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Proposed Partition Mechanism of Tetracycline

Hiroshi Terada and Toshio Inagi

Faculty of Pharmaceutical Sciences, University of Tokushima[1]

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The partition mechanism of tetracycline in an *n*-octanol/H_2O system was studied by mathematical treatment of the changes in the apparent partition coefficient determined by Colaizzi and Klink (*J. Pharm. Sci.*, **58**, 1184 (1969)) and by us, and by measuring the spectral properties of tetracycline in organic solvents. Tetracycline exists in a neutral molecular form in *n*-octanol, so it could be concluded that transfer of tetracycline from an aqueous to an organic phase is governed not by the amount of the zwitterionic form, but by the amount of the neutral molecular form in the aqueous phase. Results showed that the tetracycline molecule itself is hydrophobic, but that the concentration of the neutral molecular species in the aqueous phase is very small.

The relationship between the apparent partition coefficient and the three macroscopic ionization constants of tetracycline at a certain pH was also clarified. From this the macroscopic ionization constants were evaluated. The values agreed very well with those determined by potentiometric titration.

Biomembranes constitute hydrophobic regions, so organic solvents have sometimes been used as very simple models of biomembranes, and the mechanisms of transfer of simple substanses, such as sodium, potassium, sugars and salicylic acid across biomembranes, have been studied by measuring their partition coefficients between aqueous and organic solvent phases.[2]

Most drugs are complex substances and may exist in ionized, neutral or associated forms. Thus it is important to determine which form is active, and which is responsible for the transfer of the drug from aqueous to hydrophobic regions.[3] Investigations on the partition behavior of drugs are very helpful in elucidating these problems.

The previous paper[4] reported the partition mechanism of *p*-aminobenzoic acid and sulfonamides, which have two ionizable groups. It was concluded that transfer of these compounds from an aqueous to an organic phase is due to their acid form, since the presence of their zwitterionic form can be neglected.[5]

It seems interesting to examine the partition of drugs having more than one ionizable group, to see whether the zwitterionic species or the neutral molecular form is transferred to the organic phase. For this study we chose tetracycline as a good example of an acid with three ionizable groups. Its ionization in the aqueous phase is complex and the amount of the neutral molecular form is negligibly small.[6,7] From the pH dependence of the partition

1) Location: *Shomachi-1, Tokushima.*

2) *a*) H.L. Rosano, K. Breindel, J.H. Shulman, and A.J. Eydt, *J. Colloid Interface Sci.*, **22**, 58 (1966); *b*) H.L. Rosano, *ibid.*, **23**, 73 (1967); *c*) H.L. Rosano, P. Duby, and J.H. Schulman, *J. Phys. Chem.*, **65**, 1704 (1961); *d*) H.P. Ting, G.L. Bertrand, and D.F. Sears, *Biophys. J.*, **6**, 813 (1966); *e*) J. Perrin, *J. Pharm. Pharmacol.*, **19**, 25 (1967); *f*) C.Y. Jung, J.E. Chaney, and P.G. LeFevre, *Arch. Biochem. Biophys.*, **126**, 664 (1968).

3) *a*) T. Fujita, *J. Med. Chem.*, **9**, 797 (1966); *b*) A. Finkelstein, *Biochim. Biophys. Acta*, **205**, 1 (1970); *c*) A. Korolkovas, "Essentials of Molecular Pharmacology," John Wiley & Sons, Inc., New York, 1970, p. 158; *d*) H. Terada and S. Muraoka, *Mol. Pharmacol.*, **8**, 95 (1972); *e*) H. Terada, *Biochim. Biophys. Acta*, **387**, 519 (1975).

4) H. Terada, *Chem. Pharm. Bull.* (Tokyo), **20**, 765 (1972).

5) I.M. Klotz and D.M. Gruen, *J. Amer. Chem. Soc.*, **67**, 843 (1945).

6) L.J. Leeson, J.E. Krueger, and R.A. Nash, *Tetrahedron Letters*, **18**, 1155 (1963).

7) *a*) C.R. Stephens, K. Murai, K.J. Brunings, and R.B. Woodard, *J. Amer. Chem. Soc.*, **78**, 4155 (1956); *b*) U.W. Kesselring and L.Z. Benet, *Anal. Chem.*, **41**, 1535 (1969).

of tetracycline, Colaizzi and Klink[8] concluded that the zwitterionic species is responsible for the transfer to the organic phase from the aqueous phase. In this paper we assumed that the partition of tetracycline is governed by the amount of the neutral molecular species in the aqueous phase. Mathematical treatments of experimental results by Colaizzi and Klink[8] and by ourselves, taking account of the molecular form in the organic phase, indicated the validity of this assumption. This result seems important in elucidating the partition mechanisms of organic acids having more than one ionizable group.

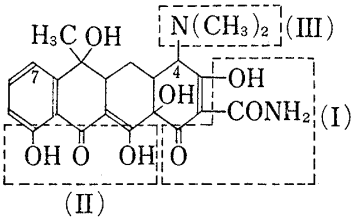

Chart. Molecular Structure of Tetracycline

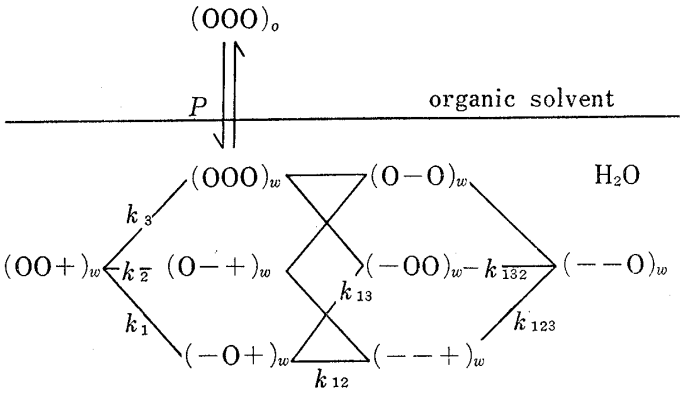

Fig. 1. Proposed Partition Mechanism and Ionization Schema of Tetracycline

Proposed Mechanism of Partition of Tetracycline

Tetracycline is thought to have three ionizable groups, a tricarbonylmethane system (I), a phenolic diketone moiety (II) and an ammonium cation (III), as shown in the Chart.[6] The complete ionization schema of tetracycline in an aqueous phase is shown in Fig. 1, where +, − and 0 represent the charges of the above ionizable groups, *i.e.*, cationic, anionic and neutral groups, respectively. In the brackets the first sign shows the charge of the tricarbonylmethane system, the second that of the phenolic diketone moiety and the third that of the ammonium cation. For example, [OO+] denotes a molecular species in which groups I and II are not ionized and III is protonated, while [− −O] denotes a molecular species in which groups I and II are both negative and III is neutral. The microscopic ionization constants (k) are also indicated in Fig. 1.

There are three macroscopic ionization constants, K_A, K_B and K_C and their relationships with the microscopic ionization constants are as follows;[9]

$$K_A = k_1 + k_2 + k_3 \tag{1}$$

$$K_A K_B = k_1 k_{12} + k_2 k_{23} + k_3 k_{31} = k_2 k_{21} + k_3 k_{32} + k_1 k_{13} \tag{2}$$

$$K_A K_B K_C = k_1 k_{12} k_{123} = k_2 k_{23} k_{231} = k_3 k_{31} k_{312} \tag{3}$$

In the case of tetracycline, the following equations are available.[6]

$$K_A \simeq k_1 = 4.68 \times 10^{-4} \tag{4}$$

$$K_B \simeq k_{12} + k_{13} = 1.78 \times 10^{-8} \tag{5}$$

$$1/K_C \simeq 1/k_{123} + 1/k_{132} = 2.46 \times 10^{-10} \tag{6}$$

So the molecular species which actually exist in the aqueous phase are [OO+], [−O+], [−OO] and [− −O], and the presence of other ionic forms can be neglected. However, as shown in Fig. 1, assuming that (i) a nonionized form of [OOO], which exists in very small amount

8) J.L. Colaizzi and P.R. Klink, *J. Pharm. Sci.*, **58**, 1184 (1969).
9) J.T. Edsall and J. Wyman, "Biophysical Chemistry," Vol. 1, Academic Press, New York, 1958, p. 494.

in the aqueous phase, is responsible for transfer of tetracycline to the hydrophobic phase, in which the only form present is [OOO] and (ii) that molecular associations between the species can be neglected, the following relationships are obtained:

$$P = (000)_o/(000)_w \tag{7}$$

$$P' = C_o/C_w$$

$$= \frac{(000)_o}{(00+)_w+(-0+)_w+(0-+)_w+(000)_w+(---+)_w+(-00)_w+(0-0)_w+(--0)_w}$$

$$= P \cdot \frac{1}{(K_A/k_3)(1+a/K_A+K_B/a+K_BK_C/a^2)} \tag{8}$$

In these equations, symbols in parentheses indicate the activities of each species, but in this study the concentration of tetracycline was always very low and so activity can be regarded as equal to concentration (C), and "a" as the activity of hydrogen ion ($-\log a$=pH). P is the true partition coefficient and P' is the apparent partition coefficient. The former is a physicochemical constant which is constant at all pH values, while the latter changes with pH. Denoting the ratio of the concentration of the molecular form (OOO) to the total concentration of tetracycline in the aqueous phase as N,

$$1/N = C_w/(000)_w$$

$$= (K_A/k_3)(1+(a/K_A)+(K_B/a)+(K_BK_C/a^2)) \tag{9}$$

$$= (K_A/k_3)(1/N') \tag{10}$$

Thus, from eq. (8),

$$\log P' = \log P + \log N \tag{11}$$

$$= \log P + \log (k_3/K_A) + \log N' \tag{12}$$

Eq. (11) indicates that the logarithm of P' is the sum of the logarithms of Pk_3/K_A and N'. N' changes with pH, but P and (k_3/K_B) are constant at all pH values. In the regions where "a" is much greater than K_A (pH$\ll$pK_A) and where "a" is much smaller than K_C (pH$\gg$pK_C), Eq. (11) can be expressed as Eqs. (13) and (14), respectively,

$$\log P' = \text{pH} + (\log P + \log k_3) \tag{13}$$

and

$$\log P' = -2\text{pH} + (\log P + \log k_3 - \log K_AK_BK_C) \tag{14}$$

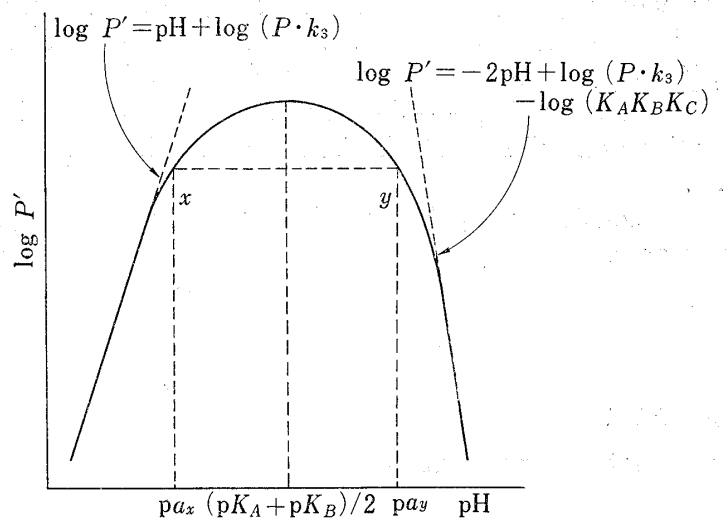

Fig. 2. Relationship between Apparent Partition Coefficient (P') and pH

The apparent partition coefficient takes the same value at points x and y, where the hydrogen ion activities are denoted as a_x and a_y respectively. ($-\log a_x$=pa_x)

It is also apparent from Eq. (9) and (11), that log P' is maximum at

$$\text{pH} = (\text{p}K_A+\text{p}K_B)/2 \tag{15}$$

Eqs. (13), (14) and (15) indicate that the apparent partition coefficient of tetracycline changes with the pH of the aqueous phase as shown in Fig. 2, and two straight lines with slopes of 1 and -2 are obtained in strongly acidic and strongly alkaline regions, respectively.

Eq. (11) indicates that the apparent partition coefficient P' is a linear function of the ratio of the concentration of tetracycline in the organic phase to that of the zwitterionic form in the aqueous phase and the slope of N' at a fixed pH is given by Eq. (16):

$$P' = N' \cdot (OOO)_o/(-O+)_w \qquad (16)$$

These relationships are also valid for other organic acids having three ionizable groups, which have more complicated ionization processes than that of tetracycline.

Experimental

Reagents——Tetracycline hydrochloride (lot No. 731212) was a gift from Dr. N. Koga, Daiichi Seiyaku Co., Ltd. *n*-Octanol was purified by shaking it with H_2O more than 10 times and was saturated with the buffer solution before use.

Measurements of Partition Coefficients——Volumes of 10 ml of $3.34\times10^{-4}-9.83\times10^{-4}$ M tetracycline in aqueous solution at pH 5.3 or 7.0 were shaken with 10 ml of *n*-octanol in a water bath at $25\pm0.1°$ for 36 hours to allow equilibration. Then the concentration of tetracycline in the aqueous phase was measured in a Hitachi Spectrophotometer, model 124, at the maximum of the spectrum in the visible region, since at this position there is little difference between the absorption spectra of tetracycline and of its epimer.[10] The buffer solutions and *n*-octanol used for partition experiments were equilibrated with the other phase before experiments. The apparent partition coefficient of tetracycline was determined from the difference between the initial concentration and that after equilibration.

Spectrophotometric Experiments——The absorption spectra of tetracycline in buffer solution, *n*-octanol and EtOH were measured in a Hitachi Spectrophotometer, model 124. The buffer solutions used were 0.2 M CH_3COONa–0.2 M HCl and 0.05 M Na_2HPO_4–0.01 M NaOH. The infrared spectrum of tetracycline was measured in a Hitachi EPU Infra-Red Spectrophotometer using NaCl cells.

Results

1. Partition Coefficient of Tetracycline

The apparent partition coefficients of tetracycline between *n*-octanol and H_2O at 25° were measured at initial concentrations of tetracycline in the aqueous phase of 3.34×10^{-4}M to 9.83×10^{-4}M at pH 5.3 and pH 7.0. The results are shown in Fig. 3. Both at pH 5.3 and pH 7.0, P' is constant, irrespective of the initial concentration of tetracycline (C_i). From the ionization constants and the concentration of tetracycline in the aqueous phase, the concentration of the zwitterionic form can be evaluated at each pH. Values of $(OOO)_o/(-O+)_w$ are plotted against P' at pH 5.3 and pH 7.0 in Fig. 4. The good linearities of both plots show the validity of Eq. (16). The slopes of the lines obtained at pH 5.3 and pH 7.0 are 0.99

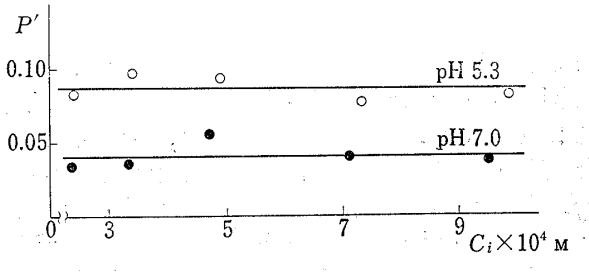

Fig. 3. Apparent Partition Coefficients of Tetracycline (P') with Various Initial Concentrations (C_i) in the Aqueous Phase

Partition coefficients were measured in an *n*-octanol/H_2O system at pH 5.3 (○) and pH 7.0 (●).

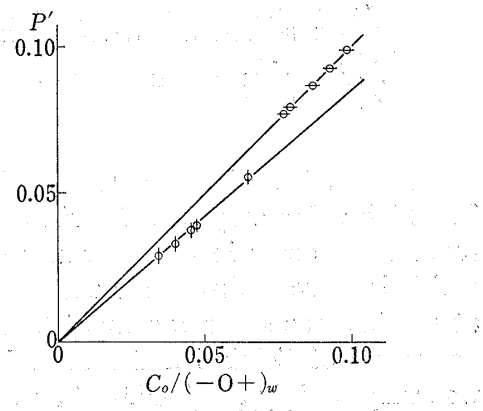

Fig. 4. Relationship between the Apparent Partition Coefficient (P') and $C_o/(-O+)_w$ of Tetracycline at pH 5.3 (⊖) and pH 7.0 (ϕ)

C_o: concentration of tetracycline in *n*-octanol
$(-O+)_w$: concentration of the zwitterionic form of tetracycline in the aqueous phase.

10) J.R.D. McCormick, S.M. Fox, L.L. Smith, B.A. Bitler, J. Reichenthal, V.E. Origoni, W.H. Muller, R. Winterbottom, and A.P. Doerschunk, *J. Amer. Chem. Soc.*, **79**, 2849 (1957).

and 0.85, respectively, which correspond to the N' values at these pH values (N' is 0.99 at pH 5.3 and 0.85 at pH 7.0).

These results indicate that there is no significant interaction between tetracycline molecules in the two phases over the concentration range examined. Colaizzi and Klink[8] measured the apparent partition coefficients of several tetracycline derivatives, including tetracycline, between *n*-octanol and H_2O at various pH values, with initial concentrations of 5.20×10^{-4}M in the aqueous phase. The above results indicate that we can use their data with ours.

Fig. 5 shows the relationship between pH and log P', determined by Colaizzi and Klink[8] and by us. In the figure the solid line shows the change of log N' with pH, calculated using the macroscopic ionization constants determined by Leeson, *et al.*[6] This curve is shown as the sum of log N' and (log P+log k_3/K_A) according to Eq. (12) regarding the latter as −1.10.

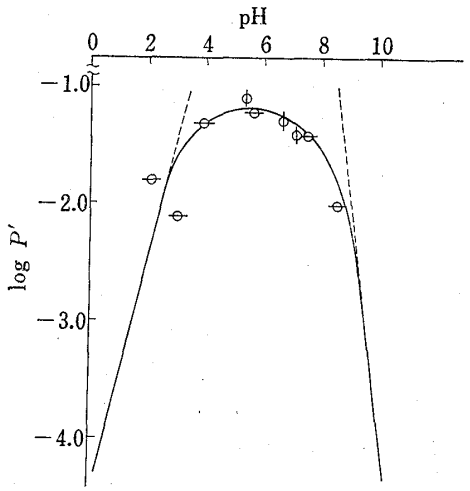

Fig. 5. Changes of the Apparent Partition Coefficient (P') of Tetracycline with pH

P' was measured in an *n*-octanol/H_2O system. The solid line indicates the curve of (log N' +log (k_3/K_A)).
⊖: determined by Colaizzi and Klink[8]
ϕ: present result

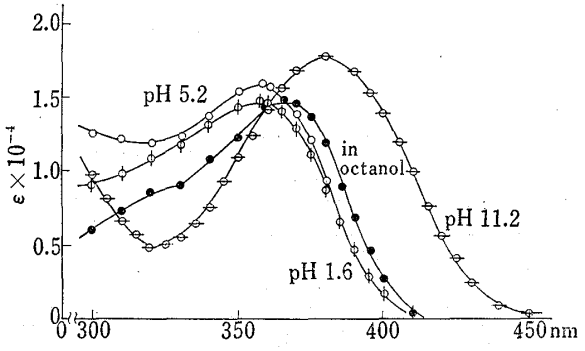

Fig. 6. Absorption Spectra of Tetracycline in H_2O and in *n*-Octanol

Concentration of tetracycline; 4.88×10^{-5}M

In Fig. 5, the log P' value at pH 5.3 is rather high but other values fit the curve very well. The high value may be epimerization at position 4 of tetracycline,[10,11] since epimerization is reported to occur in the pH range of about 2 to 6 (ref. 10). The epimer, 4-*epi*-tetracycline, which is more hydrophobic than tetracycline,[10] was formed when a solution of tetracycline at pH 5.3 was stood for about 36 hours (*cf.* Experimental). However, in the partition experiments of Colaizzi and Klink, formation of the epimer was slight because equilibration was very rapidly attained.[8] The experimental value of log P' at pH 5.3 is within the limits of experimental error, so it is uncertain at present whether it is actually due to epimerization. Moreover, even if an epimer is formed, the results in Fig. 5 indicate that the partition behavior of the epimer does not differ appreciably from that of tetracycline.

Since pK_A of tetracycline is rather small (3.33) and pK_C is large (9.61), we did not measure P' in the pH regions below pK_A and above pK_C to avoid hydrolysis. However, although the partition of tetracycline was not examined over the whole pH range, the results in Fig. 5 clearly show that P' lies on the curve of N' with a maximum value around pH 5.5 as expected from Eq. (15), thus indicating the validity of Eq. (12).

11) A.P. Doerschunk, B.A. Bitler, and J.R.D. McCormick, *J. Amer. Chem. Soc.*, 77, 4687 (1955).

Table I. Spectral Properties of Tetracycline

Medium	λmax (nm)	$\varepsilon \times 10^{-4}$ (M^{-1} cm^{-1})	λmax (nm)	$\varepsilon \times 10^{-4}$ (M^{-1} cm^{-1})
pH 1.6	358	1.47	268	1.87
pH 5.2	358	1.58	275	1.65
pH 11.2	380	1.78	267	1.54
n-octanol	366	1.47	265	1.70

2. Spectrophotometric Properties of Tetracycline

The absorption spectra of 4.88×10^{-5}M tetracycline in aqueous solution were measured at pH 1.6, pH 5.2 and pH 11.2, where the predominant molecular species of tetracycline are the cationic, the zwitterionic and the anionic forms, respectively. The spectra in the visible region are shown in Fig. 6 together with that in *n*-octanol, and the λ_{max} and molar extinction coefficient at this wavelength of each species are summarized in Table I. The spectrum of tetracycline in *n*-octanol with λ_{max} at 366 nm and 265 nm is quite different from those in aqueous solutions at the different pH values.

Ethanol was added to an aqueous solution of 4.88×10^{-5}M tetracycline at pH 5.2, in order to change the polarity of the medium. In aqueous solution of pH 5.2 the spectrum had two peaks at 358 nm and 275 nm, as shown in Table I. On addition of ethanol the former peak moved to a higher wavelength, and the latter to a lower wavelength. Increasing the percentage of ethanol, the changes became greater with increase in absorbances, and in absolute ethanol the λ_{max} values were at 366 nm and 265 nm as in *n*-octanol. Similar changes in the spectrum were observed on adding ethanol to aqueous solutions at pH 1.6 and pH 11.2. The changes of λ_{max} and the molar extinction coefficient with increasing amounts of ethanol are shown in Fig. 7 (A), (B).

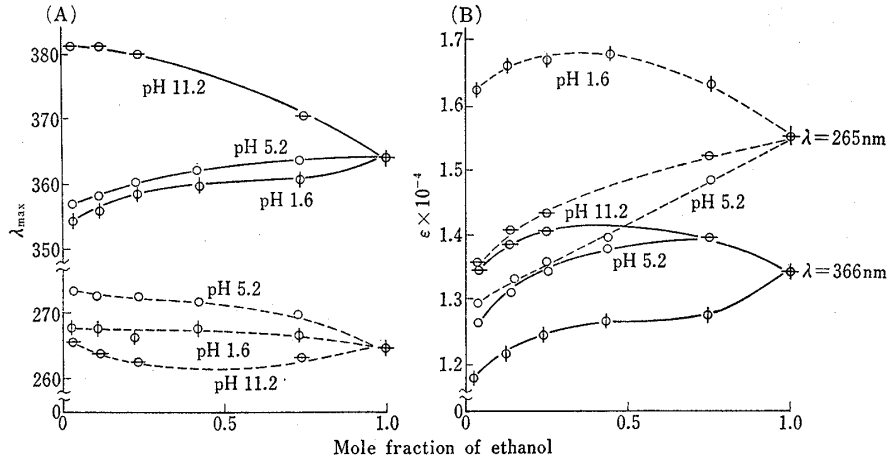

Fig. 7. Change of λ_{max} (A) and the Molar Extinction Coefficient (B) of Tetracycline with the EtOH/H_2O Ratio

The pH in the figure is that of the starting buffer before adding EtOH.
Concentration of tetracycline: 4.88×10^{-5}M

These results indicate that tetracycline is not in an ionized form in media of low polarity, such as *n*-octanol and ethanol, the only actual form in such media being the nonionized form [OOO]. This conclusion is also supported by measurement of the infrared absorption spectrum of tetracycline in chloroform, which has approximately the same dielectric constant (ε=4.7 at 25°) as that of *n*-octanol (ε=3.4 at 25°). The spectrum showed absorption at 2800 cm^{-1}, which is assigned to a nonionized dimethylamino group, but not at 3300 cm^{-1}, the position for absorption of a cationic ammonium group (not shown).

Discussion

Gastrointestinal absorption of tetracycline has received much attention,[12,13] since it is directly related to the biological activity of tetracycline and since absorption is surprisingly small compared with the total dose of tetracycline. The poor absorption is considered to be due to the polar structure of tetracycline,[13] which in aqueous solution is almost entirely in the cationic form under strongly acidic conditions, anionic forms under alkaline conditions and the zwitterionic form under neutral and weakly acidic conditions.

From detailed experiments on the effect of pH on the partition behavior of tetracycline, Colaizzi and Klink[8] ascribed the solubilization of tetracycline in lipid to the zwitterionic form, which has relatively high hydrophobicity. However, using the partition data of Colaizzi and Klink,[8] and by ourselves, and estimating the molecular form in the hydrophobic phase, we have shown that tetracycline is transferred to the organic phase *via* the neutral molecular form, which is present in only very small amount at any pH in the aqueous phase.

If we assume that the partition is governed by the amount of the zwitterionic form, and that in the organic phase the only form of tetracycline is the zwitterion as reported by Colaizzi and Klink,[8] the following relations are obtaind.

$$Q = (-\mathrm{O}+)_o/(-\mathrm{O}+)_w \tag{17}$$

$$\log P' = \log C_o/C_w = Q \cdot \frac{1}{(1+(a/K_\mathrm{A})+(K_\mathrm{B}/a)+(K_\mathrm{B}K_\mathrm{C}/a^2))} = \log Q + \log N' \tag{18}$$

Comparing Eq. (18) with Eq. (12), it is apparent that changes of the apparent partition coefficient P' expressed by Eq. (18) are the same as those expressed by Eq. (12), and the true partition coefficient with regard to the zwitterionic species, log Q, is the sum of log P and log (k_3/K_A). So, it is not possible to decide from the pH profile of the apparent partition coefficient alone, whether the zwitterion or the neutral form is responsible for the transfer from the aqueous phase to the organic phase. As described above, determination of the molecular form in a hydrophobic region is very important for elucidation of the partition mechanism.

Changes of log P' with pH reflect the ionization process of tetracycline in the aqueous phase, as shown in Eqs. (12) and (18), so the macroscopic ionization constants can be evaluated from the results in Fig. 5 using a similar method to that reported previously.[4] As shown in Fig. 2, the apparent partition coefficient has the same values at x and y, where the hydrogen ion activities are referred to as "a_x" and "a_y", respectively. We can neglect the contribution of pK_C to log P', in the region of at least 1.5 pH unit below the pH where log P' changes according to Eq. (14) (in the case of tetracycline, below pH 8.1). In this region, Eq. (9) can be written as Eq. (19).

$$P' = P(k_3/K_\mathrm{A}) \cdot \frac{1}{1+(a/K_\mathrm{A})+(K_\mathrm{B}/a)} \tag{19}$$

Where $P^\circ = P(k_3/K_A)$ from Eq. (19), and the relation between P' and "a_x" and "a_y" is,

$$\frac{1}{P'} = \frac{1}{P^0} + \frac{1}{P^0 K_\mathrm{A}} \cdot (a_x + a_y) \tag{20}$$

$$\frac{1}{P'} = \frac{1}{P^0} + \frac{K_\mathrm{B}}{P^0} \cdot \left(\frac{a_x + a_y}{a_x a_y}\right) \tag{21}$$

12) *a*) M.H. Pindell, K.M. Cull, K.M. Doran, and H.L. Dickison, *J. Pharmacol. Exptl. Therap.*, **125**, 287 (1959); *b*) J.H. Noble, L.A. Kanegis, and D.W. Hallesy, *Toxicol. Appl. Pharmacol.*, **11**, 128 (1967).
13) J.T. Doluisio and J.V. Swintosky, *J. Pharm. Sci.*, **53**, 597 (1964).

We can readily evaluate P°, K_A and K_B from Eqs. (20) and (21). The linear relationships between $1/P'$ and (a_x+a_y), and $1/P'$ and $(a_x+a_y)/a_xa_y$ of tetracycline are shown in Fig. 8(A) and (B), and from them the values of log P°, pK_A and pK_B were determined as -1.10, 3.34 and 7.67, respectively. The values of pK_A and pK_B obtained by this method are quite similar to those reported by Leeson, *et al.*,[6] determined by potentiometric titration (pK_A: 3.33, pK_B: 7.75). Using the experimental results on P' of 7-chlortetracycline at various pH values reported by Colaizzi and Klink,[8] the values of pK_A and pK_B were also evaluated by this method (pK_A: 3.28, pK_B: 7.40). These values are also in good agreement with those of Leeson, *et al.*,[6] determined by potentiometric titration (pK_A: 3.27, pK_B: 7.36).

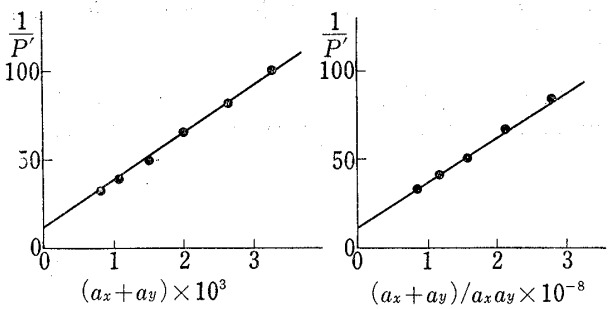

Fig. 8. Relationship between Apparent Partition Coefficient of Tetracycline (P') and (a_x+a_y) (A) and $(a_x+a_y)/a_xa_y$ (B)

a_x, a_y: refer to Fig. 2.
P' is measured in an n-octanol/H_2O system.

The macroscopic ionization constant, pK_C can be evaluated by Eq. (14) from the straight portion of the line in the alkaline region. In this study P' was not measured in the high pH region, so the value of pK_C could not be determined, but the above results show clearly that all the macroscopic ionization constants of acids having three ionizable groups can be estimated from the relationship between P' and pH.

We obtained values of log P° as -1.10 for tetracycline and -0.40 for 7-chlortetracycline. Since log P° is the sum of log P, log k_3 and pK_A, the value of log $P\cdot k_3$ becomes -4.44 for tetracycline and -3.68 for 7-chlortetracycline. To determine the true partition coefficient P, it is necessary to measure k_3 of each compound independently. Assuming that k_3 of tetracycline is the same as that of 7-chlortetracycline, the difference between log $P\cdot k_3$ of 7-chlortetracycline and that of tetracycline corresponds to the hydrophobic substituent constant of $-$Cl (π_{Cl}). The calculated value of 0.76 is approximately the same as that reported by Fujita, *et al.* (π_{Cl}: 0.71 (ref. 14)). This indicates that a $-$Cl group introduced at position 7 has no influence on the ionizability of the ammonium cation at position 4 of tetracycline substituents, possibly because position 7 is far from position 4.

In practice the effect of k_3 on K_A can be neglected (*cf.* Eq. (4)), so it can be assumed that k_3 is at least two orders less than K_A (K_A: 4.60×10^{-4}). Thus the true partition coefficient P would be more than 10 ($P\cdot k_3$: 3.68×10^{-5}). This means that the neutral molecular form of tetracycline itself is hydrophobic, but the amount of this form present is very small, which results in the low solubility of tetracycline in the lipid phase.

It is very interesting that the hydrophobicities of chemicals, determined from their partition coefficients, sometimes correlate well with their permeabilities through charged biomembranes.[15] As pointed out by Tute,[16] it is rather difficult for organic compounds, especially largely ionized molecules such as tetracycline, to traverse a charged biological interface. The mechanisms of transfer of various chemicals, ionized molecules and nonionized molecules, across charged membranes must be clarified to understand the biomembrane transport of

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drugs. There is a possibility that tetracycline penetrates biomembranes forming intermolecular ion pair in biological systems as recently predicted by Klink and Colaizzi,[17] and Uekama, *et al.*[18]

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