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Ion Pair Formation of Tetracyclines-Haloacetates and Alcohol Solvation[1]

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The distribution behavior of the tetracyclines in the presence of haloacetic acids at acidic conditions has been markedly influenced by the addition of alcohols in organic phase through the solvation of ion pair. The influences of variables, such as pH, anions, temperature on the distribution of tetracyclines into *n*-hexane containing various alcohols were studied. Stability constants, K_c, for solvated ion pairs and number of alcohol molecules associated with each ion pair, n, were determined. K_c values were highly dependent on the change in substituents of tetracyclines and on the nature of the pairing anions. A linear relationship with unit slope was found between log K_c and that of apparent distribution coefficient of intact tetracyclines. Thermodynamic parameters for ion paired chlortetracycline indicated that alcohol solvation facilitates the distribution processes of ion pair. Both values of K_c and n were influenced by the structural factors of solvating alcohols such as chain length, branching located near the OH group, and cyclic alkyl group. The solvation of alcohol with ion pair was discussed in terms of hydrophobic and hydrogen bonding.

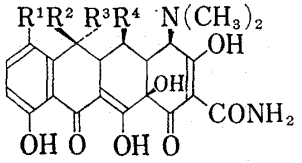
There have been increased interests in the ion pair formation of the charged species of drugs anticipating its correlation to the physiological absorbance. The tetracyclines are known to be in varying ionized forms in the physiological pH range; *i.e.*, cationic form at acidic condition, anionic form at alkaline region, and zwitterionic form at neutral pH. Colaizzi and Klink[3] have demonstrated that the zwitterionic form of the tetracyclines is the most lipid soluble form, possibly resulting from an intramolecular ion pair formation. They[4] have also shown recently that trichloroacetate (TCA) exerts a significant influence on the lipid solubility of the tetracyclines by the formation of intermolecular ion pair.

Higuchi and coworkers[5a] have reported that the addition of suitable solvating agents to organic phase enhances markedly the extraction process through the solvation of the formed ion pair. According to Higuchi's classification,[6] the tetracycline-TCA ion pair may be an example of case I. In this case the cation has largely lipophilic moiety and the small anion carries a relatively higher negative charge density per surface area. This ion pairing may be effectively solvated by proton donors such as alcohol and results in the masking of ionic character of the pair bonding.

In view of these observations, it is important to evaluate the role of alcohol in promoting distribution of ion pair complexes. In this study, the influences of variables, such as pH, anions, temperature, on the distribution of tetracyclines into *n*-hexane containing various alcohols were investigated. The tetracyclines studied are listed in Table I.

1) Main part of this study was presented at the 93rd Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1973.
2) Location: *Tanabe-dori, Mizuho-ku, Nagoya.*
3) J.L. Colaizzi and P.R. Klink, *J. Pharm. Sci.*, **58**, 1184 (1969).
4) P.R. Klink and J.L. Colaizzi, *J. Pharm. Sci.*, **62**, 97 (1973).
5) *a*) T. Higuchi, A.F. Michaelis, and J.H. Rytting, *Anal. Chem.*, **43**, 287 (1971); *b*) T. Higuchi and K. Kato, *J. Pharm. Sci.*, **55**, 1080 (1966); *c*) A.F. Michaelis and T. Higuchi, *ibid.*, **58**, 201 (1969).
6) T. Higuchi, A.F. Michaelis, T. Tan, and A. Hurwitz, *Anal. Chem.*, **39**, 974 (1967).

TABLE I. Structure of the Tetracyclines Studied



Analog	R_1	R_2	R_3	R_4
Tetracycline	H	CH_3	OH	H
Oxytetracycline	H	CH_3	OH	OH
Chlortetracycline	Cl	CH_3	OH	H
Demethylchlortetracycline	Cl	H	OH	H
Methacycline	H	$=CH_2$		OH
Doxycycline	H	CH_3	H	OH
Minocycline	$N(CH_3)$	H	H	H

Experimental

Material——Tetracycline hydrochloride, chlortetracycline hydrochloride, demethylchlortetracycline hydrochloride, and minocycline hydrochloride were favored from Japan Lederle Ltd. Oxytetracycline hydrochloride, methacycline hydrochloride, and doxycycline hydrochloride were presented from Taito Pfizer Ltd. These antibiotics which met official specifications were used without further purification. All other materials and solvents were of analytical grade.

Procedure of Distribution Study——Aqueous phase was prepared by the dissolution of tetracyclines (5.0×10^{-4} M), haloacetic acids (0.1 M), and calculated amount of NaCl which maintain constant ionic strength ($\mu=0.1$) in H_3PO_4–NaH_2PO_4 (0.05 M) buffer solution. The pH was adjusted to an appropriate value by the addition of 0.05 M NaOH or H_3PO_4 solution. Organic phase was prepared by the mixing of various amounts of alcohol and *n*-hexane and saturated with buffer solution to minimize the volume changes due to mutual miscibility. Twenty milliliters of each solution were taken in glass stoppered flask and shaken for one hour in thermostated water bath ($\pm0.1°$). The phases were separated during the standing for 30 minutes in the bath. Two ml of equilibrated aqueous phase was withdrawn and transfered into a 25 ml flask, and diluted with pH 3.0 buffer solution. The concentration of tetracyclines was determined spectrophotometrically. The concentration in organic phase was calculated by substracting the concentration in aqueous phase from the initial concentration. Apparent distribution coefficient, D', was defined as the ratio of the equilibrium concentration of organic phase to that of in aqueous phase.

Determination of the Values of K_c and n——The calculation procedure is essentially same as that of Higuchi, *et al.*[6] Aqueous phase containing 5.0×10^{-4} M tetracyclines and 0.1 M haloacetic acids at pH 3.0 was equilibrated with organic phase composed of varying amount of alcohol and *n*-hexane. At this pH, tetracyclines are predominantly in the cationic form and the other ionic species are almost negligible.[3] The stability constant for the formation of solvated ion pair complex, K_c, and the number of alcohol molecules associated to the ion pair, n, were evaluated from the plots of log D' *versus* log $(A)_0$ according to the following equation:

$$\log D' = n \log (A)_0 + \log (X^-)_w + \log K_c \qquad (1)$$

K_c is defined as:

$$K_c = \frac{(TH^+X^-A_n)_0}{(TH^+)_w(X^-)_w(A)_0^n} \qquad (2)$$

D' was experimentally determined which is defined as:

$$D' = \frac{(TH^+X^-A_n)_0}{(TH^+)_w} \qquad (3)$$

In these equations, $(TH^+)_w$ represents the concentration of tetracycline cation in aqueous phase and $(X^-)_w$, the concentration of haloacetate anion in aqueous phase; $(A)_0$ denotes the concentration of solvating alcohol in organic phase, $(TH^+X^-A_n)_0$, the concentration of solvated ion pair in organic phase.

Result and Discussions

Effect of 1-Pentanol on the Distribution of Tetracycline-TCA Ion Pairs

1-Pentanol was chosen for the preliminary studies to see the solvating effects of aliphatic primary alcohols on the distribution behavior of tetracycline-TCA ion pairs. Figure 1 shows the plots of log D' *versus* log $(A)_0$, according to equation (1), at constant TCA but varying 1-pentanol concentration. As can be seen, the plots are on straight lines with slope equal to 2 for all systems studied. This suggests that two molecules of 1-pentanol are involved with each ion pair. In organic phase, however, alcohol is largely in dimer form in these concentration range. If the solvated alcohols hold the dimerization, the binding number between ion pair and 1-pentanol monomer would be four.[6]

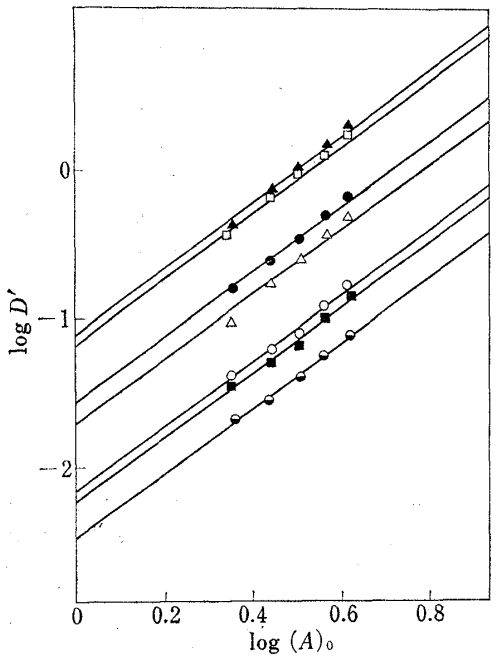


Fig. 1. Effect of Concentration of 1-Pentanol in Organic Phase on Distribution of Tetracycline-TCA Ion Pairs (pH=3.0, μ=0.1, Temp.=25°)

key: ▲, methacycline; □, doxycycline; ●, chlortetracycline; △, demethylchlortetracycline; ○, tetracycline; ■, oxytetracycline; ◒, minocycline

Table II summarizes the stability constants, K_c, and n, along with the distribution coefficients of tetracyclines in the absence of haloacetic acids, D_0. It is apparent that the value of D_0 is highly dependent on the structure of tetracyclines. For example, the introduction of Cl group onto R_1 position (see Table I) increases lipophilicity as seen in chlortetracycline and demethylchlortetracycline. When dimethylamino group is replaced on the same position, the lipophilicity of minocycline is markedly decreased. D_0 of oxytetracycline is far less than that of doxycycline and of methacycline. The significantly larger lipophilicity of the latter compounds can be attributed to the change in substituents at R_2 and R_3 positions compared with that of the parent oxytetracycline.

Figure 2 shows the plots of log K_c *versus* log D_0, which are on a straight line with a slope of unity. This simply suggests that the hydrophobic nature of the tetracyclines is directly reflected in the distribution behavior of their ion pairs.

pH-Distribution Behavior of the Tetracycline-TCA Ion Pairs

Figure 3 shows the pH profile of the apparent distribution coefficient of tetracycline-TCA ion pairs. In all cases except minocycline similar pH-log D' profile having maximum at pH

Table II. K_c and n Values for Tetracycline–TCA Ion Pairs and Apparent Distribution Coefficients of Tetracyclines at 25°

No.	Tetracycline analog	K_c	n	D_0[a)]
1	tetracycline	0.075	2	0.021
2	oxytetracycline	0.071	2	0.018
3	chlortetracycline	0.478	2	0.060
4	demethylchlortetracycline	0.338	2	0.042
5	methacycline	0.955	2	0.141
6	doxycycline	0.813	2	0.154
7	minocycline	0.036	2	0.004

a) Apparent distribution coefficient of tetracycline analog from aqueous phase (pH 3.0, μ=0.1) to organic phase composed of equal volume of 1-pentanol and n-hexane.

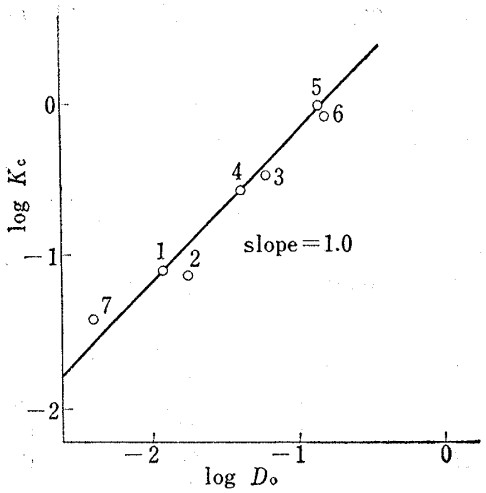


Fig. 2. Relationship between Log K_c for Tetracycline–TCA Ion Pairs and Log D_0 for Intact Tetracyclines

Numbers refer to the tetracyclines listed in Table II.

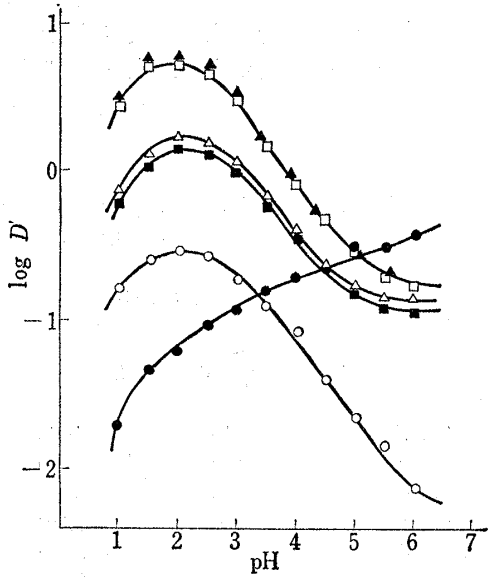


Fig. 3. pH Profiles of Apparent Distribution Coefficients of Tetracyclines in the Presence of TCA at 25°

aqueous phase: phosphate buffer (μ=0.1).
organic phase: equal volume of 1-pentanol and *n*-hexane
key: ▲, doxycycline; □, methacycline; △ chlortetracycline; ■, demethylchlortetracycline; ◎, tetracycline; ●, minocycline

2.0 were obtained. These observations are in good accordance with the results of Klink and Colaizzi.[4] It has been known that tetracyclines are in cationic form below pH 3.0[3] and TCA (pK_a=0.7)[7] is almost ionized at pH 2.0. Thus, the maximum distribution coefficient around pH 2.0 indicates that the ion pair formation is proportional to the product of the concentration of tetracycline cation and that of trichloroacetate anion.

In the case of minocycline the pH-log *D'* profile was quite unique among the tetracycline family. This may be ascribed to the additional dimethylamino group which probably limits the intermolecular ion pair formation.

Effects of Anions

Figure 4 shows the plots of log K_c for chlortetracycline-haloacetate systems against pK_a[8] of acids where K_c decreases with

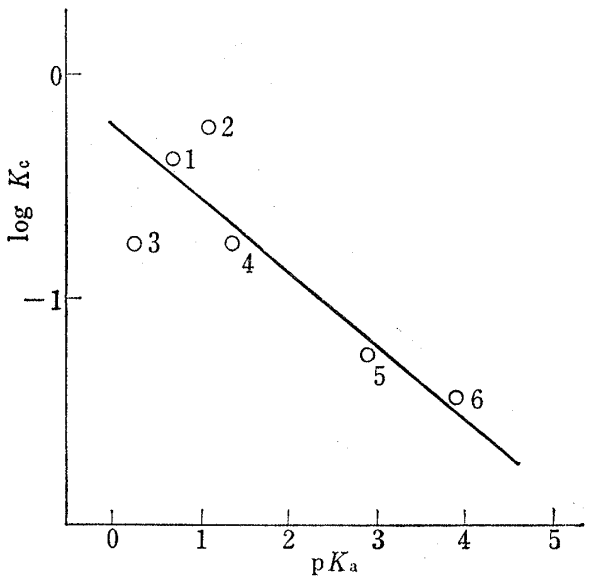


Fig. 4. Relationship between Log K_c for Chlortetracycline-Haloacetate Ion Pairs and pK_a of Pairing Acids at pH 3.0 and 25°

key: 1, trichloroacetate; 2, tribromoacetate; 3, trifluoroacetate; 4, dichloroacetate; 5, monobromoacetate; 6, monochloroacetate

7) R.C. Weast, "Handbook of Chemistry and Physics," 43rd ed., Chemical Rubber Publishing, Cleveland, Ohio, 1961, p. 1756.
8) G. Kortum, W. Vogel, and K. Andrussow, "Dissociation Constants of Organic Acids in Aqueous Solution," Butterworths, London, 1961.

pK_a. However, the K_c values for trihalogenated acetic acid systems are opposite to the general tendency. For trifluoro, trichloro, and tribromo acetate ion, K_c becomes greater in the order of the size of anion. For full interpretation on the distribution processes, other factors such as water structure, salt in effect, and/or surface tension reduction by large anion may be necessary.[9]

Table III. Thermodynamic Pramaeters for K_c and D_0 of Chlortetracycline in the Presence and Absence of 0.1 M TCA at pH 3.0 and 25°

Alcohol added in organic phase	K_c (in the presence of TCA)			D_0 (in the absence of TCA)		
	ΔG (cal/mole)	ΔH (cal/mole)	ΔS (e.u.)	ΔG (cal/mole)	ΔH (cal/mole)	ΔS (e.u.)
1-Butanol	540	−4470	−16.8	1190	697	−1.6
1-Pentanol	430	−3290	−12.5	1590	3700	7.2
1-Hexanol	370	−3030	−11.4	1930	4510	8.8
1-Heptanol	350	−2810	−10.6	2060	5850	13
1-Octanol	300	−2830	−10.5	2240	6620	15
1-Decanol	240	−2680	− 9.8	2390	7040	16
1-Dodecanol	200	−2510	− 9.1	2480	9460	21

Effects of Alcohols on the Distribution of Chlortetracycline-TCA Ion Pair

Table III summarizes the effects of primary aliphatic alcohols on the distribution of chlortetracycline in the presence and absence of TCA. Thermodynamic parameters were determined from the results on temperature dependency of distribution equilibria. In general, van't Hoff plots fell fairly on straight lines over the temperature range 10—40°. As can be seen, the distribution processes of ion paired chlortetracyclines are exothermic and exhibit negative entropy change along with favorable free energy changes for longer alcohol systems. On the other hand, in the absence of TCA they are endothermic and accompany positive entropy change, and less polar organic phases composed of longer chain alcohols show unfavorable free energy changes. These results indicate that alcohol solvation facilitates the distribution processes of ion pair while the solvation does not occur for unpaired chlortetracycline.[5c] For both of K_c and D_0, there found isoequilibrium relationships, *i.e.*, linear relationship between ΔH and ΔS values which are compensatory.

Table IV. K_c and n Values for Chlortetracycline–TCA Ion Pairs at 25°

No.	Solvating agent		K_c	n
	Alcohol	No. of carbon		
1	1-butanol	4	0.399	2
2	1-pentanol	5	0.478	2
3	1-hexanol	6	0.538	2
4	1-heptanol	7	0.546	2
5	1-octanol	8	0.563	2
6	1-decanol	10	0.671	2
7	1-dodecanol	12	0.701	2
8	cyclohexanol	6	0.621	2
9	3-methyl-1-butanol	5	0.415	1
10	2-pentanol	5	0.386	1
11	2-methyl-2-butanol	5	0.457	1
12	4-methyl-1-pentanol	6	0.467	1
13	2-ethyl-1-hexanol	8	0.473	1

9) K.S. Murthy and G. Zografi, *J. Pharm. Sci.*, 59, 1281 (1971).

The effects of various alcohols on the distribution of chlortetracycline-TCA ion pair are summarized in Table IV. K_c and n are influenced by the structure of the solvating alcohol. The steric effects can be seen from the comparison of various alcohols containing same number of carbon. Both values of K_c and n for straight chain alcohols are greater than those of branched alcohols.[10] These differences may be attributed to the steric hindrance of the branching located near the OH group of alcohol. K_c for cyclohexanol is larger than that of n-hexanol which may be ascribed to the favorably exposed OH group of cyclohexanol.

Figure 5 shows the plots of log K_c *versus* the number of carbon atoms in alcohol. It is apparent that log K_c increased with the increasing of alcohol chain, although points are not strictly on a straight line. It is assumed that largely lipophilic moiety in tetracyclines is favorable for hydrophobic association with alkyl group of alcohol. Calculation from the slope for straight chain alcohols (●) indicated that each methylene group lowered the free energy change by 390 calories. This value is fairly close to that predicted[11] for hydrophobic bonding *via* minimum contact, but is less than that of transfer of alcohol from water to organic phase.[12]

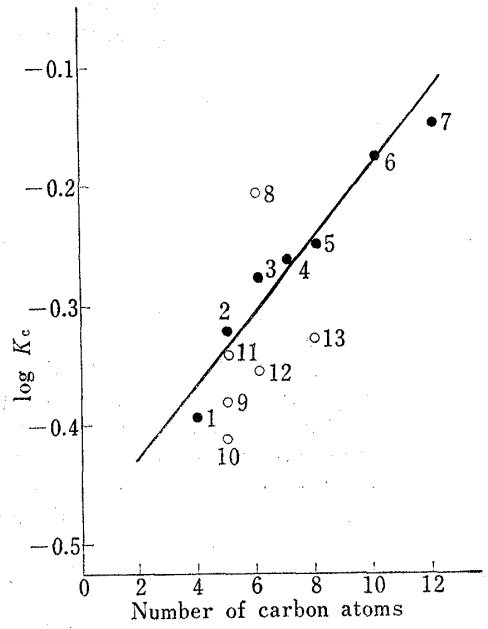


Fig. 5. Plots of Log K_c for Chlortetracycline-TCA Ion Pairs *versus* Number of Carbon Atoms in Solvating Alcohol

Numbers refer to the alcohols listed in Table IV. Closed circle represents the straight chain alcohol. Open circle represents the branched chain or cyclic alcohol.

10) R.L. Hull and J.A. Biles, *J. Pharm. Sci.*, **53**, 869 (1961); G.J. Divatia and J.A. Biles, *ibid.*, **53**, 916 (1961).
11) G. Nemethy and H. Sheraga, *J. Phys. Chem.*, **66**, 1173 (1962).
12) S.S. Davis, T. Higuchi, and J.H. Rytting, *J. Pharm. Pharmac.*, **24**, 30 P (1972).