid group, its dimethylamino group being electrically neutral at above pH 5,[4,5] and its amount is easily determined spectrophotometrically.

In a previous paper,[6] we reported the ion-pair partition of the methyl orange anion ($M^-$) with alkali metal cations such as $Na^+$, $K^+$ and $Cs^+$ in the octanol/water system. As an extension of this study, we studied its ion-pair partition with organic amines such as aminoalkanols $N^+H_3(CH_2)_nOH$ and alkylamines $N^+H_3(CH_2)_{n-1}CH_3$, $n$ being the total number of $CH_2$ and $CH_3$. Results of this study would also be useful for understanding the binding mechanism of anionic drugs to proteins, because these amines could be a model of cationic amino acid residues such as lysine.

## Results

**Model of Ion-Pair Partition of Methyl Orange** As direct transfer of $M^-$ into the octanol phase and protonation of $M^-$ in both aqueous and octanol phases under neutral pH region are negligible, almost all the $M^-$ is transferred from the aqueous to the octanol phase through the formation of ion-pair complexes with counter cations $X^+$ and $Z^+$.[4,5] Here, $X^+$ and $Z^+$ are the alkali metal cations, $Na^+$ and $K^+$ which are present as components of the buffer solution, and the amine cations, $N^+H_3(CH_2)_nOH$ and $N^+H_3(CH_2)_{n-1}CH_3$, respectively. The partition coefficients of the ion-pairs $M^-X^+$ and $M^-Z^+$ ($P_{MX}$ and $P_{MZ}$, respectively), are shown by Eqs. 1 and 2,

$$P_{MX}=[MX]_o/[MX]_w \tag{1}$$

$$P_{MZ}=[MZ]_o/[MZ]_w \tag{2}$$

where brackets represent concentration, the activity coefficients of these ion species being assumed to be 1.0.

The ion-pair formation constants of $M^-$ with $X^+$ and $Z^+$ in the aqueous ($K_{MX}$ and $K_{MZ}$), and octanol ($K'_{MX}$ or $K'_{MZ}$) phases are expressed by Eqs. 3—6.

$$K_{MX}=[MX]_w/[M^-]_w[X^+]_w \tag{3}$$

$$K_{MZ}=[MZ]_w/[M^-]_w[Z^+]_w \tag{4}$$

$$K'_{MX}=[MX]_o/[M^-]_o[X^+]_o \tag{5}$$

$$K'_{MZ}=[MZ]_o/[M^-]_o[Z^+]_o \tag{6}$$

Furthermore, the apparent partition coefficient $P'$ of methyl orange is expressed by Eqs. 7 and 8, in which $C_w$ and $C_o$ are referred to as the total methyl orange concentrations of the aqueous and organic phases, respectively.

$$P'=C_o/C_w \tag{7}$$

$$=\frac{[MH]_o+[M^-]_o+[MX]_o+[MZ]_o}{[MH]_w+[M^-]_w+[MX]_w+[MZ]_w} \tag{8}$$

In these equations, existence of the neutral form MH of methyl orange in both phases, and the anionic form $M^-$

in the organic phase was taken into consideration, but that of the cationic form of methyl orange was ignored. From Eqs. 1—8, $P'$ can be shown by Eq. 9, when $\mathrm{pH} \gg \mathrm{p}K_a$ of methyl orange,

$$P' = \frac{(Q + P_{MX}K_{MX}[X^+]_w) + P_{MZ}K_{MZ}[Z^+]_w}{(1 + K_{MX}[X^+]_w) + K_{MZ}[Z^+]_w} \tag{9}$$

where

$$Q = P_{MX}K_{MH}[H^+]_w + P_{M^-} \tag{10}$$

$Q$ is $([MH]_o + [M^-]_o)/[M^-]_w$ and can be determined from the intercept of the linear relation between $P'$ and $[X^+]_w$, when $Z^+$ is absent. In Eq. 10, $P_{MH}$ and $P_{M^-}$ are the partition coefficients of MH and $M^-$, respectively, and $K_{MH}$ is the association constant of $M^-$ with $H^+$ to form the neutral form MH, *i.e.*, $K_{MH} = 1/K_a$. Denoting $(1 + K_{MX}[X^+]_w)$ as $R$ and $(Q + P_{MX}K_{MX}[X^+]_w)$ as $S$, Eq. 9 becomes Eq. 11, and is represented by Eq. 12.

$$P' = \frac{S + P_{MZ}K_{MZ}[Z^+]_w}{R + K_{MZ}[Z^+]_w} \tag{11}$$

$$\frac{P' - (S/R)}{P'} \cdot \frac{1}{[Z^+]_w} = \frac{P_{MZ}K_{MZ}}{R} \cdot \frac{1}{P'} - \frac{K_{MZ}}{R} \tag{12}$$

Thus, a plot of $(P' - (S/R))/P'[Z^+]_w$ against $1/P'$ gives a straight line with a slope of $P_{MZ}K_{MZ}/R$ and an intercept of $K_{MZ}/R$, and the ion-pair formation constant $K_{MZ}$ and ion-pair partition coefficient $P_{MZ}$ can be determined from the intercept and slope, respectively, of this plot.

Apparent partition coefficient $P'$ at various $[Z^+]_w$ can be determined under conditions where $[X^+]_w$ and $[Z^+]_w$ do not change appreciably during the partition process and there is no actual direct transfer of methyl orange either in its anionic or protonated neutral forms in the partition equilibrium system. To achieve these conditions, $[X^+]_w$ and $[Z^+]_w$ should be much higher than the methyl orange concentration in the aqueous phase, and the pH value of buffer solution should be about 1.5 lower than $\mathrm{p}K_a$ of amines.[3] As $\mathrm{p}K_a$ values of the amino group of $N^+H_3(CH_2)_nOH$ and $N^+H_3(CH_2)_{n-1}CH_3$ are about 9.5 and 10.5,[7] respectively, they are completely in the cationic forms at pH less than 7.0 and as the $\mathrm{p}K_a$ of dimethylamino group of methyl orange is about 3.4,[4] methyl orange takes completely anionic form at above pH 5. Under these conditions the transfer of methyl orange into the octanol phase by forming ion-pair complexes can be regarded to proceed by the mechanism shown in Fig. 1. The ion-pair extraction constant $E_{MZ}$ is actually expressed by Eq. 13, because the transfer of MX is expected to be much smaller than that of MZ.[3,5,6]

$$E_{MZ} = [MZ]_o/[M^-]_w[Z^+]_w = P_{MZ}K_{MZ} \tag{13}$$

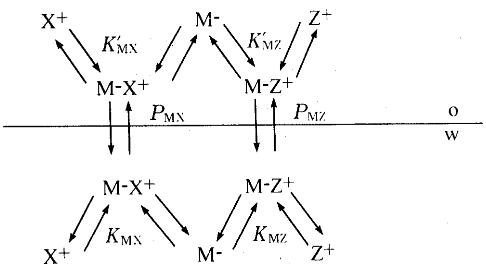

Fig. 1. Possible Model of Partition of Methyl Orange Anion ($M^-$) with Counter Cations $X^+$ and $Z^+$ between Aqueous (w) and Octanol (o) Phases

**Change in the Partition of Methyl Orange with Amine Concentrations at Various Temperatures** First, we carried out partition of methyl orange at various concentrations of aminoalkanols and alkylamines under conditions in which the concentration of methyl orange was much less than those of amines at about pH 7 and at 25 °C. Figure 2 shows dependence of the apparent ion-pair partition coefficient of methyl orange $P'$ on the aminoalkanol $N^+H_3(CH_2)_nOH$ concentration with $n$ being 2, 3 and 5. In all cases, $P'$ increased with increase in the aminoalkanol concentration $[Z^+]_w$ and $P'$ was greater with greater $n$. Values of $S$ and $R$ were determined in the partition of methyl orange in the presence of $X^+ (= K^+$ or $Na^+)$, but in the absence of $Z^+$. Plots of $(P' - (S/R))/P'[Z^+]_w$ *vs.* $1/P'$ according to Eq. 12 showed linear relationship with all the aminoalkanols examined (Fig. 3), indicating achievement of the ion-pair partition equilibrium shown in Fig. 1. Similar results were obtained with alkyl amines of $n$ being 3, 4 and 5 (data not shown). From these linear relationships, the ion-pair partition coefficient $P_{MZ}$ and ion-pair formation constant $K_{MZ}$ of methyl orange anion

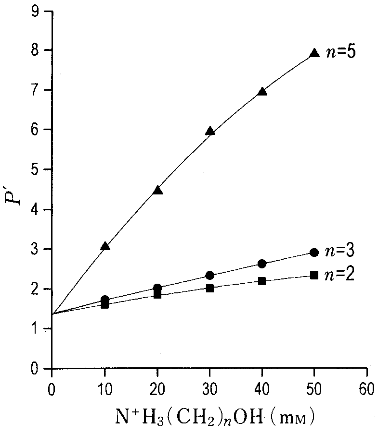

Fig. 2. Change in the Apparent Coefficient $P'$ of Methyl Orange with the Concentration of Aminoalkanols $N^+H_3(CH_2)_nOH$ at 25 °C

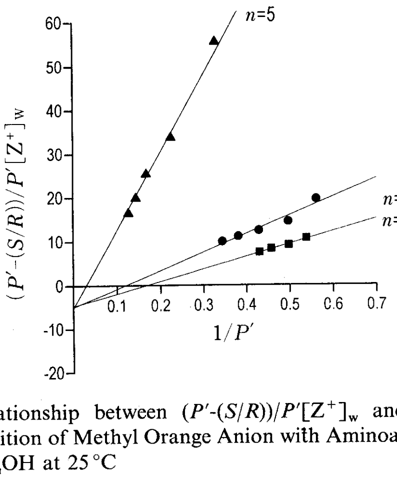

Fig. 3. Relationship between $(P'\text{-}(S/R))/P'[Z^+]_w$ and $1/P'$ in the Ion-Pair Partition of Methyl Orange Anion with Aminoalkanol Cations $N^+H_3(CH_2)_nOH$ at 25 °C

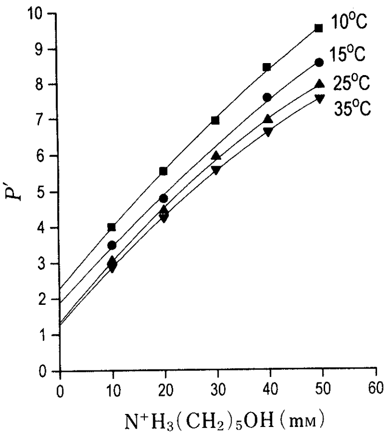

Fig. 4. Apparent Partition Coefficient $P'$ of Methyl Orange in the Presence of $N^+H_3(CH_2)_5OH$ at Various Temperatures

Table I. Values of $K_{MZ}$, $P_{MZ}$ and $E_{MZ}$ for Ion-Pair Partition of Methyl Orange with Aminoalkanols[a)]

| $n$ | Temperature (°C) | $\log K_{MZ}$ ($M^{-1}$) | $\log P_{MZ}$ | $\log E_{MZ}$ ($M^{-1}$) |
|---|---|---|---|---|
| 2 | 10 | $0.68\pm0.03$ | $0.95\pm0.03$ | $1.63\pm0.01$ |
| | 15 | $0.73\pm0.02$ | $0.85\pm0.02$ | $1.58\pm0.01$ |
| | 25 | $0.85\pm0.03$ | $0.60\pm0.10$ | $1.45\pm0.06$ |
| 3 | 10 | $0.68\pm0.01$ | $1.19\pm0.01$ | $1.87\pm0.01$ |
| | 15 | $0.76\pm0.01$ | $1.07\pm0.01$ | $1.83\pm0.01$ |
| | 25 | $0.85\pm0.02$ | $0.93\pm0.01$ | $1.78\pm0.02$ |
| 5 | 10 | $0.71\pm0.04$ | $1.70\pm0.02$ | $2.41\pm0.05$ |
| | 15 | $0.73\pm0.02$ | $1.61\pm0.04$ | $2.34\pm0.02$ |
| | 25 | $0.85\pm0.06$ | $1.55\pm0.05$ | $2.40\pm0.01$ |
| | 35 | $0.95\pm0.02$ | $1.44\pm0.01$ | $2.39\pm0.01$ |

a) Z represents aminoalkanols $N^+H_3(CH_2)_nOH$. The mean values with S.D. of at least three runs are shown.

Table II. Values of $K_{MZ}$, $P_{MZ}$ and $E_{MZ}$ for Ion-Pair Partition of Methyl Orange with Alkylamines[a)]

| $n$ | Temperature (°C) | $\log K_{MZ}$ ($M^{-1}$) | $\log P_{MZ}$ | $\log E_{MZ}$ ($M^{-1}$) |
|---|---|---|---|---|
| 3 | 10 | $0.90\pm0.02$ | $1.58\pm0.05$ | $2.48\pm0.07$ |
| 4 | 10 | $0.93\pm0.01$ | $2.08\pm0.01$ | $3.02\pm0.01$ |
| | 15 | $1.03\pm0.03$ | $1.97\pm0.06$ | $2.99\pm0.03$ |
| | 25 | $1.13\pm0.03$ | $1.83\pm0.02$ | $2.96\pm0.02$ |
| 5 | 10 | $1.05\pm0.01$ | $2.57\pm0.01$ | $3.62\pm0.01$ |
| | 15 | $1.09\pm0.04$ | $2.49\pm0.04$ | $3.58\pm0.01$ |
| | 25 | $1.17\pm0.03$ | $2.34\pm0.06$ | $3.51\pm0.03$ |

a) Z represents alkylamines $N^+H_3(CH_2)_{n-1}CH_3$. The mean values with S.D. of at least three runs are shown.

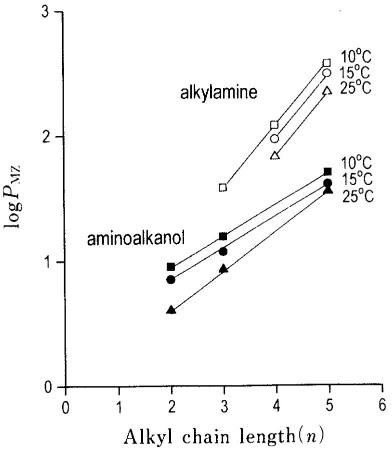

Fig. 5. Relationship between $\log P_{MZ}$ and Alkyl Chain Length of Aminoalkanols or Alkylamines

with aminoalkanol and alkylamine cations were determined.

Values of $P'$ were further determined at various temperatures. With all the aminoalkanols and alkylamines examined, $P'$ decreased with increase in temperature. The results with aminopentanol ($n=5$) are shown in Fig. 4 as an example. In all cases, linear relationships between $(P'-(S/R))/P'[Z^+]_w$ and $1/P'$ were observed as in Fig. 3. Values of $P_{MZ}$ and $K_{MZ}$ of methyl orange anion were determined with all the aminoalkanols and alkylamines at various temperatures, and these values together with the ion-pair extraction constant $E_{MZ}$ are summarized in Table I for aminoalkanols and in Table II for alkylamines.

With aminoalkanols, $K_{MZ}$ was almost independent of their chain length $n$, and increased with rising temperature. In contrast, $P_{MZ}$ increased with increase in $n$, but decreased with rise in temperature (Table I). Similar tendencies were observed with alkylamines (Table II). At certain $n$ and temperature, both $K_{MZ}$ and $P_{MZ}$ of alkylamines were always greater than those of the corresponding aminoalkanols, hence the extraction constant $E_{MZ}$ of alkylamines was greater than that of the corresponding aminoalkanols. The value of $\log P_{MZ}$ increased linearly with $n$ in both aminoalkanols and alkylamines shown in Fig. 5. Their relations at 10 °C are represented in Eqs. 14 and 15 for aminoalkanols and alkylamines, respectively.

$$\log P_{MZ}(\text{aminoalkanols}) = 0.24n + 0.51 \tag{14}$$

$$\log P_{MZ}(\text{alkylamines}) = 0.50n + 0.07 \tag{15}$$

The slope of the linear relationship slightly increased with increase in temperature for aminoalkanols, but was almost the same for alkylamines.

From the values of $P_{MZ}$, $K_{MZ}$ and $E_{MZ}$ at various temperatures, the thermodynamic parameters free energy change $\Delta G^{\ominus}$, entropy change $\Delta S^{\ominus}$ and enthalpy change $\Delta H^{\ominus}$ at 25 °C of $P_{MZ}$, $K_{MZ}$ and $E_{MZ}$ were determined by Eqs. 16, 17 and 18, respectively, and these values are summarized in Tables III, IV and V. In these equations, the equilibrium constant $K$ represents $K_{MZ}$, $P_{MZ}$ or $E_{MZ}$.

$$\Delta G^{\ominus} = -RT\ln K \tag{16}$$

$$\left(\frac{\partial(-RT\ln K)}{\partial T}\right)_p = -\Delta S^{\ominus} \tag{17}$$

$$\left(\frac{\partial \ln K}{\partial(1/T)}\right)_p = -\frac{\Delta H^{\ominus}}{R} \tag{18}$$

The values of $\Delta G^{\ominus}$ with $K_{MZ}$ for both aminoalkanols and alkylamines were negative, but those of $\Delta S^{\ominus}$ and $\Delta H^{\ominus}$ were positive. All the thermodynamic parameters of $P_{MZ}$ took negative values, and values of $\Delta S^{\ominus}$ of $P_{MZ}$ and $E_{MZ}$ increased with increase in $n$. Similarly, $\Delta H^{\ominus}$ of $P_{MZ}$ increased with $n$, and this change was more significant with aminoalkanols.

TABLE III. Thermodynamic Parameters for $K_{MZ}$ with Aminoalkanols and Alkylamines at 25 °C

| $Z^+$ | $n$ | $\Delta G^{\ominus}$ (kJ·mol$^{-1}$) | $\Delta S^{\ominus}$ (J·K$^{-1}$·mol$^{-1}$) | $\Delta H^{\ominus}$ (kJ·mol$^{-1}$) |
|---|---|---|---|---|
| $N^+H_3(CH_2)_nOH$ | 2 | $-4.8\pm0.1$[a] | $77.2\pm9.7$ | $18.2\pm2.9$ |
| | 3 | $-4.8\pm0.1$ | $75.6\pm2.7$ | $17.7\pm0.8$ |
| | 5 | $-4.8\pm0.1$ | $72.8\pm9.7$ | $16.9\pm2.9$ |
| $N^+H_3(CH_2)_{n-1}CH_3$ | 4 | $-6.5\pm0.2$ | $92.7\pm14.9$ | $21.1\pm4.3$ |
| | 5 | $-6.7\pm0.2$ | $67.7\pm15.0$ | $13.5\pm4.3$ |

*a*) Errors are estimated from the standard deviations of $K_{MZ}$.

TABLE IV. Thermodynamic Parameters for $P_{MZ}$ with Aminoalkanols and Alkylamines at 25 °C

| $Z^+$ | $n$ | $\Delta G^{\ominus}$ (kJ·mol$^{-1}$) | $\Delta S^{\ominus}$ (J·K$^{-1}$·mol$^{-1}$) | $\Delta H^{\ominus}$ (kJ·mol$^{-1}$) |
|---|---|---|---|---|
| $N^+H_3(CH_2)_nOH$ | 2 | $-3.4\pm0.1$[a] | $-125.1\pm14.0$ | $-40.7\pm4.1$ |
| | 3 | $-5.3\pm0.1$ | $-73.8\pm6.7$ | $-27.3\pm2.0$ |
| | 5 | $-8.8\pm0.1$ | $-26.2\pm3.1$ | $-16.6\pm1.0$ |
| $N^+H_3(CH_2)_{n-1}CH_3$ | 4 | $-10.5\pm0.1$ | $-55.2\pm8.3$ | $-26.9\pm2.4$ |
| | 5 | $-13.3\pm0.3$ | $-37.8\pm7.1$ | $-24.4\pm7.1$ |

*a*) Errors are estimated from the standard deviations of $P_{MZ}$.

TABLE V. Thermodynamic Parameters for $E_{MZ}$ with Aminoalkanols and Alkylamines at 25 °C

| $Z^+$ | $n$ | $\Delta G^{\ominus}$ (kJ·mol$^{-1}$) | $\Delta S^{\ominus}$ (J·K$^{-1}$·mol$^{-1}$) | $\Delta H^{\ominus}$ (kJ·mol$^{-1}$) |
|---|---|---|---|---|
| $N^+H_3(CH_2)_nOH$ | 2 | $-8.3\pm0.2$[a] | $-40.1\pm14.2$ | $-20.2\pm4.0$ |
| | 3 | $-10.1\pm0.1$ | $2.1\pm8.9$ | $-9.5\pm2.6$ |
| | 5 | $-13.7\pm0.1$ | $42.0\pm3.2$ | $-1.2\pm1.0$ |
| $N^+H_3(CH_2)_{n-1}CH_3$ | 4 | $-17.2\pm0.2$ | $37.4\pm6.6$ | $-5.9\pm1.9$ |
| | 5 | $-20.2\pm0.1$ | $34.9\pm15.0$ | $-9.7\pm4.3$ |

*a*) Errors are estimated from the standard deviations of $E_{MZ}$.

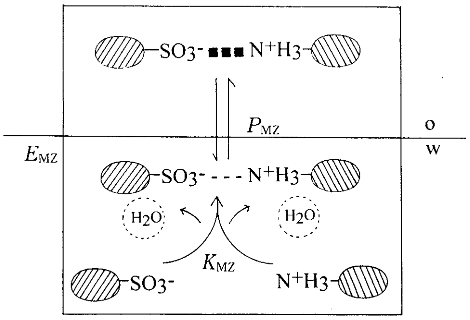

Fig. 6. Schematic Model of Ion-Pair Partition Process of Methyl Orange with Aminoalkanols and Alkylamines

▨—$SO_3^-$ represents methyl orange anion and $N^+H_3$—▨ represents aminoalkanol and alkylamine cations. The bold discontinuous line in the ion-pair complex in the octanol phase indicates stronger ionic attraction than that in the aqueous phase.

## Discussion

We examined the effects of aminoalkanol and alkylamine cations on the partition of methyl orange anion between octanol and aqueous phases. According to our present model shown in Fig. 1, the ion-pair partition of methyl orange consists of two processes: formation of an ion-pair complex with the counter cation $Z^+$ in the aqueous phase ($K_{MZ}$), and the transfer of the ion-pair complex to the octanol phase ($P_{MZ}$). Total degree of transfer of the ion-pair complex is shown by the extraction constant $E_{MZ}$. These values were determined by Eq. 12 under conditions where direct transfers of the molecular species $M^-$ and its neutral form MH, the counter cation $X^+$ which was present as a buffer component, and the cationic forms of aminoalkanols and alkylamines $Z^+$ as well as their neutral forms could be ignored. In fact, these conditions were achieved in this study under present experimental conditions. Thus, we hereafter discussed these two processes mainly from a thermodynamic point of view.

**Ion-Pair Formation** The $K_{MZ}$ values of aminoalkanols were essentially the same at certain temperatures (Table I) and similar tendencies were found for alkylamines (Table II). These results indicate that hydrophobicity of the amines is not appreciably associated with formation of the ion-pair complex with methyl orange anion. It is noteworthy that the entropy changes $\Delta S^{\ominus}$ of $K_{MZ}$ with all the aminoalkanols and alkylamines were positive (Table III). As hydration of ions results in the negative $\Delta S^{\ominus}$, the positive $\Delta S^{\ominus}$ of $K_{MZ}$ is regarded as a result of dehydration of $M^-$ and $Z^+$ by formation of the ion-pair complex $M^-Z^+$.

**Ion-Pair Partition** Values of $P_{MZ}$ and $E_{MZ}$ of the methyl orange anion complexed with alkylamines were always greater than the corresponding values of aminoalkanols. In Eqs. 14 and 15, increase of $\log P_{MZ}$ per $CH_2$ group was 0.24 with aminoalkanols, but was 0.5 with alkylamines at 10 °C. The value of 0.5 with alkylamines well agreed with that reported with the neutral alkylamines[8] and other alkyl compounds.[9] The small value with aminoalkanols is noted, which is attributable to presence of the OH group. Similarly, the increment of $\log P_{MZ}$ per $CH_2$ was 0.5 with alkylamines at other temperatures. In contrast, the value was smaller with aminoalkanols, and became greater with higher temperatures (*cf.* Tables I and II).

Results of Table IV show that all the $\Delta G^{\ominus}$, $\Delta S^{\ominus}$ and $\Delta H^{\ominus}$ of $P_{MZ}$ were negative, indicating that the partition is driven by enthalpy. This can be explained by taking account of the difference between the dielectric constants of water and octanol. The electrostatic attractive force ($F(r)$) between monovalent ions is expressed by Eq. 19,

$$F(r)=\frac{e^2}{4\pi\varepsilon r^2} \tag{19}$$

where $\varepsilon$, $e$ and $r$ are the dielectric constant of a solvent, unit charge of an electron and the distance between ions, respectively. Equation 19 shows that $F(r)$ is inversely proportional to the dielectric constant of the environment.[10] As the dielectric constant of *n*-octanol is much smaller than that of water (10.3 and 80.4 at 20 °C,[7] respectively), the electrostatic interaction for the ion-pair complex in the octanol phase should be much greater than that in the aqueous phase. Therefore, the enthalpy $\Delta H^{\ominus}$ of $P_{MZ}$, which reflects the difference between the electrostatic force of the ion-pair complex in the octanol phase and that in the aqueous phase, was negative. The negative value of $\Delta S^{\ominus}$ of $P_{MZ}$ could be a result of the decrease in freedom of molecular motion, due to increase of the electrostatic attraction between methyl orange anion and the counter ion in the octanol phase.

A possible model of the ion-pair partition of methyl orange with aminoalkanols and alkylamines is depicted schematically in Fig. 6, indicating that the ion-pair partition is divided into two processes: ion-pair formation and transfer of the ion-pair. We have evaluated these two processes separately in a quantitative manner. It has been demonstrated that this model is very useful for further understanding of the interaction between anionic drugs and proteins.

## Experimental

**Materials** Methyl orange was obtained from Merck & Co. (Darmstadt). *n*-Octanol was the first grade product of Nacalai Tesque Inc. (Kyoto) and was saturated with water before use. The other reagents including alkylamines and aminoalkanols were of the highest grade commercially available.

**Measurement of Partition Coefficient** The aqueous phase contained $5 \times 10^{-5}$ M of methyl orange and an aminoalkanol $NH_2(CH_2)_nOH$ ($n=2$, 3 and 5) or an alkylamine $NH_2(CH_2)_{n-1}CH_3$ ($n=3$, 4 and 5) at various concentrations. The concentration of the alkylamine or aminoalkanol was always much greater than that of methyl orange to keep the amine concentration in the aqueous phase essentially constant before and after partition equilibrium. The pH of the aqueous phase was adjusted to 6.80 with 0.1 M sodium phosphate or potassium phosphate buffer solution with alkylamines, and to 6.45 with 0.1 M sodium phosphate buffer with aminoalkanols.

To the buffer solution (4—5 ml), which had been saturated with *n*-octanol and contained fixed amounts of methyl orange and an amine, the octanol (2—5 ml) that had been saturated with water was added, and these two phases were shaken gently in a water bath at a constant temperature for about 6 h; the mixture was then allowed to stand for 12 h at the same temperature to achieve partition equilibrium. As a next step, the concentration of methyl orange in the aqueous phase was determined spectrophotometrically in a Shimadzu spectrophotometer, model UV-180, at 464 nm. The concentration of methyl orange in the octanol phase ($C_o$) was determined from the difference between the initial concentration of methyl orange in the aqueous phase ($C_i$) and that after equilibrium ($C_w$) according to Eq. 20, where $V$ represents the volume of the aqueous (subscript w) and the organic (subscript o) phases.

$$C_o = (C_i - C_w)(V_w / V_o) \tag{20}$$

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