Kinetic and Thermodynamic Aspects of *In Vitro* Interphase Transfer of Tetracyclines I: Influence of Hydroxyl Group Substitution

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Abstract □ The influence of hydroxyl group substitution on the kinetic and thermodynamic aspects of the interphase transfer of three tetracycline derivatives was studied in a two-phase *in vitro* system composed of an aqueous pH 5.5 buffer and 1-octanol. Data are reported on the kinetic and thermodynamic parameters of activation, the net thermodynamic parameters for interphase transfer, and the contribution of hydroxyl group substituents to the energy changes associated with partitioning. For each derivative, ΔH, ΔS, $T\,\Delta S$, and ΔF were positive with enthalpy dominating the entropic energy contribution. Introduction of a hydroxyl group at C-5 on the tetracycline nucleus promoted partitioning through an entropy-dominated decrease in the "apparent" free energy of partitioning, whereas shifting the hydroxyl group to C-6β retarded partitioning due to an enthalpy-dominated gain in the apparent free energy.

Keyphrases □ Tetracyclines—partitioning in aqueous–octanol system, kinetics and thermodynamics, effect of hydroxyl group □ Partitioning—tetracyclines in aqueous–octanol system, kinetics and thermodynamics, effect of hydroxyl group □ Kinetics—partitioning of tetracyclines in aqueous–octanol system □ Thermodynamics—partitioning of tetracyclines in aqueous–octanol system □ Antibacterials—tetracyclines, partitioning in aqueous–octanol system, kinetics and thermodynamics, effect of hydroxyl group

Previous papers (1, 2) concerned the influence of hydrophobic methyl group substitution and interface composition on the kinetic and thermodynamic aspects of the interphase transfer of sulfonamides in a liquid two-phase *in vitro* system. This report concerns the influence of hydrophilic hydroxyl group substitution on the kinetic and thermodynamic parameters for the interphase transfer of tetracyclines. Tetracycline, oxytetracycline, and doxycycline were studied because they differ from each other only in the number and placement of hydroxyl groups on the tetracycline nucleus.

EXPERIMENTAL

The apparatus, the method of performing the diffusion experiments, and the procedure for determining the partition coefficients were discussed previously (1).

Materials—The following reagents were the highest quality obtainable: tetracycline hydrochloride[1] (98% potency), oxytetracycline hydrochloride[1] (91% potency), doxycycline hyclate[1] (86% potency), 1-octanol, monobasic and dibasic sodium phosphates, sodium chloride, sodium hydroxide, and hydrochloric acid.

Diffusion Experiments—To study tetracycline diffusion, 5.2×10^{-4} M solutions[2] of each tetracycline derivative were prepared in aqueous phosphate buffer adjusted to pH 5.5 and ionic strength 0.15. The studies were performed at 23, 30, and 37° using water baths maintained at ±0.5°. Absorbance was measured with a UV spectrophotometer at 365 nm for tetracycline, 360 nm for oxytetracycline, and 355 nm for doxycycline.

[1] Pfizer and Co., Brooklyn, N.Y.; potency supplied by company.

[2] Although tetracycline and oxytetracycline were used as hydrochloride salts, doxycycline was used as the hyclate, which is the hydrochloride hemiethanolate hemihydrate. Therefore, doxycycline contributes a small quantity of ethyl alcohol ($\sim 10^{-4}$ M) to the aqueous buffer phase whereas tetracycline and oxytetracycline do not. This variable may have a small influence on the thermodynamic parameters observed for doxycycline.

RESULTS AND DISCUSSION

General Considerations—The structures of the three tetracyclines, their reported macrodissociation constants (3, 4), and their apparent partition coefficients in pH 5.5 phosphate buffer–1-octanol are summarized in Table I.

The complete ionization scheme for the tetracyclines is complex as a consequence of the three acidic moieties present, resulting in a dissociation sequence including 15 microionization steps (4, 5). A pH value of 5.5 was selected for the aqueous buffer solution to ensure that the zwitterionic form of the tetracyclines would be the predominant species, although other species are present for each derivative at this pH (4–6). Partitioning has been attributed to the zwitterionic form because maximum partitioning was observed in the general pH range of maximum zwitterionic concentration (5, 6). However, other reports (7) indicated that the unionized species of tetracycline is the dominant form for partitioning.

Because their pK values are the same, the concentration of various species at pH 5.5 should be the same for tetracycline and doxycycline. For oxytetracycline, the concentration of species will be slightly varied. Selection of pH 5.5 also minimized the epimerization of the various tetracyclines at the C-4 dimethylamino group commonly observed at lower pH values (8).

Tetracycline, oxytetracycline, and doxycycline were selected to examine the influence of hydroxyl group substitution and its position in these molecules on the interphase transfer characteristics. As shown in Table I, tetracycline differs from doxycycline only in a shift of the hydroxyl group from C-6β in the former to C-5 in the latter; this shift results in a ninefold increase in the apparent partition coefficient at pH 5.5. Oxytetracycline differs from tetracycline and doxycycline by the addition of a hydroxyl group; with respect of oxytetracycline, tetracycline and doxycycline are dehydroxylated at C-5 and C-6β, respectively.

Kinetic and Thermodynamic Data—Methods for obtaining the kinetic and thermodynamic data for the interphase transfer of tetracyclines in the two-phase system were discussed previously (1, 2). General mechanisms postulated for the interphase transfer of sulfonamides were used to interpret the data for the tetracyclines.

The activated complex for interphase transfer consists of an association of a tetracycline molecule with 1-octanol and water molecules. The octanol molecules interact primarily through hydrogen bonding and secondarily through hydrophobic interaction between the nonpolar moieties of the tetracycline and octanol molecules. The water molecules associate through hydrogen bonding and the formation of "icebergs" (9) of water molecules around the nonpolar groups of the tetracycline. Differences in the magnitude of the thermodynamic parameters for the interphase transfer of the three derivatives are attributed to variations in solute–solvate interactions arising from the extent of hydrogen bond and hydrophobic interactions among the tetracycline, octanol, and water molecules. As mentioned previously, it is unresolved whether the zwitterionic species, the unionized species, or both forms of the tetracyclines are capable of forming the activated complex and partitioning across the interface.

The kinetic and thermodynamic parameters of activation for the interphase transfer of the tetracyclines at 37° are summarized in Table II. Slight variations in structure lead to profound changes in the kinetic and thermodynamic parameters of activation. For example, since tetracycline and doxycycline differ only in the site of a hydroxyl substituent C-6β or C-5, respectively, and their pK values are the same, variation in the kinetic and thermodynamic parameters for these compounds may be attributed to the influence of the site of the hydroxyl group.

Doxycycline shows a greater k_f/k_b ratio and more positive $\Delta H_f^{\ddagger}$, $\Delta S_f^{\ddagger}$, $\Delta H_b^{\ddagger}$, and $\Delta S_b^{\ddagger}$ values than does tetracycline. This behavior is probably the effect of: (*a*) the larger continuous, uninterrupted hydrophobic surface on doxycycline, resulting from shifting the hydroxyl group from C-6β

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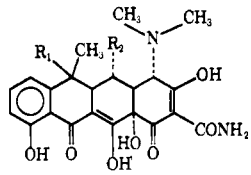

Table I—Structures and Physical Data for the Tetracyclines

Tetracycline	R_1 (C-6β)	R_2 (C-5)	Apparent Dissociation Constants at 25° (3) pK_1	pK_2	pK_3	Apparent Partition Coefficient at pH 5.5[a] 23°	30°	37°
Tetracycline hydrochloride	OH	H	3.3	7.7	9.7	0.075	0.083	0.092
Oxytetracycline hydrochloride	OH	OH	3.3	7.3	9.1	0.101	0.116	0.136
Doxycycline hyclate	H	OH	3.4	7.7	9.7	0.789	0.810	0.837

[a] Expressed in terms of the ratio of solute concentrations in octanol–aqueous pH 5.5 phosphate buffer phases as obtained from interphase transfer experiments.

Table II—Kinetic and Thermodynamic Parameters of Activation for the Interphase Transfer of Tetracyclines in a Two-Phase System at 37°

Tetracycline	k[a], hr^{-1} k_f	k_b	ΔH^*, cal/mole ΔH_f^*	ΔH_b^*	ΔS^*, cal/mole degree ΔS_f^*	ΔS_b^*	ΔF^*, cal/mole ΔF_f^*	ΔF_b^*	E_a, cal/mole E_{a_f}	E_{a_b}
Tetracycline hydrochloride	0.038	0.413	8,435	5683	−54.2	−58.3	25,237	23,767	9,051	6,299
Oxytetracycline hydrochloride	0.016	0.118	8,415	4622	−56.0	−64.2	25,770	24,539	9,031	5,238
Doxycycline hyclate	0.211	0.252	10,551	9783	−44.0	−46.1	24,181	24,071	11,167	10,399

[a] With the t test for independent means, there are significant differences between the k_f values for tetracycline *versus* oxytetracycline and oxytetracycline *versus* doxycycline ($p < 0.01$); there are also significant differences between the k_b values for tetracycline *versus* oxytetracycline and oxytetracycline *versus* doxycycline ($p < 0.01$). The data are based on nine determinations for each tetracycline.

in tetracycline to C-5 in doxycycline, which promotes greater hydrophobic interaction between solute and 1-octanol molecules in doxycycline; and (*b*) the greater potential for intramolecular hydrogen bonding of the C-5 hydroxyl group as compared to the C-6β hydroxyl substituent, which decreases hydrogen bonding between solute and solvate molecules in doxycycline. These factors alter the thermodynamic parameters of activation resulting from desolvation and resolvation of the tetracycline derivatives in forming the activated complex for interphase transfer.

Of course, the specific kinetic and thermodynamic parameters (Table II) depend on the cell design and stirring rate used in the interphase transfer experiments. The interphase transfer rates are influenced by the thickness of the aqueous diffusion layer, which, in turn, depends on the agitation rate (10).

By using the concept of the partition coefficient energy (1, 11), the differences in thermodynamic parameters of activation for forward minus backward transfer (*e.g.*, ΔF_f^* minus ΔF_b^*), termed net thermodynamic parameters (*e.g.*, ΔF), was calculated (Table III). The net thermodynamic parameters can be obtained directly, without consideration of the thermodynamic parameters of activation, by determining the partition coefficients at various temperatures and applying an Arrhenius treatment of the data. The relationships among the partition coefficient, rate constants, and net thermodynamic parameters of interphase transfer are given in Eqs. 1 and 2:

$$K_w^0 = \frac{k_f}{k_b} = \frac{e^{-\Delta F_f^*/RT}}{e^{-\Delta F_b^*/RT}} = e^{-\Delta F/RT} \qquad \text{(Eq. 1)}$$

$$K_w^0 = \frac{k_f}{k_b} = e^{(-\Delta H/RT + \Delta S/R)} \qquad \text{(Eq. 2)}$$

For all three tetracycline derivatives, the net free energy, ΔF, of interphase transfer is positive for partitioning between aqueous pH 5.5 and 1-octanol phases. Therefore, the net transfer of these tetracyclines from aqueous to 1-octanol phases is not favored ($K_w^0 < 1$). The net enthalpy, ΔH, and net entropy, ΔS, are also positive for each tetracycline derivative. In all cases, however, ΔH dominates the entropic energy, $T\,\Delta S$, and makes the major contribution to the positive ΔF. These results contrast with the previous observation for the more hydrophobic sulfonamide derivatives that the addition of methyl groups resulted in an entropy-dominated decrease in ΔF (1).

Table III—Net Thermodynamic Parameters and Apparent Partition Coefficients for the Interphase Transfer of Tetracyclines in a Two-Phase System at 37°

Tetracycline	$K_w^{0\,a,b}$	$\Delta H^{b,c}$, cal/mole	$\Delta S^{b,c}$, cal/mole degree	$T\,\Delta S^{b,c}$, cal/mole	$\Delta F^{b,c}$, cal/mole
Tetracycline hydrochloride	0.092	2752	4.1	1271	1470
Oxytetracycline hydrochloride	0.136	3793	8.2	2542	1231
Doxycycline hyclate	0.837	768	2.1	651	110

[a] As defined by Eqs. 1 and 2. [b] With the t test for independent means, there are significant differences between the values for tetracycline *versus* oxytetracycline and oxytetracycline *versus* doxycycline ($p < 0.01$). The data are based on nine determinations for each tetracycline. [c] Net values representing the difference between activation parameters for forward transfer minus back transfer. Values are calculated from the data in Table II.

The number and placement of hydroxyl groups appear to affect partitioning and net thermodynamic parameters significantly for the tetracycline derivatives. When doxycycline is modified to tetracycline by shifting the hydroxyl group from C-5 to C-6β, the ΔH, ΔS, and ΔF values increase markedly. This effect appears to be the consequence of the increased hydrophilic contribution of the hydroxyl group when substituted at C-6β rather than C-5, as discussed previously. When tetracycline is then modified to oxytetracycline by adding another hydroxyl group, ΔH and ΔS values increase again.

The positive ΔH and ΔS values observed for the three tetracycline derivatives suggest a net liberation of solvate molecules in forming the activated complex for interphase transfer.

"Apparent" Contribution of Hydroxyl Group Substitution to Energy Changes for Partitioning of Tetracyclines—To estimate the contribution of the C-5 and C-6β hydroxyl groups to the energy changes for partitioning of tetracyclines at pH 5.5, the method of Hansch (12, 13) was used. The contribution of individual substituents is measured by comparing the partitioning of the "parent" compound to that of a derivative differing by only a single substituent group. Equations 3 and 4 were used to calculate the data summarized in Table IV:

$$\pi = \log (K_w^{0''}/K_w^{0'}) = \Delta F_{G\ \Delta F}/-2.303RT \qquad \text{(Eq. 3)}$$

$$\Delta F_{G\ \Delta F} = \Delta F'' - \Delta F' \qquad \text{(Eq. 4)}$$

where π represents the log of the substituent group contribution factor (the log of $K_w^{0''}/K_w^{0'}$); $K_w^{0''}$ and $K_w^{0'}$ denote the partition coefficients for the derivative and parent compounds, respectively; and $\Delta F_{G\ \Delta F}$ represents the substituent group free energy contribution. The thermodynamic data in Table IV were obtained using the form of Eq. 4 and the data of Table III in which the appropriate net thermodynamic parameter for the parent drug was subtracted from the corresponding parameters for the derivative. When comparing the three derivatives, only a qualitative interpretation of the data is possible since the slight variation in pK values for oxytetracycline compared to tetracycline and doxycycline results in a small variation in the ratio of ionized to unionized species at pH 5.5. This change in species composition may in itself alter the apparent partition coefficient and, thus, the thermodynamic parameters observed. Because of this limitation, the data in Table IV are denoted as "apparent" values.

Table IV—Apparent [a] Contribution of Hydroxyl Groups to Net Thermodynamic Parameters for the Interphase Transfer of Tetracyclines in a Two-Phase System at 37°

Functional Group	π [b]	$\Delta F_{G\ \Delta F}$ [c], cal/mole	$\Delta H_{G\ \Delta H}$ [c], cal/mole	$\Delta S_{G\ \Delta S}$ [c], cal/mole degree	$T\Delta S_{GT\ \Delta S}$ [c], cal/mole
C-5 hydroxyl [d]	0.170	−239	1041	4.1	1271
C-6β hydroxyl [e]	−0.789	1121	3025	6.1	1891

[a] Apparent values because the composition of species is not constant for each tetracycline at pH 5.5. [b] The log of substituent constant calculated using Eq. 3. [c] The functional group free energy, enthalpy, entropy, and entropic energy of partitioning calculated using Eq. 4 and the net thermodynamic parameters of Table III; the net value of one derivative is subtracted from a second derivative to obtain the functional group thermodynamic contribution. [d] Obtained by subtracting tetracycline data from oxytetracycline data. [e] Obtained by subtracting doxycycline data from oxytetracycline data.

Substitution of a hydroxyl group at C-5 promotes partitioning by decreasing the apparent free energy of partitioning through an entropy-dominated effect. Shifting the hydroxyl group to C-6β, however, inhibits partitioning due to an enthalpy-dominated gain in the apparent free energy of partitioning. This small variation in position of the hydroxyl group from C-5 to C-6β results in substantial changes in the energy contributions of the hydroxyl group, as estimated by subtracting the C-5 hydroxyl data in Table IV from the C-6β data. For this shift in hydroxyl group, ΔF = 1360 cal/mole, ΔH = 1984 cal/mole, ΔS = 2.0 cal/mole degree, and $T\ \Delta S$ = 620 cal/mole. These observations suggest the fundamental importance of desolvation and resolvation processes in forming the activated complex for interphase transfer. In moving the hydroxyl group from C-5 to C-6β, a shift that probably accentuates the hydrophilic contribution of the hydroxyl group and diminishes the hydrophobic interaction of the tetracycline derivative with 1-octanol molecules in forming the activated complex, a ninefold decrease in the apparent partition coefficient is observed.

As shown in Tables III and IV, the addition of a hydrophilic substituent such as a hydroxyl group to a tetracycline derivative does not necessarily decrease the apparent partition coefficient. When tetracycline is converted to oxytetracycline by adding another hydroxyl group at C-5, the apparent partition coefficient increases because of a negative apparent $\Delta F_{G\ \Delta F}$ contribution. However, in converting doxycycline to oxytetracycline by adding an additional hydroxyl group at C-6β, the apparent partition coefficient decreases because of a positive apparent $\Delta F_{G\ \Delta F}$ contribution. For both examples, an additional hydroxyl group is substituted on the tetracycline derivative, but the effect on the apparent partition coefficient varies due to the fundamental influence on solute–solvate interactions in forming the activated complex for interphase transfer.

The data provide additional support for the mechanisms of solute transfer previously proposed (1, 2). While the simple model system used does not simulate the complexity of biological membranes, the similarity in intermolecular forces governing the transfer of solute across liquid–liquid and liquid–membrane interfaces suggests that studies such as these may furnish insight into *in vitro* and *in vivo* interphase transfer processes.

REFERENCES

(1) G. E. Schumacher and J. B. Nagwekar, *J. Pharm. Sci.*, **63**, 240 (1974).

(2) *Ibid.*, **63**, 245 (1974).

(3) W. C. Barringer, W. Shultz, G. M. Sieger, and R. A. Nash, *Am. J. Pharm.*, **146**, 179 (1974).

(4) L. J. Leeson, J. E. Krueger, and R. Nach, *Tetrahedron Lett.*, **18**, 1155 (1963).

(5) J. L. Colaizzi and P. R. Klink, *J. Pharm. Sci.*, **58**, 1184 (1969).

(6) T. F. Chin and J. L. Lach, *Am. J. Hosp. Pharm.*, **32**, 625 (1975).

(7) H. Terada and T. Inagi, *Chem. Pharm. Bull.*, **23**, 1960 (1975).

(8) E. G. Remmers, G. M. Sieger, and A. P. Doerschuk, *J. Pharm. Sci.*, **52**, 752 (1963).

(9) H. S. Frank and M. W. Evans, *J. Chem. Phys.*, **13**, 507 (1945).

(10) S. A. Howard, M. A. Farvar, A. Suzuki, and W. I. Higuchi, *J. Pharm. Sci.*, **58**, 1325 (1969).

(11) J. T. Davies, *J. Phys. Colloid Chem.*, **54**, 185 (1950).

(12) C. Hansch and A. Steward, *J. Med. Chem.*, **7**, 691 (1964).

(13) G. L. Flynn, *J. Pharm. Sci.*, **60**, 345 (1971).

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Kinetic and Thermodynamic Aspects of *In Vitro* Interphase Transfer of Tetracyclines II: Influence of Divalent Metal Salts

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Abstract □ With a two-phase *in vitro* model composed of an aqueous pH 5.5 buffer and 1-octanol, the kinetics of the interphase transfer of tetracycline derivatives were examined in the presence and absence of calcium and magnesium salts, and the contribution of some functional group substituents to the "apparent" free energy changes for partitioning of tetracyclines was evaluated. Only small changes were observed in k_f, k_b, K_w^0, and apparent functional group free energy changes, $\Delta F_{G\ \Delta F}$, in the presence of divalent metals as compared to values observed in the absence of these metals. Introduction of the C-6β hydroxyl group on the tetracycline nucleus decreased the apparent K_w^0 because of a positive apparent $\Delta F_{G\ \Delta F}$ contribution, whereas introduction of C-5 hydroxyl, C-6α methyl, or C-7 chloro groups increased the apparent K_w^0 through negative apparent $\Delta F_{G\ \Delta F}$ contributions.

Keyphrases □ Tetracyclines—partitioning in aqueous-octanol system, kinetics and thermodynamics, effect of divalent metal salts □ Partitioning—tetracyclines in aqueous-octanol system, kinetics and thermodynamics, effect of divalent metal salts □ Kinetics—partitioning of tetracyclines in aqueous-octanol system □ Thermodynamics—partitioning of tetracyclines in aqueous-octanol system □ Metal salts, divalent—effect on partitioning of tetracyclines in aqueous-octanol system □ Antibacterials—tetracyclines, partitioning in aqueous-octanol system, kinetics and thermodynamics, effect of divalent metal salts

The absorption of a tetracyclines is depressed in the presence of antacids and dairy products (1). Yet no evidence has been reported that calcium and magnesium ions, commonly present in antacid preparations, are capable of

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