*International Journal of Pharmaceutics*, 59 (1990) 271–279
Elsevier



IJP 02022

# Solubility and stability characteristics of a series of methotrexate dialkyl esters

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(Received 25 August 1989)
(Modified version received 20 October 1989)
(Accepted 1 November 1989)

*Key words*: Methotrexate dialkyl ester; pH-solubility relationship; pH-stability relationship; Co-solvent solubility-stability relationship; Dimethylformamide/water co-solvent

## Summary

A series of methotrexate (MTX) dialkyl esters were examined for their solubility in an aqueous and a co-solvent medium (20%) (Co-solvent solubility-stability relationship; Dimethylformamide/water co-solvent (DMF)-aqueous buffer) as a function of pH. The pH-solubility relationship showed an unusual increase at pH values above the $pK_a$ of these weak bases. The stability of this series of compounds was also studied in the same 20% DMF medium. The rate constant for diester loss decreased as the series was ascended and additional stability resulted from the branching of the diisopropyl ester. Also, the stability of the MTX methyl ester was studied as a function of pH, with the resulting pH-stability profile showing a typical V-shaped pattern. The maximum aqueous stability of dimethyl ester was shown to be between pH 4 and 5.

## Introduction

Methotrexate (MTX) has been shown to be an effective systemic agent in the treatment of psoriasis (Weinstein and Frost, 1971). A homologous series of MTX dialkyl esters has been prepared for their evaluation as potential topical anti-psoriatic agents. The synthesis and physicochemical characterization of MTX dialkyl esters have been reported in a previous communication (Fort and Mitra, 1987). A study of their cutaneous transport and bioconversion across hairless mouse skin is the subject of another report (Fort and Mitra, 1989). In order for these compounds to be utilized in any topical delivery system, particularly since an aqueous component may be present, it is necessary to evaluate the aqueous solubility and stability of these compounds. In this paper, the aqueous and dimethylformamide (DMF)-aqueous co-solvent solubility of a series of MTX diesters, as well as their stability in the same co-solvent media, are described. Additionally, a complete pH-stability profile of a representative diester has been examined, namely, MTX dimethyl ester (DM-MTX).

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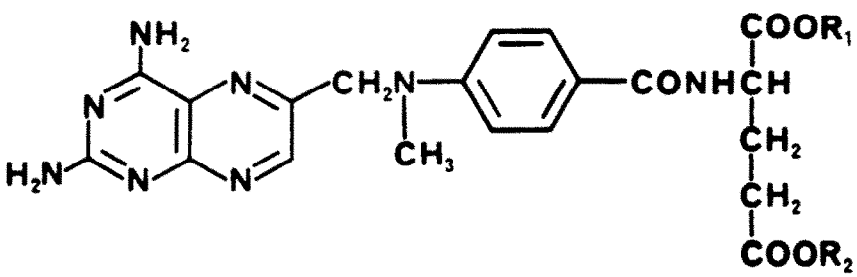
## Materials and Methods

*Solubility in DMF-water diffusion medium*

The saturation solubility of MTX and its diesters (Fig. 1) in 20% DMF/phosphate buffer (pH 7.4) solution, a vehicle employed in in vitro skin transport studies, was obtained in the following manner. Into 25-ml screw-capped culture tubes were placed 10 ml of the solvent and excess solid MTX or diester. After 24–48 h agitation at 37 ± 0.5 °C in a Dubnoff Metabolic Shaking Incubator (Precision Scientific), the suspension was vortexed and transferred to a warm 10 ml glass syringe, followed by filtering through a 0.45 μm polycarbonate filter (Nucleopore). Appropriate dilutions in the same solvent medium were made and the final concentration of the prodrugs was determined by an HPLC method described elsewhere (Fort and Mitra, 1989). Determinations were performed in triplicate.

*pH-solubility studies of MTX and diesters*

The solubility of each of the diesters in 20% DMF/aqueous buffer was determined as a function of pH at intervals of 0.5 pH unit within the range pH 2.5–8. The solubility of MTX was determined from pH 2.5 to 7.5 at 1 pH unit intervals. Again, triplicate determinations were carried out. The pH measurements were made with an Accumet model 825 MP pH meter equipped with a glass electrode (Fisher). The pH meter was standardized using certified pH 4 and 7 buffer solutions (Fisher) at the temperature of the study. The buffers utilized throughout the pH range were either combinations of acetic acid/sodium acetate or phosphoric acid/sodium phosphate buffers, with the concentration of acetate and phosphate employed being 0.03 M each, adjusted to a constant ionic strength of 0.1125 with sodium chloride. The diester solubility was determined, using the same procedure as described previously.




| Compound | $R_1$ | $R_2$ | Ester chain length |
|---|---|---|---|
| Methotrexate (MTX) | H | H | 0 |
| Dimethyl methotrexate (DMMTX) | $CH_3$ | $CH_3$ | 2 |
| Diethyl methotrexate (DEMTX) | $CH_2CH_3$ | $CH_2CH_3$ | 4 |
| Dipropyl methotrexate (DPMTX) | $CH_2CH_2CH_3$ | $CH_2CH_2CH_3$ | 6 |
| Diisopropyl methotrexate (DIPMTX) | $CH(CH_3)_2$ | $CH(CH_3)_2$ | 6 |
| Dibutyl methotrexate (DBMTX) | $CH_2(CH_2)_2CH_3$ | $CH_2(CH_2)_2CH_3$ | 8 |

Fig. 1. Chemical structures of methotrexate and its dialkyl esters.

*Stability of diesters in diffusion medium (20% DMF)*

The stability of diesters in the diffusion medium was ascertained at 37°C. Appearance of the corresponding degradation products was also monitored. A solution of the diester (100 μm) (10 ml in a 25 ml screw-capped culture tube) was allowed to undergo hydrolysis in the diffusion medium. Two 50-μl samples were taken from each tube every 24 h for at least five half-lives. One sample was analyzed for diester content, the other for resulting monoester and MTX by HPLC (Fort and Mitra, 1989).

*pH-stability profile of DMMTX*

The pH-stability profile of dimethylmethotrexate (DMMTX) was determined at 37°C at unit pH intervals over the range pH 1–11. The effect of buffer catalysis at each pH was also determined and the buffer-independent rate constants were obtained from the intercepts of plots of rate constants vs. buffer concentration. For each pH an appropriate buffer of reasonable buffering capacity was utilized. A list of buffers employed under various pH conditions is shown in Table 1. For each data point, triplicate determinations were performed. The reactions were followed for at least five half-lives. The pH standardization of each solution was performed at 37°C with standard commercial solutions. The solution pH remained essentially unaltered during the time course of the experiment. Each stability determination was performed by placing 10 ml of the appropriate buffer solution adjusted to the proper pH and ionic strength into a 25 ml screw-capped culture tube. To this solution, already equilibrated at 37°C, 100 μl of a methanolic stock solution of DMMTX was added to produce an initial concentration of 10μM. The solution was vortexed and an initial 50 μl sample was taken. Subsequently, at appropriate time intervals as determined by preliminary experiments 50 μl of the samples were withdrawn and frozen immediately in dry ice. When the experiment was completed, each of the samples was analyzed by HPLC whereby 50 μl of a methanolic solution of methyl paraben was added as an internal standard. Then 50 μl of the mixture was injected onto the HPLC column. For fast reaction where sample freezing was not possible, 25-μl samples were injected immediately without prior freezing.

TABLE 1

*Buffers employed for pH-rate profile of DMMTX*

| pH | Buffer species |
|---|---|
| 1 | HCl/KCl |
| 2 | $H_3PO_4/NaH_2PO_4$ |
| 3 | $H_3PO_4/NaH_2PO_4$ |
| 4 | HOAc/NaOAc |
| 5 | HOAc/NaOAc |
| 6 | $NaH_2PO_4/Na_2HPO_4$ |
| 7 | $NaH_2PO_4/Na_2HPO_4$ |
| 8 | $NaH_2PO_4/Na_2HPO_4$ |
| 9 | $H_3BO_3/NaH_2BO_3$ [a] |
| 10 | $H_3BO_3/NaH_2BO_3$ |
| 11 | NaOH/KCl |

[a] $Na_2B_4O_7 \cdot H_2O$.

## Results and Discussion

*Solubility in DMF-water diffusion medium*

The solubility of the diesters in the 20% DMF/phosphate buffer (pH 7.4) solution medium used throughout the skin transport experiments is listed in Table 2 and graphically illustrated in Fig. 2. The solubility profile initially shows an increase in solubility on going from MTX to DMMTX, and then descends for the remaining members of the series. It is also noted that DIPMTX, a branched-chain analog, exhibits greater solubility

TABLE 2

*Melting point and solubility of MTX and its diesters in diffusion medium*

| Compound | Solubility (M) ($\times 10^3$) | Melting point [b] |
|---|---|---|
| MTX | 7.12 (0.11) [a] | dec. 185–204 |
| DMMTX | 8.64 (0.45) | 168–172 |
| DEMTX | 5.07 (0.27) | 127–131 |
| DPMTX | 2.80 (0.08) | 131–136 |
| DIPMTX | 4.42 (0.53) | 147–152 |
| DBMTX | 0.35 (0.03) | 150–153 |

[a] SD for 3 determinations given in parentheses.
[b] Taken from Fort and Mitra (1987).

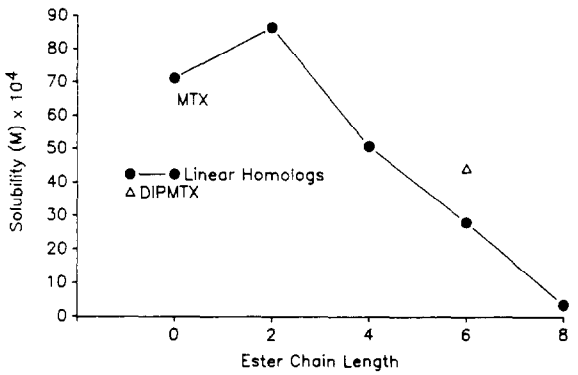



Fig. 2. Solubility of MTX and MTX dialkyl esters in diffusion medium (20% DMF/pH 7.4 buffer) as a function of alkyl chain length.

than the straight-chain compound DPMTX. The solubility data may be rationalized by examining the melting point characteristics of the compounds presented in Table 2. The initial increase in solubility on progression from MTX to DMMTX may be explained by the fact that the crystalline forces involved in MTX lead to stacking of the molecules by $\pi$-$\pi$ interactions of pteridine and hydrogen bonding of the glutamate which result in decomposition rather than true melting of MTX at high temperatures. These forces are somewhat relieved due to dimethyl esterification. The effect of increasing chain length beyond dimethyl shows an expected decrease in melting point; however, this is unaccompanied by a corresponding increase in solubility. Thus, beyond the dimethyl homolog there appears to be a lesser degree of solubility enhancement from the effects of relieved intracrystalline forces. Apparently, increased hydrophobicity prevails over decreased crystallinity.

The branched-chain DIPMTX consistently showed greater solubility than DPMTX which may be due to the same factors as discussed above. The main difference between the rank order of solubility in this mixed solvent as compared to pure aqueous systems over the entire pH range is that the DMF component greatly increases the contribution of the solute-solvent interaction over the solute-solute forces in the crystalline solid. Thus, melting point effects are less pronounced in mixed solvent systems than in pure aqueous medium. The greater affinity of the solutes for the mixed solvent is evident, since the solubilities in this solvent are consistently higher than those in pure water.

*pH-solubility studies of MTX and diesters*

The relationship of aqueous solubility with pH for the MTX diesters exhibited unusual profiles. The series of compounds depicted in Fig. 1 are weak bases with the $N^1$ on the pteridine ring having a $pK_a$ of the protonated nitrogen at around

TABLE 3

*Solubility vs pH relationship for MTX and MTX diesters*

| pH | Solubility (M) ($\times 10^5$) | | | | | |
|---|---|---|---|---|---|---|
| | MTX | DMMTX | DEMTX | DPMTX | DIPMTX | DBMTX [a] |
| 2.5 | 35.63 (2.51) [b] | 20.68 (1.06) | 37.66 (0.47) | 10.53 (1.57) | 25.93 (2.48) | 6.44 (0.12) |
| 3.0 | – | 10.61 (2.74) | 17.25 (1.36) | 4.15 (0.06) | 14.80 (1.20) | 6.92 (1.85) |
| 3.5 | 26.36 (0.44) | 7.73 (0.23) | 11.67 (0.91) | 4.23 (0.06) | 10.03 (0.46) | 2.28 (0.23) |
| 4.0 | – | 4.66 (0.16) | 7.10 (0.44) | 1.39 (0.09) | 6.39 (0.45) | 1.90 (0.65) |
| 4.5 | 30.35 (0.18) | 2.94 (0.13) | 5.28 (0.13) | 1.57 (0.37) | 4.14 (0.32) | 0.72 (0.08) |
| 5.0 | – | 2.60 (0.08) | 3.50 (0.19) | 0.65 (0.01) | 4.00 (0.24) | 1.26 (0.47) |
| 5.5 | 172.47 (7.55) | 2.78 (0.07) | 4.13 (0.03) | 0.85 (0.03) | 4.09 (0.34) | 0.65 (0.10) |
| 6.0 | – | 4.36 (0.20) | 5.52 (0.15) | 1.32 (0.10) | 8.48 (0.76) | 1.68 (0.35) |
| 6.5 | 745.59 (7.02) | 6.85 (0.09) | 31.81 (4.12) | 4.85 (0.13) | 9.98 (0.48) | 3.31 (0.30) |
| 7.0 | – | 13.61 (1.06) | 19.03 (0.54) | 5.04 (0.18) | 8.98 (0.86) | 2.58 (0.30) |
| 7.5 | 1619.3 (20.06) | 17.41 (1.98) | 23.48 (1.96) | 4.43 (0.34) | 10.78 (1.57) | 3.50 (0.37) |
| 8.0 | – | 9.50 (3.01) | 22.13 (2.74) | 3.91 (0.82) | 9.78 (1.58) | 3.59 (0.97) |

[a] $\times 10^6$.

[b] SD for 3 determinations, in parentheses.

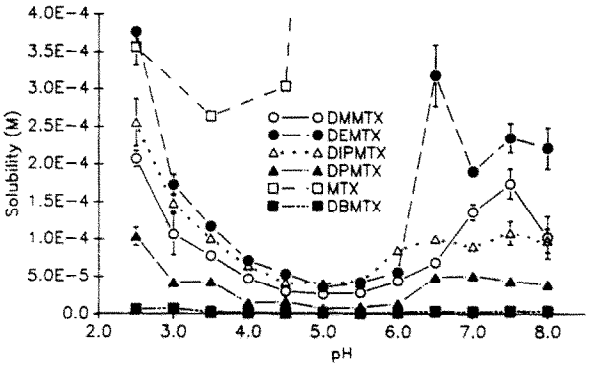



Fig. 3. Solubility of MTX and MTX dialkyl esters as a function of pH in pure aqueous solution.

5.71 (Poe, 1977). These compounds would be anticipated to show a pH-dependent aqueous solubility relationship where the compound would exhibit a lower intrinsic solubility at higher pH values and an increased solubility in the lower pH range. The mathematical relationship illustrating this pH dependence for a weak base is expressed by the following equation (Amidon, 1981):

$$S_t = S_0\left(1 + \frac{[H^+]}{K_a}\right) \quad (1)$$

where $S_t$ represents the total solubility as a function of pH, and $S_0$ is the intrinsic solubility of the unionized free base.

The above relationship does not hold entirely true in the case of these diesters. The solubility data have been summarized in Table 3 and schematically deplicted in Fig. 3. The solubility-pH relationship for MTX is qualitatively similar to that reported by Wallace et al. (1978). However, for the diesters, the solubility appears to exhibit a V-shaped profile within the range pH 2.5–8. All of the compounds in this series, i.e., from DMMTX to DBMTX including the branched-chain DIPMTX, show the same qualitative relationship with a minimum solubility at about pH 5. The increase in solubility from pH 5 to 2.5 can most likely be explained in traditional terms, namely that the molecule becomes more ionized in this region and hence has increasing solubility. The increase in solubility above pH 5 will require a more complicated explanation. In the higher pH region, a peculiar increase to a maximum followed by subsequent decrease is also observed. For example, with some of the esters, i.e., DMMTX, there is a solubility maximum at pH 7.5 and then a decrease at pH 8. The high pH region yields an unusual profile and this relative maximum is even more uncommon.

The diester placed in solution could presumably undergo a chemical reaction which would be very rapid in the basic region and the resulting product could be a more soluble species. Moreover, any such hydrolytic product would have to possess the same HPLC retention time as the diesters (if this degradation product peak was masked by MTX-diester peak on HPLC) and have significant absorbance at 254 nm, enabling detection. According to Fig. 4, which shows one of the known degradation pathways of MTX itself, it is possible that the amine on $C^4$ of the pteridine ring can undergo hydroxyl attack leading to the imine-ol/amide tautomer. The favored amide tautomer of the diester degradation product with a $pK_a$ of around 7.68 (Poe, 1977) could conceivably ionize, resulting in a higher solubility in the higher pH via regions. The only way to confirm the occurrence of this phenomenon was via the synthesis of the

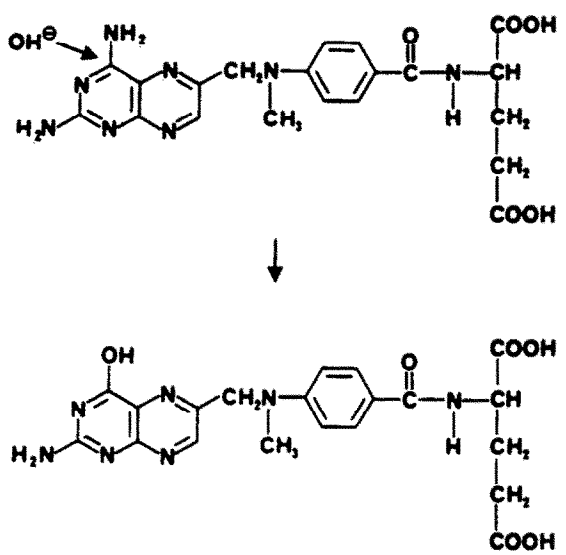



Fig. 4. Mechanism for the degradation of MTX in alkaline solution at high temperature (85 °C). Nucleophilic attack by hydroxide forming $N^{10}$-methylfolic acid.

dimethyl ester of this degradation product (dimethyl $N^{10}$-methylfolic acid) (Fort and Mitra, 1989).

When the dimethyl ester of $N^{10}$-methylfolate was injected onto the HPLC column with and without DMMTX under analytical conditions specific for DMMTX, the compound was shown to give a peak distinguishable from DMMTX. This same peak was not observed in any of the DMMTX solubility samples. Therefore, it was concluded that it was probably not the formation of this compound in the solubility medium that was causing or contributing to the unusual solubility profile.

Other interesting aspects of the solubility profiles are that the order of solubility over most of the pH range (from highest to lowest) was DEMTX, DIPMTX, DMMTX, DPMTX, and DBMTX. Although melting points are a very crude indicator of crystal binding forces, it can be suggested that the melting point effect is operative only for DMMTX and DEMTX, the first and second members of the series. Beyond four carbons in these linear-chain compounds, the solubility drops, presumably because of increased hydrophobicity of the longer carbon chains. With DIPMTX, the melting point is not as low as that observed with DPMTX, and therefore, its solubility can be explained by the fact that a branched-chain molecule has less hydrophobicity exposed to the solvent medium than does the straight-chain isomer. Because of the branching, the molecule appears to the solvent more like DEMTX than DPMTX. Hence, its rank in the overall solubility order could be somewhat explained.

Until further experiments have been performed, it can only be left to speculation as to why the pH-solubility profile in the alkaline region shows such a strange shape for this series of compounds. The possibility exists that some type of aggregates is formed from individual diester monomers in the alkaline region. On examining the structure of the diester, one can see that, at least with the lower members of the series, the molecule does not possess an amphiphilic structure, a characteristic that may lead to micelle formation. However, aggregation may occur in the case of the higher members of the series, where the alkyl chains are extended and constitute the nonpolar part of the micelle, while the remainder of the complex molecule would represent the relatively polar region of the aggregate. The problem with this theory is that the solubility trend is seen throughout the series. Therefore, the only rational type of aggregate formation should be based on common characteristics of the molecule in the heterocyclic region. The pteridine ring system of several of the diester molecules can stack and align with one another, forming an aggregate. The basis of the alignment would be $\pi$-$\pi$ interactions and London dispersion forces between two or more hydrophobic faces of the heterocycle, as observed with nucleosides, and purine and pyrimidine bases (Martel, 1985). This same type of interaction is operative with drugs having flat ring systems that intercalate into DNA generating anticancer activity (Myers, 1982). However, such micelle formation may not be readily observed experimentally, since the mechanism of aggregation would not necessarily involve alignment of these molecules at a surface. Fiber optic Doppler anemometry may be used in the future to determine such micelle formation.

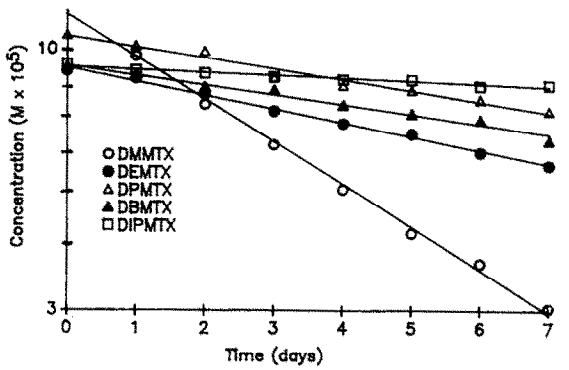



Fig. 5. Loss of dialkyl esters in 20% DMF diffusion medium with respect to time at 37 °C.

*Stability of diesters in diffusion medium (20% DMF)*

Each diester utilized in diffusion studies was evaluated with respect to its stability in the previously described diffusion medium. The main purpose for undertaking this study was to assess the degree to which the formation of monoesters in the donor solution of a transport study was due to

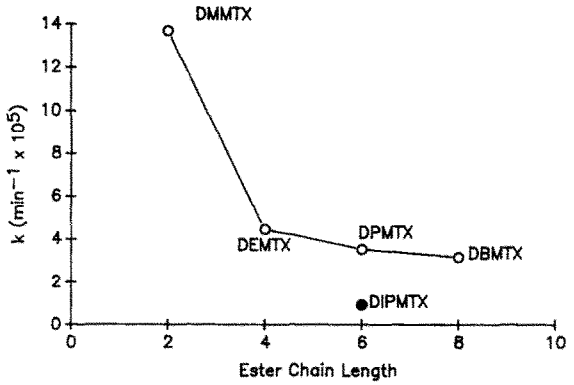



Fig. 6. Pseudo-first order rate constant as a function of ester chain length for MTX dialkyl esters.

chemical hydrolysis. In addition to the rate of loss of diesters, the relative proportions of the α and γ monoesters formed were also determined.

Fig. 5 and 6 depict plots of the loss of various diesters as a function of time and pseudo-first order rate constants vs chain length relationship, respectively. The pseudo-first order kinetics were obeyed for at least five half-lives during which reactions were monitored.

The data in Table 4 show that the rate constants decline with chain length, a sharp fall occurring with branching as in the case of DIPMTX. When reaction solutions were periodically analyzed for monoesters, it was observed that in every case the γ monoester predominated over the α isomer. With the exception of DMMTX and DIPMTX, the diesters are hydrolysed to the corresponding α and γ monesters in the proportions of

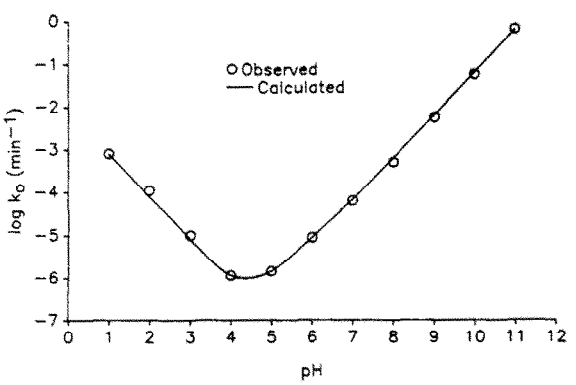



Fig. 7. pH-stability profile for DMMTX at 37 °C and μ 0.5.

TABLE 4

*First-order rate constants for diester loss in diffusion medium*

| Compound | Rate constant ($min^{-1}$) ($\times 10^5$) | Proportion (%) of isomers | |
|---|---|---|---|
| | | α | γ |
| DMMTX | 13.70 | 19.54 | 80.46 |
| DEMTX | 4.45 | 28.28 | 71.72 |
| DPMTX | 3.53 | 28.61 | 71.39 |
| DIPMTX | 0.93 | 37.19 | 62.81 |
| DBMTX | 3.15 | 28.47 | 71.53 |

1 : 3. The dimethyl ester is slightly more selective for the γ isomer, while the selectivity is considerably reduced in the case of DIPMTX.

*pH-stability profile for DMMTX*

In order to characterize the stability of the MTX dialkyl ester series in various pH media, a representative of the series, viz., the dimethyl ester, was chosen.

In order to determine the rate constants for a given pH at zero buffer concentration, four different buffer concentrations, i.e., 0.04, 0.08, 0.12 and 0.16 M were used, all at $\mu = 0.5$. From the rate constants at each buffer concentration, and the resulting linear relationship between rate constant and buffer concentration, it was possible to evaluate the buffer-independent rate constants by extrapolation to zero buffer concentration. The buffers used for each pH have already been described. The degree of buffer catalysis varied from buffer to buffer. These results are summarized in Table 5. All buffer-independent rate constants derived from these data are also included in Table 5.

The buffer exhibiting the strongest catalytic effect is borate. In the range pH 6–8, phosphate buffer catalysis was observed. The buffer species exhibiting the greatest catalytic effect in this pH region is the $HPO_4^{2-}$. In the acidic range, i.e., between pHs 2 and 3, phosphate is also observed to be catalytic, albeit to a lesser extent than in the neutral range. From the above data, the $H_3PO_4$ species is shown to be the main catalytic species. Acetate as a catalyst shows the least influence on DMMTX degradation, with the acetate anion

TABLE 5

*Dependence of first-order rate constants on buffer type*

| pH | Buffer | Intercept ($k_0$) – buffer-independent rate constant ($min^{-1}$) | Slope (catalytic constant) ($M^{-1}\ min^{-1}$) | $r$ |
|---|---|---|---|---|
| 1 | Hydrochloric acid | $8.05\times10^{-4}$ | | |
| 2 | Phosphate | $1.00\times10^{-4}$ | $9.23\times10^{-5}$ | 0.9969 |
| 3 | Phosphate | $1.03\times10^{-5}$ | $1.68\times10^{-5}$ | 0.9569 |
| 4 | Acetate | $1.13\times10^{-6}$ | $7.43\times10^{-6}$ | 0.9325 |
| 5 | Acetate | $1.46\times10^{-6}$ | $1.80\times10^{-5}$ | 0.9691 |
| 6 | Phosphate | $9.38\times10^{-6}$ | $9.52\times10^{-5}$ | 0.9642 |
| 7 | Phosphate | $6.32\times10^{-5}$ | $3.18\times10^{-4}$ | 0.9973 |
| 8 | Phosphate | $5.04\times10^{-4}$ | $1.74\times10^{-3}$ | 0.9886 |
| 9 | Borate | $5.53\times10^{-3}$ | $1.58\times10^{-3}$ | 0.9909 |
| 10 | Borate | $5.67\times10^{-2}$ | $7.78\times10^{-2}$ | 0.9829 |
| 11 | Sodium hydroxide | $6.50\times10^{-1}$ | | |

species showing the most catalytic effects of this acid/base pair.

The pH dependence of the loss of DMMTX as a function of time at 37 °C and an ionic strength of 0.5 can be described mathematically by considering the following possible reactions.

| Reactions | Rate constants | |
|---|---|---|
| $DMMTX^+ + H^+ \rightarrow$ products | $k_1$ | (2) |
| $DMMTX^+ + H_2O \rightarrow$ products | $k_2'$ | (3) |
| $DMMTX + H_2O \rightarrow$ products | $k_3'$ | (4) |
| $DMMTX + OH^- \rightarrow$ products | $k_4$ | (5) |

In the first two of the above expressions, the reactive DMMTX species is positively charged due to the ionization of the $N^1$-pteridinyl nitrogen ($pK_a = 5.71$) (Poe, 1977).

The overall velocity of the reaction can then be represented by Eqn 6.

$$v = \frac{-d[DMMTX]_T}{dt} = k_{obs}[DMMTX] \quad (6)$$

where

$$[DMMTX]_T = [DMMTX^+] + [DMMTX] \quad (7)$$

The rate can then be expressed at any given pH as:

$$\begin{aligned} \text{Rate} &= k_{obs}[DMMTX]_T \\ &= k_1 f_{DM+}[DMMTX]_T[H^+] \\ &\quad + k_2' f_{DM+}[DMMTX]_T[H_2O] \\ &\quad + k_3' f_{DM}[DMMTX]_T[H_2O] \\ &\quad + k_4 f_{DM}[DMMTX]_T[OH^-] \end{aligned} \quad (8)$$

where $f_{DM+}$ and $f_{DM}$ denote the fractions of ionized and unionized forms of DMMTX, respectively. For simplicity, the rate constants, $k_2$ and $k_3$ are defined as follows:

$$k_2 = k_2'[H_2O] \quad (9)$$

and

$$k_3 = k_3'[H_2O] \quad (10)$$

From these expressions, the final equation for the observed rate constant can be expressed by Eqn 11:

$$k_{obs} = \frac{k_1[H^+]^2 + k_2[H^+] + k_3K_a + k_4K_a[OH^-]}{[H^+] + K_a} \quad (11)$$

In order to solve for the four rate constants, i.e., $k_1$–$k_4$, the data in Table 5 can be used. Eqn 11 can be simplified in the acidic and basic regions. In order to obtain the values of $[OH^-]$ and $[H^+]$ at each pH, the following equations are utilized. According to Harned and Hamer (1933), at 37 °C:

$$a_{H^+}a_{OH^-} = 2.40\times10^{-14} \quad (12)$$

TABLE 6

*Rate equation constants for the hydrolysis of DMMTX*

| $k_1$ ($M^{-1}\ min^{-1}$) | $k_2$ ($min^{-1}$) | $k_3$ ($min^{-1}$) | $k_4$ ($M^{-1}\ min^{-1}$) |
|---|---|---|---|
| $5.68\times10^{-3}$ | $2.47\times10^{-7}$ | $8.82\times10^{-6}$ | 189.30 |

$$[H^+][OH^-] = 4.56 \times 10^{-14} \quad (13)$$

$$[H^+]\gamma = a_{H^+} \quad (14)$$

$$[OH^-]\gamma = a_{OH^-} \quad (15)$$

where $a_{H^+}$ and $a_{OH^-}$ represent the activities of $H^+$ and $OH^-$, respectively, and $\gamma$ is the mean ionic activity coefficient, having a value of 0.725 at 37 °C, and $\mu = 0.5$ (Harned and Hamer, 1933). As a result:

$$\log[H^+] = 0.14 - pH \quad (16)$$

and

$$\log[OH^-] = pH - 13.48 \quad (17)$$

Once $k_1$ and $k_4$ have been evaluated from the intercepts at the pH extremes, $k_2$ and $k_3$ can be readily obtained by inserting these values into Eqn 11 for pH 4 and 5, followed by solving for these two unknowns from the two equation. The resulting values of the four rate constants are listed in Table 6. The experimentally observed pH-rate profile is illustrated in Fig. 7 along with the theoretical curve calculated based on the four determined rate constants.

From the pH-stability profile, it can be stated that the most stable pH region for DMMTX is between 4 and 5. The greatest measured stability was shown at pH 4, generating a half-life of 1.17 years at 37 °C and $\mu = 0.5$.

The $\alpha$-monoester linkage has been consistently found to be more susceptible to hydrolysis than the $\gamma$-linkage. One explanation might be that the tetrahedral intermediate resulting from a nucleophilic attack on the carbonium ion of the $\alpha$-ester group generates a partial negative charge on the carbonyl oxygen. This partial negative charge can be stabilized by the neighboring amide proton. Such stabilization of the tetrahedral intermediate is not possible for the $\gamma$-ester group, since it is not adjacent to the amide group.

## Acknowledgements

This work was supported in part by a grant from Lederle Laboratories and in part by a Faculty Development Award to A.K.M. by Merck, Sharp and Dohme Research Laboratories. J.J.F. is an American Foundation for Pharmaceutical Education Fellow. Instrumentation support was provided in part by NIH Biomedical Research Support Grant RR05586 and in part by NIH Grant NS 25284.

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