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Physicochemical properties and chromatographic behavior of a homologous series of methotrexate-α,γ-dialkyl ester prodrugs

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Summary

A homologous series of 5 α,γ-dialkylester prodrugs of methotrexate (dimethyl through dipentyl) have been synthesized utilizing an acid-catalyzed direct esterification procedure. A high-performance liquid chromatographic (HPLC) method for separating each diester from its corresponding α- and γ-monoester mixture and methotrexate utilizing a pH 3 buffer solution/acetonitrile combination has been developed. The physicochemical properties of each diester including their chromatographic capacity factors and octanol/dimethyl formamide-water partition coefficients have been determined as well as the correlation between these two parameters. The effect of chain length and mobile phase composition on the capacity factors is shown. The methylene group contribution to both capacity factors and partition coefficients have been calculated. Also, the thermodynamic significance of these findings, based on free energy calculations, is discussed. From the data obtained in these experiments evolves a discussion of the possible application of these compounds to the topical treatment of psoriasis.

Introduction

Methotrexate (MTX), a folic acid antagonist, has been widely employed in the treatment of various neoplasms including choriocarcinoma (Hartz, 1963) and acute lymphoblastic leukemia (Farber et al., 1948) either as a single entity or in combination with other antineoplastic agents. At the end of 3 decades, the compound still remains the principal drug for the therapy of severe psoriasis and psoriatic arthritis (Weinstein and Frost, 1971). Although systemically administered MTX is very effective in clearing out widespread severe psoriatic plaques, the risk of short- and long-term toxic effects on the cells of bone marrow, gastrointestinal mucosa and particularly hepatocytes has precluded the use of this drug in the great majority of patients with minimal psoriasis (Weinstein and Frost, 1971, Schein and Winokur, 1975).

In an effort to enhance the therapeutic efficacy and to minimize toxicity associated with the systemic MTX therapy, topical application of the

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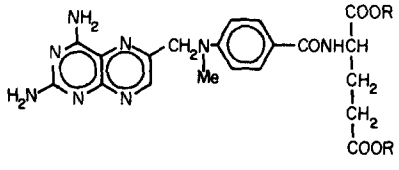
drug to the affected areas of the skin has been considered. However, clinical trials of topical MTX therapy have been uniformly disappointing (Van Scott and Reinertson, 1954; Nurse, 1963; Stewart et al., 1972) despite data which suggest that MTX acts directly on the psoriatic plaque rather than systemically at a distant site (Weinstein et al., 1981). The clinical ineffectiveness of the topical MTX therapy in psoriasis might be due to a combination of factors which lead to lower-than-adequate drug levels at the diseased epidermal tissue site. Such MTX levels may not be able to inhibit DNA synthesis in the rapidly proliferating cells of the psoriatic epidermis. Two main contributing factors appear to be (1) less-than-adequate drug absorption into the epidermal layer due to high resistance of the lipoidal cornified outer skin layer (stratum corneum) to the MTX dianion and (2) rapid clearance of MTX from viable epidermis to dermis followed by rapid uptake into the blood capillaries. The primary reason for poor cutaneous transport may be due to the fact MTX is a highly polar molecule, owing to the two carboxylic acid groups (pK_a's of 3.36 and 4.70) on its glutamic acid moiety (Poe, 1977).

By synthesizing a homologous series of diesters of MTX, it is possible to impart a higher degree of lipophilicity, by masking the carboxylic acid groups and thereby contributing to the overall hydrophobicity of the molecule.

This study describes the synthesis and purification of 5 diesters of MTX (Fig. 1) and subsequent development of a high-performance liquid chromatographic (HPLC) method for the simultaneous determination of each diester–monoester combination as well as MTX. Also, the chromatographic capacity factor has been determined for each diester in a number of aqueous buffer/acetonitrile (AcN) mobile phases, and the octanol/DMF-water partition coefficients for each diester have been determined and subsequent correlations made between these values.



	R
MTX	-H
I	$-CH_3$
II	$-CH_2CH_3$
III	$-(CH_2)_2CH_3$
IV	$-(CH_2)_3CH_3$
V	$-(CH_2)_4CH_3$

Fig. 1. Chemical structure of MTX and α,γ-dialkyl esters investigated in this study.

Materials and Methods

^{1}H-NMR spectra were obtained using a Varian FT-80 spectrometer. Chemical ionization mass spectra were obtained from a Finnegan 4000 mass spectrometer. Melting points were obtained using a Thomas Hoover Unimelt capillary device and are uncorrected. HPLC was done with a Waters model 510 solvent delivery system equipped with a Waters U6K injector, Waters Lambda Max model 481 variable-wavelength LC spectrophotometer, a Fisher Recordall series 5000 strip chart recorder, and a Shimadzu C-R3A Chromatopac electronic integrator. The column was a Waters Resolve 5 μm spherical C-18 reversed phase system. pH measurements were done on a Fisher Accumet model 825 MP pH meter. Temperature control in partition coefficient determination was accomplished with a Sargent Welch Thermonitor (Model ST). Column fractions were collected with an Isco Golden Retriever model 328 fraction collector. Samples for injection were stirred to homogeneity using a Thermolyne Maxi Mix stirrer.

Chemicals

MTX was generously provided by Lederle Laboratories, Code 1260, RNX3101, Pearl River, New York. All other chemicals and/or solvents were either analytical reagent grade or reagent grade. All HPLC solvents were HPLC grade.

Synthesis of MTX-α,γ-dialkyl esters

The compounds I, II, III, IV, and V, were synthesized by a direct esterification procedure adapted from Rosowsky's synthesis of these com-

pounds (1973), whereby the parent methotrexate is reacted at room temperature with the corresponding alcohol employing HCl as a catalyst.

I-α,γ-dimethyl methotrexate. 500 mg of methotrexate was added to a 500-ml round bottom flask with 100 ml of methanol and 0.14 ml of concentrated HCl with constant stirring. The flask was fitted with a drying tube. The reaction was run for 16 h. At completion, the mixture was evaporated to dryness with a rotary evaporator under reduced pressure. 150 ml of 0.25 M sodium bicarbonate was added to the dried residue and the suspension filtered. The filtrate was extracted into chloroform (3 × 100 ml), rinsed to neutrality with 200 ml sodium chloride saturated solution and evaporated to dryness. The residue and filtered product were dissolved in a minimum of 4/1-chloroform/methanol and added to a silica column (71 g), previously equilibrated with solvent, and eluted with a gradient of 95/5, 90/10, 85/15, 80/20, 75/25 chloroform/methanol (200 ml each). Tubes 21–60 contained pure I (329 mg, 62% yield), which was dried overnight under vacuum. This yielded a bright yellow crystalline solid. Monitoring of column separation was done with thin-layer chromatography using silica plates and chloroform/methanol-4/1. Identity of I was confirmed with NMR and mass spectrometry. Purity of the final material was confirmed by TLC and HPLC. ^{1}H-NMR (DMSO-d_6) δ 1.70–2.60 (m,4H); 3.21 (s,3H); 3.53 (s,3H); 3.53 (s,3H); 3.58 (s,3H); 4.43 (q,1H); 4.81 (s,2H); 6.81 (d,2H); 7.42 (broad s,4H); 7.78 (d,2H); 8.21 (d,1H); 8.55 (s,1H). CI-MS(CH_4) m/e 483(m + 1) (3.12% of base).

II-α,γ-diethyl MTX. 500 mg of MTX was added to 75 ml of absolute ethanol to which was added 1.6 ml of concentrated HCl. The reaction mixture was stirred at room temperature for 72 h and after a workup similar to I, was isolated from a silica column (71 g) using the same solvent gradient as I. Tubes 29–60 contained pure II (292 mg, 52% yield), a bright yellow crystalline solid. This was dried overnight under vacuum and used. Purity and identity were ascertained in a manner similar to I. ^{1}H-NMR (DMSO-d_6) δ: 1.15 (t,3H); 1.18 (t,3H); 1.70-2.60 (m,4H); 3.21 (s,3H); 4.13 (m,4H); 4.43 (q,1H); 4.81 (d,2H); 7.42 (broad s,4H); 7.78 (d,2H); 8.21 (d,1H); 8.55 (s,1H). CI-MS(CH_4) m/e 511(M + 1) (9.79% of base).

III-α,γ-dipropyl MTX. 500 mg of MTX was added to 100 ml of *n*-propanol and 0.14 ml of concentrated HCl and reacted as I. The crude material was obtained as with I. Purification employed column separation on silica (71 g) with dichloromethane/methanol 70/30 as the eluent. Fractions 1–11 contained pure III. After solvent evaporation, the product (339.2 mg, 56.53% yield) was observed to be bright yellow and waxy in appearance. It was dried overnight under vacuum and used. ^{1}H-NMR (DMSO-d_6) δ: 0.85 (t,6H); 1.55 (m,4H); 1.70-260 (m,4H); 3.21 (s,3H); 3.95 (m,4H); 4.43 (q,1H); 4.81 (s,2H); 6.81 (d,2H); 7.42 (broad s,4H); 7.78 (d,2H); 8.21 (d,1H); 8.55 (s,1H). CI-MS(CH_4) m/e 539(m + 1) (11.71% of base).

IV-α,γ-dibutyl MTX. 500 mg of MTX was added to 75 ml of *n*-butanol and 1.6 ml of concentrated HCL similar to II except that the reaction was stopped after 48 h. The crude product was obtained as with II and then purified on a silica column (71 g) utilizing chloroform/methanol 95/5 as the eluent. After evaporation of solvent, the product (311 mg, 50% yield), which was of waxy appearance, was dried overnight under vacuum and used. ^{1}H-NMR (DMSO-d_6) δ: 0.85 (t,6H); 1.15–1.68 (m,8H); 1.70–2.60 (m,4H); 3.21 (s,3H); 3.95 (m,4H); 4.43 (q,1H); 4.81 (s,2H); 7.42 (broad s,4H); 7.78 (d,2H); 8.21 (d,1h); 8.55 (s,1H). CI-MS(CH_4) m/e 567(M + 1) (14.57% of base).

V-α,γ-dipentyl MTX. 500 mg of MTX was added to 100 ml of *n*-amyl alcohol and 1.6 ml of concentrated HCl. The reaction was run for 72 h. The crude product was obtained as with II and IV and was then purified on a silica column (71 g) using chloroform/methanol 95/5 as the eluent. After evaporation of solvent, the yellow waxy material (307 mg, 47% yield) was further dried overnight under vacuum and used. ^{1}H-NMR(DMSO)-d_6) δ: 0.8 (m,10H); 1.1–1.68 (m,8H); 1.70-2.60 (m,4H); 3.21 (s,3H); 3.95 (m,4H); 4.43 (q,1H), 4.81 (s,2H); 6.81 (d,2H); 7.42 (broad s,4H); 7.78 (d,2H); 8.21 (d,1H); 8.55 (s,1H). CI-MS(CH_4) m/e 595(M + 1) (3.91% of base).

Capacity factor determination

Compounds I–V were analyzed utilizing varying mixtures of a pH 3 (0.05 M monobasic potas-

sium phosphate) buffer and acetonitrile proportions in which the buffer was adjusted to pH 3 with 85% phosphoric acid. The organic modifier ranged from 30 to 70% of acetonitrile. The flow rate of the HPLC instrument was set at 1.0 ml/min giving a pressure varying from 700 to 1100 psi depending on the acetonitrile concentration. The chart speed on both the strip chart recorder and the integrator was set at 2.5 mm/min. Each of the compounds was dissolved in the mobile phase being used at the time and injected. The mobile phases were filtered using a 0.45-μm nylon filter and degassed prior to use. Usual injection volumes were 5 μl. The compounds' retention times were recorded by the integrator. For each mobile phase, the void volume was determined by injecting 15 μl of distilled water, and the time of deflection on the integrator was recorded. The detector was set at 270 nm. Capacity factors (k') were calculated as shown later.

Partition coefficient determination

5–10 mg of each ester was dissolved with stirring in 155 ml of a DMF–water (50–50) mixture which had been previously equilibrated with 1-octanol overnight. After dissolution was achieved, the solution was filtered through a 0.45-μm nylon filter using a Millipore suction filtration apparatus. The filtered solutions were divided into 50 ml portions and added to each of 3 water-jacketed (450 ml) beakers mounted on magnetic stirrers, with stir bars spinning in each cell. These cells were maintained at 25°C. At this time 50-μl samples were taken from each beaker, representing the initial diester concentration. Then, the 1-octanol which was equilibrated with the DMF–water was added (50 ml) to each beaker. Samples (50 μl) were then taken from the lower (DMF–water) phase every hour up to 8 h. Depending on the diester, it was noted that no further change in concentration occurred after 6–8 h. Initial samples and each hourly sample were analyzed by adding 50 μl of a 0.06 mg/ml solution of procaine HCl internal standard or a dilution thereof to the sample, mixing for 10 s, and injecting 10 μl into the HPLC using the appropriate mobile phase. Concentrations were determined by comparison with standard curves of peak area ratios using the pure diester as the reference. The linearity and precision of the assay in this determination are listed in Tables 1 and 2.

TABLE 1

Standard curve data for MTX diesters in partition coefficient determination

Compound	Slope	Intercept	r
I	13.4308	0.0102	0.999
II	20.9349	0.0061	1.000
III	18.9708	0.0172	0.999
IV	11.4927	0.0146	0.999
V	18.6346	0.0066	1.000

Standard curves were constructed to bracket the range of concentrations assayed in the PC studies. In the case of those compounds which partitioned extensively into octanol, the standard curve covered several orders of magnitude.

TABLE 2

Precision data for HPLC assay in PC determination

Compound	High concentration (mg/ml)	Low concentration (mg/ml)
I	2.64×10^{-4} at 0.0472	7.07×10^{-4} at 0.0395
II	4.16×10^{-4} at 0.0611	1.15×10^{-4} at 0.0317
III	2.52×10^{-4} at 0.0484	1.73×10^{-4} at 0.0109
IV	9.61×10^{-4} at 0.0464	2.00×10^{-4} at 0.0025
V	8.62×10^{-4} at 0.0527	7.07×10^{-5} at 0.0008

The high concentration was the initial diester concentration in DMF–H_2O while the low concentration was the diester concentration in DMF–H_2O after equilibrium with octanol. Values are S.D.'s for 3 determinations.

Results and Discussion

Melting points of compounds I–V and MTX are shown in Table 3. An explanation for the effect of chain length on melting point requires the consideration of the influence of two factors. The first of these is the chain length itself (the increased effect of London forces and molecular weight). For a homologous series of straight-chain alkanes or carboxylic acids, it has been observed (Noller, 1957) that the melting point increases with alkyl chain length, but in this series, the

odd-numbered members show a lower melting point than the even numbered ones due to less efficient crystal packing of the odd-numbered chains. Thus, the melting point proceeds upward with chain length in a zig-zag manner. The second factor is the effect alkyl substitution has on the crystalline structure of the parent molecule. It has been observed that n-7-alkyl substitution of dimethyl xanthines results in progressive lowering of the melting point with increased chain length (Guttman and Higuchi, 1957). This phenomenon is a result of steric interference with crystal packing.

Both of these effects are seen in the series from MTX to V. MTX has such high crystalline forces that it decomposes before fusion is achieved. Proceeding from I to III a decline in melting point is observed due to interference with crystal packing. From III to V, the effect just described is outweighed by increasing Van der Waals forces and the melting points begin to increase again in an alternating fashion.

The chromatographic capacity factors and the partition coefficient (PC) data for compounds I–V have been listed in Table 4. The chromatographic system employing mixtures of pH 3 buffer and acetonitrile as the mobile phase was developed in an attempt to analyze each of the 5 dialkyl esters of MTX in the presence of their hydrolytic degradation products. The diesters of MTX can hydrolyze via either α- or γ-monoesters to the parent drug MTX. It was observed that in order to obtain baseline separations (resolution ≥ 1.5) among MTX, monoesters and diesters as well as to effect reasonably short retention times ($t_r < 15$ mins), progressively higher percentages of the organic modifier were needed as the ester chain length was increased. A representative chromatogram is seen in Fig. 2. The k' values (capacity factors) were calculated from the chromatographic retention times of each of the MTX-diesters and appropriate void volumes (Snyder and Kirkland, 1979).

Fig. 3 depicts the plot of logarithm of capacity factors (k') as a function of the number of added carbon atoms on the diester promoiety. Excellent linearity ($r > 0.99$) has been observed between the two parameters for all the mobile phase compositions used. The slopes of these lines as presented in Table 5 represent the π values or hydrophobic parameters analogous to those described by Hansch and Leo (1979) from oil-water partition coefficients. However, the π values calculated for the chromatographic system would be different from the values calculated by Hansch and Leo (1979) because the free energy of transfer of one methylene group from AcN/aqueous mobile phase

TABLE 3

Melting points of the various methotrexate dialkyl esters

Compounds	Melting point, °C
MTX	dec. 185–204
I	168–172
II	127–131
III	94– 98
IV	150–153
V	141–145

dec, decomposition.

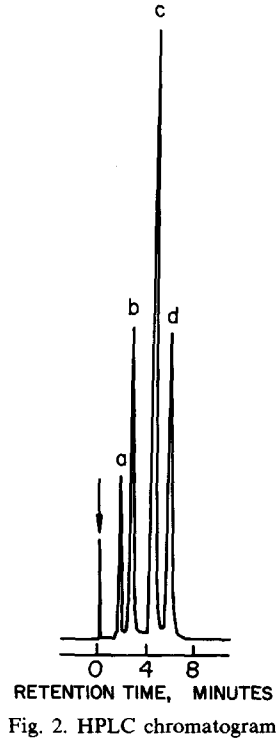


Fig. 2. HPLC chromatogram of compound II in the presence of (a) MTX (b) its monoethyl ester and (c) internal standard, procaine.

TABLE 4

PCs and capacity factors (k') for various MTX dialkyl esters in a variety of pH 3/AcN mobile phases

	Compound I	Compound II	Compound III	Compound IV	Compound V
PC	0.22(0.01)	0.93(0.03)	3.27(0.06)	17.64(1.49)	72.43(2.53)
70/30	3.89(0.024)	–	–	–	–
60/40	1.11(0.050)	2.67(0.064)	8.1(0.098)	13.44(0.046)	–
55/45	1.00(0.111)	2.12(0.005)	5.00(0.005)	12.00(0.012)	21.61(0.088)
50/50	0.88(0.019)	1.62(0.016)	3.50(0.033)	6.62(0.032)	12.22(0.030)
45/55	0.62(0.016)	1.25(0.019)	2.38(0.032)	3.75(0.009)	8.28(0.016)
40/60	0.86(0.014)	1.29(0.005)	2.14(0.026)	4.14(0.014)	5.16(0.005)
35/65	0.66(0.009)	1.00(0.005)	1.43(0.010)	2.86(0.010)	3.68(0.009)
30/70	0.62(0.010)	0.84(0.005)	1.18(0.009)	1.81(0.009)	2.92(0.036)

Parenthetical values are S.D.'s (3 determinations).
Omitted values (–) were for retained compounds.

to bonded octadecyl silane stationary phase would be different from the energy required to transfer such a group from pure water to octanol. Similar chromatographic indices have been previously described. (Tomlinson et al., 1981).

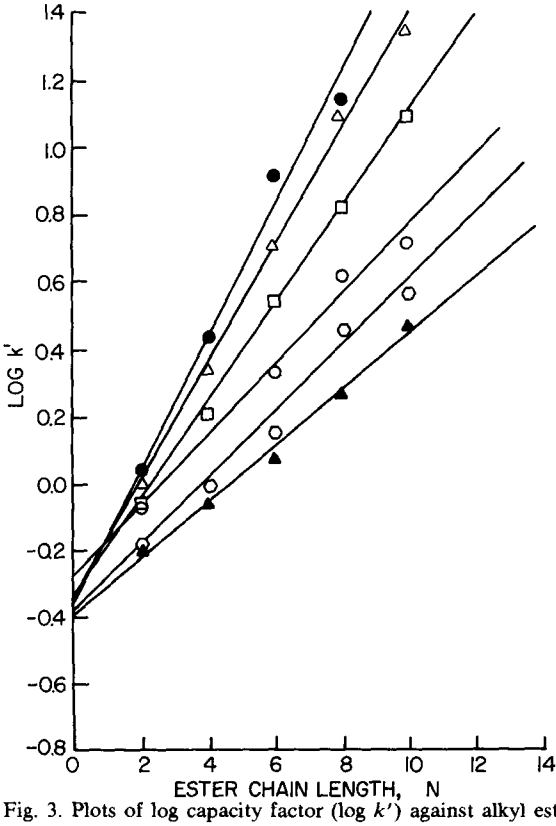


Fig. 3. Plots of log capacity factor (log k') against alkyl ester chain length with various (pH 3/AcN) combinations. (●), 60/40, (△), 55/45, (□), 45/55, (○), 40/60, (○), 35/65, (▲), 30/70.

All π values calculated for various mobile phase compositions were less than 0.5, a number obtained by Hansch et al. (1963) for 2- and 3-alkyl-substituted phenoxy acetic acids. The relationship between the π values and the volume percent AcN (% v/v) in the mobile phase is linear according to Eqn. 1.

$$\pi = 0.327\ (8.37 \times 10^{-5}) - 0.0036\ (2.21 \times 10^{-4})\%\ (v/v)\ AcN \qquad (1)$$

$$r = 0.989,\ n = 7$$

TABLE 5

Results from plot of $-k'$ vs ester chain length

Mobile phase (pH 3/AcN)	Slope (π)	Intercept	r	n
60/40	0.187 (0.018)	−0.306 (0.099)	0.991 [b]	4 [c]
55/45	0.17 (3.5×10^{-5})	−0.338 (0.039)	0.998	5
50/50	0.145 (0.0032)	−0.349 (0.021)	0.999	5
45/55	0.136 (0.0057)	−0.464 (0.038)	0.997	5
40/60	0.103 (0.0078)	−0.280 (0.052)	0.992	5
35/65	0.098 (0.0073)	−0.388 (0.048)	0.992	5
30/70	0.084 (0.0046)	−0.399 (0.031)	0.995	5

Parenthetical values are S.D.s; r = coefficient of linear correlation; n = number of data points.

This represents linearity in the range investigated experimentally. The intercept does not imply linearity at low vol% AcN (Karger et al., 1976; Tanaka et al., 1978).

Dialkyl MTX molecules at pH 3 will be ionized to a large extent due to protonation of the pteridinyl nitrogen (the no. 1 ring position) which has a pK_a of 5.71 (Poe, 1977). This ionization is apparently important since unless the mobile phase was adjusted to an apparent pH lower than the pteridine pK_a, the diesters were retained for a long period of time, making analysis impractical. AcN, being a highly polarizable compound, can significantly interact with the high π electron density of the pteridine moiety of the diesters. Increasing the volume fraction of AcN would be expected to decrease the π value due to enhanced affinity of the analytes for the mobile phase relative to the stationary phase. However, the interaction of the pteridine with the mobile and stationary phases is constant throughout the homologous series. The strength of hydrophobic interactions of the bonded silanol groups of the stationary phase with the solute molecules would be different from the strength of such interactions between solute molecules and octanol. Some degree of interaction of the solute molecules with the underivatized silanol groups of the stationary phase (Kaliszan, 1981) is possibly leading to some adsorption as opposed to partitioning.

$\Delta(\Delta G_t)$, the free energy of transfer of a methylene group on a dialkyl MTX molecule from the aqueous AcN mixed solvents to the bonded octadecylsilane stationary phase can be calculated according to Eqn. 2 (Flynn, 1971).

$$\Delta(\Delta G_t) = -2.303\ \pi RT \qquad (2)$$

where π values represent the slopes of logarithm of capacity factors vs carbon chain length plots, R is the molar gas constant having a value of 1.987 cal/K/mol and T is the absolute temperature. The calculated $\Delta(\Delta G_t)$ values for all the mobile phase compositions studied have been summarized in Table 6. It is evident from these results that an increasing AcN fraction in the mobile phase tends to decrease the magnitude of the free energy of transfer of a methylene group from the mixed solvent system to the stationary phase, primarily due to the greater relative affinity of the analytes for the mobile phase as previously discussed.

TABLE 6

Free energies of methylene group transfer from various mobile phases to column

Mobile phase (pH 3/AcN)	$\Delta(\Delta G_t)$ (cal/mol)
60/40	−254.46
55/45	−233.32
50/50	−197.73
45/55	−185.46
40/60	−140.87
35/65	−133.23
30/70	−114.14

$\Delta(\Delta G_t)$ values are calculated from Eqn. 2

Fig. 4 depicts the relationships for the logarithm of capacity factors with the volume percent of AcN in the mobile phase for different mobile phase compositions. Only 3 mobile phases are shown for clarity. The non-linearity may be the result of a gradual change in effective acidity of the mobile phase with corresponding change in

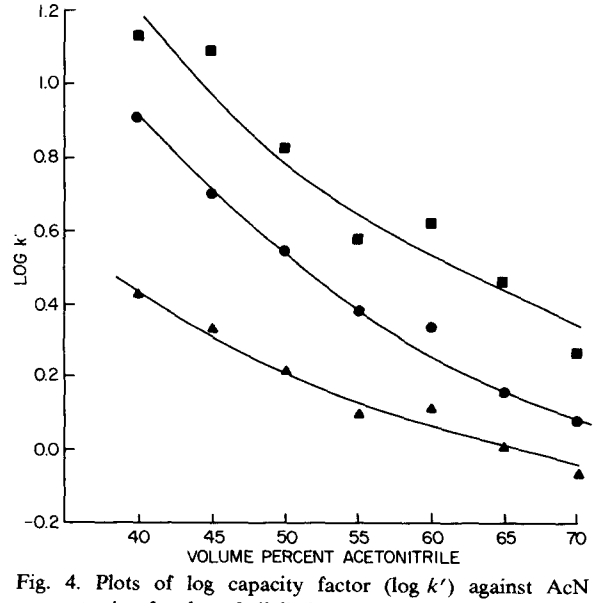


Fig. 4. Plots of log capacity factor (log k') against AcN concentration for three 3 dialkyl esters. (▲), compound II, (●), compound III, (■), compound IV.

volume fraction of AcN in the mobile phase. When the chromatographic parameter R_Q, defined as $\log[(t_r - t_0/t_r]$, was used instead of log k', it was shown that the relationship can be linearized (Toon et al., 1984). However, the parameter R_Q has questionable thermodynamic significance and will not be employed to linearize the MTX diester chromatographic retention data.

The relationship between the logarithm of the PCs (log PC) for MTX diesters and ester chain length (N) is represented in the following relationship:

$$\log PC = 0.316\ (7.42 \times 10^{-3})N - 1.307\ (4.92 \times 10^{-2}) \quad (3)$$

$r = 0.999,\ n = 5$

Excellent linearity is observed between the two parameters over a carbon chain length of 2–10. The PCs were determined in an octanol/DMF–water (50–50) system since aqueous solubilities of dialkyl MTX esters are too low to allow any reasonable PC measurements. The PCs were calculated according to Eqn. 4.

$$PC = (C_I - C_T)/C_T \quad (4)$$

where C_I is the initial concentration of MTX diester in the polar phase, and C_T is the concentration at distribution equilibrium. No apparent degradation of MTX diesters in DMF–water (50–50) was noted during the 6–8-h time period.

The π value obtained from the slope of the logarithms of PCs against ester chain lengths is only 0.316 which is lower than 0.5, a value for π calculated by Hansch et al. (1963) for 2- and 3-alkyl-substituted phenoxy acids in an octanol/water system. The difference in π-values can be attributed to the higher degree of interaction between the MTX diesters and the DMF–water systems as compared to pure aqueous systems. DMF–water can be considered a more polarizable solvent than water alone giving rise to π-interactions between the pteridine moiety on the diester and the amide moiety of DMF (another highly polarizable group). The free energy of transfer of a methylene group from DMF–water into octanol has been calculated from the π value according to Eqn. 2. The value has been found to be −430.24 cal/mol. The determined PCs show a large difference from the values determined by Rosowsky (1973) possibly due to the following reason. The experimental methods described by Rosowsky involved agitation of the two immiscible phases for only 60 s followed by analysis of the DMF-water phase. Therefore, the obtained results represent a non-equilibrium kinetic phenomenon rather than a true thermodynamic process because it requires approximately 6–8 h for the distribution equilibrium to be established.

Combining the PC data with the k' data from Table 4 for each of the several pH 3/acetonitrile mixtures, it can be observed that good linear correlation exists between log PC and log k' (Table 7). Linearity is observed from 2–10 carbons with no apparent deviation. The relationship would very likely allow extrapolation to compounds containing more than 10 carbons in this homologous series, of course, up to the limit of detection of the HPLC system *. The correlation between log k' and log PC may be explained by some electrostatic interaction with the stationary phase, due possibly to a monolayer of solvent adsorbed to the stationary phase (Hafkenscheid and Tomlinson, 1983). Also, there is a degree of hydrogen bonding of solute with octanol as well as hydrophobic interaction. This combination effect may represent the amphiphilicity that is required for a molecule to effectively enter into the skin (Higuchi, 1977). There is some evidence of the dual nature of the interaction in the octanol phase. When the determination of PCs was attempted from water to iso-octane, no partitioning took place even after several hours. That is, these compounds have no affinity for the pure hydrocarbon solvent. Thus, the interaction is not a purely hydrophobic effect.

The major thermodynamic justification for transfer from the DMF–water phase to the octanol phase, and the transfer from the mobile

* All calculations are based on the amount of compound remaining in the polar DMF–water phase. This concentration is likely to be very low with very long chains.

TABLE 7

Results from plot of log PC vs log k'

Mobile phase (pH 3/AcN)	Slope	Intercept	r	n
60/40	1.632 (0.323)	−0.756 (0.244)	0.981	4
55/45	1.838 (0.078)	−0.678 (0.066)	0.997	5
50/50	2.170 (0.090)	−0.544 (0.060)	0.998	5
45/55	2.303 (0.137)	−0.224 (0.005)	0.995	5
40/60	3.006 (0.218)	−0.437 (0.098)	0.992	5
35/65	3.189 (0.189)	−0.047 (0.065)	0.995	5
30/70	3.742 (0.170)	+0.201 (0.044)	0.997	5

phase to the stationary octadecylsilane phase is entropic (Tomlinson, 1983; Beezer, 1983; Guy and Honda, 1984). The positive ΔS of transfer may be attributed to the arrangement of diester molecules in the polar DMF–water phase via hydrogen bonding and π–π electronic interactions which is of a highly ordered nature. The transfer from this highly polar phase into the octanol medium may release solvent molecules form their highly ordered arrangement.

It should be noted that prodrugs of MTX having very long ester chain lengths ($n > 10$) are of more interest from a thermodynamic standpoint than from a view of therapeutic applicability. The compounds will have even less aqueous solubility, as well as greater tendency to be accumulated in lipoidal material of the stratum corneum, making transport impossible.

Conclusion

As determined from the experimental evidence presented here, α,γ-dialkyl esters of MTX can easily be detected and quantitated using appropriate mixtures of a pH 3 buffer and acetonitrile. With this chromatographic system, the compounds can be detected in the presence of hydrolytic degradation products, including the parent drug MTX, allowing kinetic studies to be performed easily.

PCs were also determined for the esters and were shown to be linearly correlated with the capacity factors in a log–log plot for each of the mobile phases utilized. Reasons for deviation from ideal values were proposed as well, owing to the greater complexity of these systems than octanol/water systems.

As has been stated by other authors (Yamana et al., 1977; Hafkenscheid and Tomlinson, 1983; Toon et al., 1984), the capacity factor determined from a suitable chromatographic system should be used as the parameter for characterizing relative hydrophobicity or hydrophilicity of the drug molecule since good correlation between log *PC* and log k' exists. This particular observation as well as the fact that the PC can be a good correlator for a compound's biological activity, provide further reasons for undertaking such chromatographic studies.

A comprehensive study considering the role of chemical and enzymatic hydrolyses of MTX diesters and their transport across hairless mouse skin is now underway and will be reported later.

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