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In vitro hydrolysis of polyunsaturated fatty acid *N*-acyloxymethyl derivatives of theophylline

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Abstract

A series of fatty acid *N*-acyloxymethyl derivatives of theophylline was prepared to determine the effects of varying the nature of the fatty acid on their in vitro rates of hydrolysis using porcine liver esterase and human plasma. The carboxylic acids examined include acetic (Ac), valeric (VA), nonanoic (NA), stearic (SA), oleic (OA), linoleic (LA), γ-linolenic (GLA), α-linolenic (ALA), eicosapentaenoic (EPA), elaidic (EA) and linoelaidic acid (LEA). When exposed to porcine liver esterase the NA acid derivative was hydrolyzed about 80 times faster than the triple bond GLA and ALA derivatives, which were hydrolyzed about four times as fast as the double bond LA derivative, which was hydrolyzed three times as fast as the single bond OA derivative. In human plasma the VA acid derivative was hydrolyzed over 100 times faster than the GLA derivative. Both the trans fatty acid EA and LEA derivatives were hydrolyzed slower than their cis counterparts. In both porcine esterase and human plasma, the derivatives displayed the classical parabolic relationship when the logarithm of the rate constant, k, was plotted versus the lipophilicity index, log K. Although the unsaturated fatty acid derivatives with three or more double bonds are not hydrolyzed at the maximum rate, they are hydrolyzed at markedly faster rates than the corresponding saturated or mono-unsaturated derivatives and as such still may serve as a delivery vehicle for other drugs.

Keywords: Theophylline; Prodrugs; Fatty acids; *N*-acyloxymethyl theophylline derivatives; Enzymatic hydrolysis; Human plasma

1. Introduction

The rationale of making lipophilic prodrugs of conventional drugs by derivatizing with carboxylic acids is well known. The resulting increased lipophilicity may increase the efficacy of

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the parent drug by yielding better penetration into cells or across the skin, improved absorption from the gut or better transport across the blood-brain barrier (Stella and Pochopin, 1992). In general, the pharmaceutical industry has only utilized relatively short chain saturated carboxylic acids as the lipophilic moieties and appears to be unaware of the additional beneficial properties that exist when long chain polyunsaturated fatty acids (PUFA) are used (Horrobin, 1996). Indeed, to date, the use of PUFAs as derivatizing agents for the delivery of drugs has been largely unexplored.

However, a number of studies have been conducted looking at the improvement in oral bioavailability of drugs by making long chain saturated fatty acid ester derivatives. For example, palmitate and stearate esters of testosterone increased the duration, and bioavailability for several weeks (Stella and Pochopin, 1992; Charman and Porter, 1996).

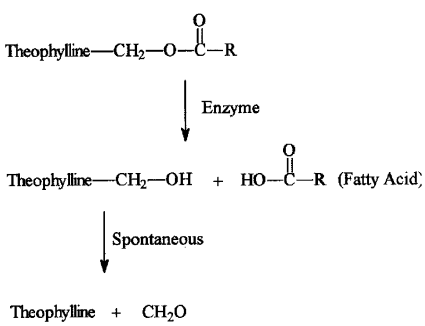
We previously reported the in vitro hydrolysis using porcine liver esterase and human plasma of a variety of *N*-γ-linolenoyloxyalkyl derivatives of theophylline (Burke et al., 1997). We chose the *N*-acyloxyalkyl approach as a means of evaluating the fatty acid prodrug derivatives because of the increased flexibility in that both the alkyl and acyl portion (fatty acid) can be varied systematically and whereby esterase results in the eventual release of theophylline as shown in Scheme 1. In this way the rate of hydrolysis can be specifically designed to suit either an oral, intravenous or transdermal application.

Theophylline was chosen as the drug for two reasons. Firstly, it is a widely used treatment for the management of asthma, however, a relatively short half-life and narrow therapeutic range make it necessary to administer the drug often. Therefore, a slow release theophylline preparation that would eliminate round-the-clock dosing every 6 h would be desirable in the treatment of asthma. As such, 7,7′-Succinylditheophylline has been prepared and shows a slow dissolution rate yet fast hydrolysis yielding a slow-release form of theophylline for potential use with asthma (Bodor et al., 1978). Secondly, the mode of action of theophylline in the treatment of asthma is similar in psoriasis, i.e. elevating cAMP levels, however, oral doses of theophylline required to treat psoriasis mean that systemic toxic blood levels are reached. To overcome this problem it is desirable to deliver theophylline topically. Moreover, short chain carboxylic acid *N*-acyloxymethyl derivatives have been reported as potential prodrugs of theophylline for the treatment of psoriasis (Sloan and Bodor, 1982).

In our previous study, γ-linolenic acid (GLA) was the only fatty acid studied since it has a range of desirable anti-inflammatory, anti-cancer and cardiovascular actions (Horrobin, 1992). By maintaining GLA as the fatty acid moiety we demonstrated that as the SA hindrance of the linking moiety between GLA and theophylline increased, the rate of ester hydrolysis decreased.

As an extension of this work, we have prepared *N*-acyloxymethyl derivatives of theophylline keeping the linking moiety between theophylline and the fatty acid constant but varying the nature of the fatty acid. We chose to link the PUFAs to 7-(hydroxymethyl)theophylline (Scheme 1) as these derivatives eliminate the complication of chirality that we observed previously for the other linking groups. Moreover, the GLA derivative with this methylene linker was hydrolyzed, the fastest using both human plasma and porcine liver esterase, hence, it was anticipated the kinetic analysis will be facilitated using this linking group.

This study examined the effect of varying the number, position and configuration of the double bonds in fatty acid moieties of *N*-acyloxymethyl theophylline derivatives on the rate of hydrolysis using human plasma and porcine esterase. The fatty acids esterified were stearic (SA, 18:0), oleic (OA, 18:1ω9), elaidic (EA, 18:1ω9trans), linoleic



Scheme 1.

(LA, 18:2ω6), linoelaidic (LEA, 18:2ω6trans), γ-linolenic (GLA, 18:3ω6), α-linolenic (ALA, 18:3ω3) and eicosapentaenoic acid (EPA, 20:5ω3). The short chain carboxylic acids, acetic (Ac), valeric (VA) and nonanoic (NA) acid derivatives were examined as well.

2. Materials and methods

2.1. Apparatus

The HPLC consisted of a Beckman System Gold™ (Beckman Instruments, Palo Alto, CA) system comprised of a model 126 pump, model 507 autosampler, and a model 168 variable wavelength diode array detector. The analysis was performed using a reversed-phase ODS column (Beckman, 5 μ, 25 cm $\times$ 4.6 mm) with a C18 guard column with methanol-water as the mobile phase at a flow rate of 1 ml/min and uv detection at 254 nm. The mobile phase ranged from 40 to 100% methanol-water (vol/vol) depending on the derivative examined.

^{1}H NMR spectra were recorded with a Bruker AC 250 MHz spectrometer using $CDCl_3$ as the solvent. Chemical shifts are given in δ (ppm) values measured downfield from the tetramethylsilane internal standard.

Accurate mass measurements were determined by high resolution liquid secondary ion mass spectrometry (LSIMS) in positive ion mode using a Micromass AutoSpec Oatof Mass Spectrometer with resolution 8000, voltage scanning and 6 s/scan. The reference compound was polyethylene glycol (PEG) 600 and the matrix was 1:1 glycerol/*meta*-nitrobenzyl alcohol plus 0.1% trifluoroacetic acid.

2.1.1. Chemicals

Theophylline, paraformaldehyde, dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (4-DMAP), valeric acid, NA acid and pyridine were purchased from Aldrich (Milwaukee, WI). SA, OA, LA, ALA, EA and linoelaidic acid (LEA) were obtained from Nu-Chek Prep (Elysian, MN). GLA acid and EPA methyl ester was supplied by Callanish (Breasclete, Outer Hebrides, UK). All solvents were obtained from Fisher Scientific (Ottawa, Ont.) and were HPLC grade.

2.2. Synthesis

2.2.1. Preparation of 1-(theophyllin-7-yl)methyl stearate, **1**

In a 50 ml round bottom flask was placed 0.75 g (2.6 mmol) of SA acid, 0.50 g (2.4 mmol) of 7-(hydroxymethyl)theophylline (Sloan and Bodor, 1982) and 0.29 g (2.4 mmol) of 4-DMAP in 15 ml of $CHCl_3$. In all 0.54 g (2.6 mmol) of DCC was then dissolved in 10 ml of $CHCl_3$ and added dropwise to the flask. The reaction was monitored by TLC in 5% Methanol (MeOH)/$CHCl_3$ and after 4 h was complete. The $CHCl_3$ was evaporated and the residue purified by flash column chromatography using 5% methanol in chloroform to give 1-(theophyllin-7-yl)methyl stearate as a waxy white solid. 1.14 g (82%). ^{1}H NMR ($CDCl_3$) δ 7.85 (s, 1H, N=CH), 6.22 (s, 2H, N–CH_2–O), 3.62 (s, 3H, CH_3), 3.42 (s, 3H, CH_3), 2.43–2.30 (t, 2H, O=C–CH_2), 1.68–1.55 (m, 2H, O=C–CH_2–C$\underline{H}_2$), 1.35–1.20 (m, 18H, CH_2), 1.95–1.85 (t, 3H, CH_3). HR-LSIMS MH^+ 477.3436 $\pm$ 0.0003 $C_{26}H_{45}N_4O_4$ requires 477.3441.

The following 1-(theophyllin-7-yl)methyl carboxylic acid esters were prepared in a similar manner.

1-(Theophyllin-7-yl)methyl oleate, **2**: 1.03 g (88%). ^{1}H NMR ($CDCl_3$) δ 7.85 (s, 1H, N=CH), 6.23 (s, 2H, N–CH_2–O), 5.40–5.20 (m, 2H, CH=CH), 3.62 (s, 3H, CH_3), 3.40 (s, 3H, CH_3), 2.40–2.25 (t, 2H, O=C–CH_2), 2.10–1.95 (m, 4H, CH–C$\underline{H}_2$), 1.70–1.50 (m, 2H, O=C–CH_2C$\underline{H}_2$), 1.40–1.15 (m, 20H, CH_2), 0.95–0.80 (t, 3H, CH_3). HR-LSIMS MH^+ 475.3275 $\pm$ 0.0003 $C_{26}H_{43}N_4O_4$ requires 475.3284.

1-(Theophyllin-7-yl)methyl linoleate, **3**: 0.72 g (53%). ^{1}H NMR ($CDCl_3$) δ 7.80 (s, 1H, N=CH), 6.20 (s, 2H, N–CH_2–O), 5.40–5.20 (m, 4H, CH=CH), 3.62 (s, 3H, CH_3), 3.40 (s, 3H, CH_3), 2.75–2.60 (t, 2H, CH–C$\underline{H}_2$–CH), 2.42–2.25 (t, 2H, O=C–CH_2), 2.15–1.95 (m, 4H, CH–C$\underline{H}_2$), 1.70–1.55 (m, 2H, O=C–CH_2C$\underline{H}_2$), 1.45–1.15 (m, 14H, CH_2), 0.95–0.80 (t, 3H, CH_3). HR-LSIMS MH^+ 473.3107 $\pm$ 0.0007 $C_{26}H_{41}N_4O_4$ requires 473.3128.

1-(Theophyllin-7-yl)methyl α-linolenate, **4**: was described previously (Burke et al., 1997).

1-(Theophyllin-7-yl)methyl α-linolenate, **5**: 1.45 g (83%). ^{1}H NMR ($CDCl_3$) δ 7.85 (s, 1H, N=CH), 6.20 (s, 2H, N–CH_2–O), 5.42–5.20 (m, 6H, CH=CH), 3.58 (s, 3H, CH_3), 3.42 (s, 3H, CH_3), 2.85–2.75 (t, 4H, CH–C$\underline{H}_2$–CH), 2.40–2.30 (t, 2H, O=C–CH_2), 2.15–1.95 (m, 4H, CH–C$\underline{H}_2$), 1.65–1.52 (m, 2H, O=C–CH_2C$\underline{H}_2$), 1.37–1.20 (m, 8H, CH_2), 1.00–0.93 (t, 3H, CH_3). HR-LSIMS MH^+ 471.2966 ± 0.0003 $C_{26}H_{39}N_4O_4$ requires 471.2971.

1-(Theophyllin-7-yl)methyl elaidate, **6**: 1.42 g (86%). ^{1}H NMR ($CDCl_3$) δ 7.85 (s, 1H, N=CH), 6.20 (s, 2H, N–CH_2–O), 5.40–5.20 (m, 2H, CH=CH), 3.60 (s, 3H, CH_3), 3.40 (s, 3H, CH_3), 2.45–2.30 (t, 2H, O=C–CH_2), 2.05–1.85 (m, 4H, CH–C$\underline{H}_2$), 1.65–1.50 (m, 2H, O=C–CH_2C$\underline{H}_2$), 1.35–1.15 (m, 20H, CH_2), 0.95–0.80 (t, 3H, CH_3). HR-LSIMS MH^+ 475.3278 ± 0.0006 $C_{26}H_{43}N_4O_4$ requires 475.3284.

1-(Theophyllin-7-yl)methyl linoelaidate, **7**: 1.37 g (66%). ^{1}H NMR ($CDCl_3$) δ 7.85 (s, 1H, N=CH), 6.20 (s, 2H, N–CH_2–O), 5.42–5.35 (m, 4H, CH=CH), 3.62 (s, 3H, CH_3), 3.42 (s, 3H, CH_3), 2.70–2.60 (t, 2H, CH–C$\underline{H}_2$–CH), 2.40–2.25 (t, 2H, O=C–CH_2), 2.05–1.85 (m, 4H, CH–C$\underline{H}_2$), 1.65–1.50 (m, 2H, O=C–CH_2C$\underline{H}_2$), 1.40–1.15 (m, 14H, CH_2), 0.90–0.75 (t, 3H, CH_3). HR-LSIMS MH^+ 473.3114 ± 0.0002 $C_{26}H_{41}N_4O_4$ requires 473.3128.

1-(Theophyllin-7-yl)methyl eicosapentaenoate, **8**: Prior to esterification with 7-(hydroxymethyl)theophylline, EPA methyl ester was saponified to give the free acid. 0.91 g (67 %). ^{1}H NMR ($CDCl_3$) δ 7.86 (s, 1H, N=CH), 6.23 (s, 2H, N–CH_2–O), 5.44–5.25 (m, 10H, CH=CH), 3.59 (s, 3H, CH_3), 3.42 (s, 3H, CH_3), 2.85–2.72 (t, 10H, CH–C$\underline{H}_2$–CH), 2.39–2.32 (t, 2H, O=C–CH_2), 2.12–2.01 (m, 4H, CH–C$\underline{H}_2$), 1.75–1.63 (m, 2H, O=C–CH_2C$\underline{H}_2$), 1.00–0.94 (t, 3H, CH_3). HR-LSIMS MH^+ 495.2967 ± 0.0003 $C_{28}H_{39}N_4O_4$ requires 495.2971.

1-(Theophyllin-7-yl)methyl nonanoate, **9**: 0.34 g (68%). ^{1}H NMR ($CDCl_3$) δ 7.85 (s, 1H, N=CH), 6.21 (s, 2H, N–CH_2–O), 3.58 (s, 3H, CH_3), 3.40 (s, 3H, CH_3), 2.31–2.35 (t, 2H, O=C–CH_2), 1.56–1.59 (m, 2H, O=C–CH_2C$\underline{H}_2$), 1.21–1.28 (m, 10H, CH_2), 0.83–0.86 (t, 3H, CH_3). HR-LSIMS MH^+ 351.2035 ± 0.0003 $C_{17}H_{27}N_4O_4$ requires 351.1954.

1-(Theophyllin-7-yl)methyl valerate, **10**: 0.39 g (93%). ^{1}H NMR ($CDCl_3$) δ 7.85 (s, 1H, N=CH), 6.21 (s, 2H, N–CH_2–O), 3.58 (s, 3H, CH_3), 3.40 (s, 3H, CH_3), 2.32–2.35 (t, 2H, O=C–CH_2), 1.53–1.61 (m, 2H, O=C–CH_2C$\underline{H}_2$), 1.26–1.31 (m, 2H, C$\underline{H}_2$$CH_3$), 0.84–0.88 (t, 3H, CH_3). HR-LSIMS MH^+ 295.1403 ± 0.0003 $C_{13}H_{19}N_4O_4$ requires 295.1406.

1-(Theophyllin-7-yl)methyl acetate, **11**: 0.28 g (77%). ^{1}H NMR ($CDCl_3$) δ 7.84 (s, 1H, N=CH), 6.20 (s, 2H, N–CH_2–O), 3.57 (s, 3H, CH_3), 3.40 (s, 3H, CH_3), 2.09 (t, 3H, O=C–CH_3). HR-LSIMS MH^+ 253.0938 ± 0.0008 $C_{10}H_{13}N_4O_4$ requires 253.0936.

*2.3. Apparent lipophilicity indices of theophylline methyl fatty acid esters **2**–**11***

The lipophilic indices of the *N*-acyloxymethyl theophylline fatty acid esters were determined using reversed-phase HPLC as described above. The mobile phase was 4:1 methanol/water. Esters **2**–**11** demonstrated the following retention times (min) **2**, 148.1; **3**, 88.8; **4** and **5**, 57.7; **6**, 170.9; **7**, 107.2; **8**, 52.5; **9**, 9.1; **10**, 4.0 and **11**, 3.1 (solvent front, 2.5). The lipophilic index (log K) is calculated from the equation:

$$\log K = \log[(t_r - t_0)/t_0] \quad (1)$$

where t_r is the retention time of the ester and t_0 is the retention time of the elution solvent.

2.4. Determination of porcine esterase and human plasma hydrolysis rates

Porcine esterase (170 units/mg protein) was obtained from Sigma (Mississauga, Ont.). Esters **2**–**11** were prepared by dissolving each of the compounds in ethanol to give a 0.01 M concentration. An appropriate aliquot was then added to phosphate buffer, 19.72 ml, 0.025 M, pH = 7.4 with an ionic strength of 0.5 (KCl) in a shaker water bath thermostated at 37°C. The porcine esterase was diluted 100-fold with the phosphate buffer and 200 μl added to the buffered ester solutions to give a final volume of 20.0 ml, 4×10^{-5} M ester concentration and 1.3 units of enzyme/ml. The Theo-m-SA, **1**, derivative was

insoluble in ethanol and was dissolved in acetonitrile to give 2.2×10^{-2} M. A 200 μl aliquot was added to 19.6 ml of buffer and 200 μl of enzyme to give a final volume of 20.0 ml, 2.3×10^{-5} M concentration and 1.3 units of enzyme/ml. At appropriate time intervals a 500 μl aliquot was removed and added to 500 μl of ethanol to quench the reaction.

For the hydrolysis experiments in plasma, a total of 120 ml of human plasma from 30 volunteers was mixed together, aliquoted into 10 ml vials and frozen until needed. To 4.98 ml of 80% human plasma, made up with phosphate buffer, at 37°C allowed to equilibrate in shaker bath for 5 min was added 20 μl of the 0.01 M ester, **2**–**11**, stock solution to give a final concentration of 4×10^{-5} M. At appropriate time intervals a 250 μl aliquot was removed and added to 500 μl of ethanol, vortexed, centrifuged using an Eppendorf Microfuge at 10000 rpm for 10 min and 100 μl of the clear supernatant analyzed by HPLC. The Theo-m-SA, **1**, derivative dissolved in acetonitrile was added to the plasma to give a final concentration of 2.0×10^{-5} M.

The amount of ester remaining was determined by reverse-phase HPLC as described above. Pseudo-first order rate constants for the hydrolysis were determined from the slopes of the linear plots of the logarithm of residual theophylline PUFA prodrug versus time.

3. Results and discussion

*3.1. Synthesis of N-acyloxymethyl theophylline carboxylic acid esters, **1**–**11***

The general structure of the *N*-acyloxymethyl theophylline carboxylic acid derivatives prepared are shown in Fig. 1. The objectives of this report were to determine the effect of varying the nature of the acyl group (i.e. varying R) on the rate of hydrolysis catalyzed by porcine esterase and human plasma. The derivatives were all readily prepared from the condensation of the fatty acid with 7-(hydroxymethyl)theophylline using DCC and 4-DMAP.

*3.2. Kinetics of esters, **1**–**11**, using porcine liver esterase*

With the exception of Theo-m-SA all derivatives displayed first-order kinetics which indicates that the initial concentration was below the saturation level for porcine liver esterase. The rate constants and half-lives are given in Table 1. These theophylline derivatives are quantitatively converted to theophylline (Burke et al., 1997) which is consistent with the observation that the rate of theophylline formation is based only on the initial ester cleavage with a very short lived 7-(hydroxymethyl)theophylline intermediate (Scheme 1). Moreover, autoxidation and/or chemical hydrolysis did not occur during the assay conditions as the polyunsaturated Theo-m-GLA was stable in pH = 7.4 buffer alone up to 7 h.

The short chain carboxylic acid derivatives, Theo-m-VA and Theo-m-NA were hydrolyzed significantly faster than the longer chain fatty acid derivatives. On the other hand, Theo-m-Ac was hydrolyzed slower than the derivatives containing PUFA moieties.

In order to compare the rates of hydrolysis of the various fatty acid derivatives it is useful to utilize lipophilicity indices (or log K) as determined by reversed-phase HPLC. Log K is linearly related to log P, the *n*-octanol/water partition coefficient which is related to the number of carbons and degree of unsaturation in the derivatizing moiety. Plotting log k using porcine liver esterase for the theophylline derivatives, **1**–**11**, against log K as shown in Fig. 2, a parabolic relationship is obtained. It is known that the biological properties of a series of homologous compounds show regularities of increase and decrease. For many compounds lengthening of a saturated carbon side chain from one (methyl) through five to nine carbons (pentyl to nonyl) produces an increase in the pharmacological effect; however, further lengthening results in a decrease in the potency of the parent compound. This parabolic relationship is generally attributed to an increased lipophilicity of the molecule, which permits penetration into cell membranes, however, as the chain length increases lowered water solubility of the derivative becomes prob-

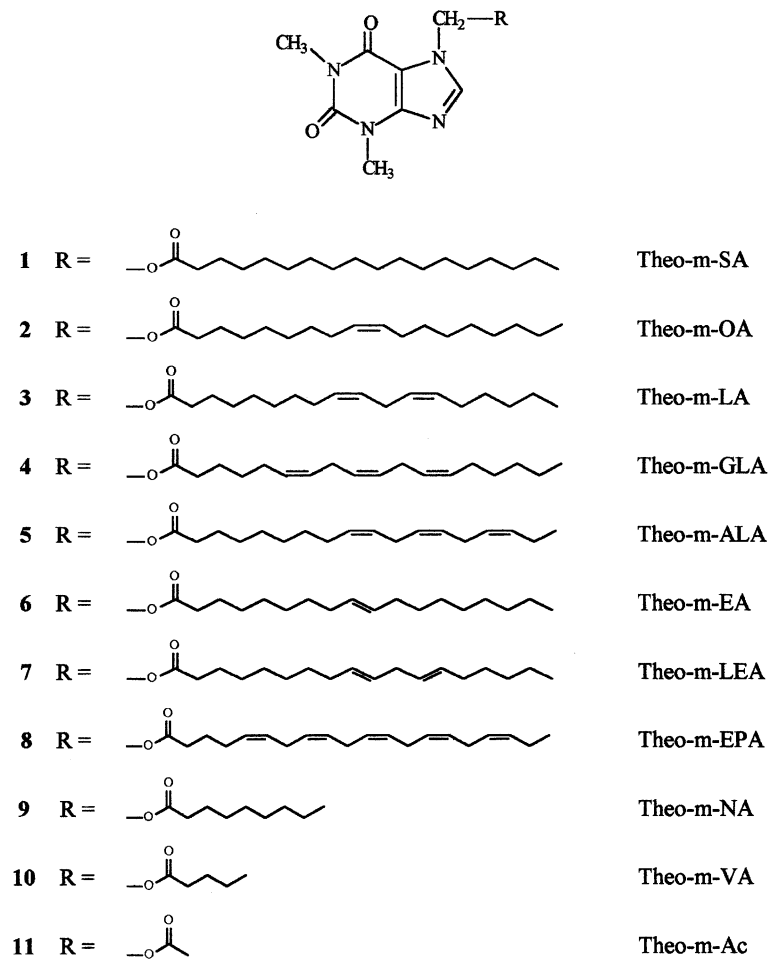


Fig. 1. *N*-acyloxymethyl theophylline esters.

lematic in its transport through aqueous media (Sinkula and Yalkowsky, 1975; Silverman, 1992).

Regularities of increase and then decrease in the rate of ester hydrolysis have been observed for *N*-acyloxymethyl saturated C2–C10 carboxylic acid derivatives of phenytoin in rat intestine homogenates, as well as rat and human plasma (Yamaoka et al., 1983). Moreover, skin penetration studies of lipophilic derivatives of indomethacin (Bonina et al., 1991) and acetaminophen (Nghiem et al., 1987) also showed this parabolic relationship. Our results are, therefore, consistent and provide yet another example of the phenomenon of regularities of increase then decrease in the biological activity as the derivatizing chain length is increased.

However, our results are still surprising in that for the derivatives with the same number of carbons in the fatty acid moiety, **1**–**7**, the rates of hydrolysis increased as the degree of unsaturation increased. Theo-m-GLA and Theo-m-ALA were hydrolyzed about four times as fast as Theo-m-LA which was hydrolyzed three times as fast as Theo-m-OA. Interestingly both the trans fatty acid derivatives, Theo-m-EA and Theo-m-LEA, were hydrolyzed slower than their cis counterparts Theo-m-OA and Theo-m-LA, respectively. These differences in the rates of hydrolyses of the derivatives with the same number of carbons in the PUFA promoiety but differing number of double

Table 1
Porcine esterase and 80% human plasma rate constants, half-lives and lipophilicity index (log K) for *N*-acyloxymethyl theophylline esters, **1–11**

Compound		Porcine esterase (1.3 U/ml)[a]		80% Human plasma[a]		log K[b]
		k (min^{-1})	$t_{1/2}$ (min)[c]	k (min^{-1})	$t_{1/2}$ (min)[c]	
Theo-m-SA	**1**	—	No hydrolysis	0.00119	580	—
Theo-m-OA	**2**	0.00660	105	0.00428	162	1.77
Theo-m-LA	**3**	0.0212	32.7	0.0107	64.9	1.54
Theo-m-GLA	**4**	0.0779	8.9	0.0254	27.3	1.34
Theo-m-ALA	**5**	0.0888	7.8	0.0180	38.4	1.34
Theo-m-EA	**6**	0.00128	540	0.00297	233	1.83
Theo-m-LEA	**7**	0.0145	47.9	0.00554	125	1.62
Theo-m-EPA	**8**	0.0417	16.6	0.0231	30.0	1.30
Theo-m-NA	**9**	6.93	0.10	0.642	1.08	0.42
Theo-m-VA	**10**	1.81	0.38	3.68	0.188	−0.22
Theo-m-Ac	**11**	0.00207	335	0.45	1.54	−0.62

[a] pH = 7.4, 37°C.
[b] Determined by reversed-phase HPLC, see Section 2.
[c] Calculated via $kt_{1/2} = 0.693$.

bonds can be explained by examining the volume and linear parameter of the promoiety. For instance, it has been shown, for the hydrolysis of cytarabine-N^4-carboxylate and succinamate ester prodrugs by porcine liver esterase, that the steric parameters such as the van der Waals volume of the ester side chain, molecular bulkiness and linear dimensions influence the rate (Kawaguchi et al., 1996). This study correlated the rate of hydrolysis of the ester prodrugs with an increase in the molecular bulkiness of the promoiety (methyl to octyl ester) but a decrease in the rate as the linear parameter increased (cholesteryl ester). The authors suggest that for porcine liver esterase there is a need for a bulky ester group to increase the prodrug molecular bulkiness and hence the rate bearing in mind that an increase in the linear parameter outside the boundary of the pocket of the active site retards hydrolysis significantly. Since we obtained a similar order of hydrolysis with our acetate, valerate and nonanoate theophylline derivatives the observation of the importance of the molecular bulkiness and length is very relevant in explaining the hydrolysis rates of our theophylline PUFA derivatives.

Therefore, while it is not the aim of this study to determine volumes and linear parameters of these theophylline derivatives the observed rates of hydrolysis certainly can be explained in a qualitative way. PUFAs such as GLA and ALA are bent and twisted like a portion of a coil in a spring. In relative terms they are bulkier than the less unsaturated and less twisted LA. Moreover, because GLA and ALA are bent to a greater extent their linear dimensions would be less than LA. Consequently both their bulkiness and linear parameters are favorable for Theo-m-GLA and Theo-m-ALA to give enhanced rates of hydrolysis, relative to the less unsaturated derivatives. Similar arguments can be made for the Theo-m-LA versus Theo-m-OA and Theo-m-OA versus Theo-m-SA. Analogous reasoning explains the enhanced rates of hydrolyses for the cis derivatives over their trans counterparts which are more linear and hence less bulky compared to the cis derivatives. Since Theo-m-EPA was hydrolyzed slower than Theo-m-GLA and Theo-m-ALA but faster than Theo-m-LA it may be that, although EPA is bent and twisted to a greater extent than ALA or GLA, the linear parameter is important due to the presence of the two additional carbons.

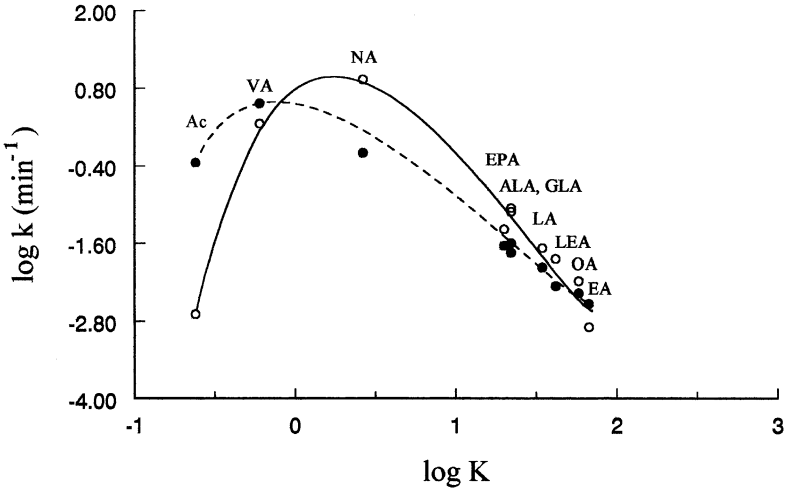


Fig. 2. Log k versus log K for theophylline derivatives **2**–**11** using (○) porcine esterase (pH = 7.4, 37°C) and (●) using 80% human plasma (pH = 7.4, 37°C). The letters represent the carboxylic acid moieties given in Fig. 1.

*3.3. Kinetics of esters, **1**–**11**, using human plasma*

As with porcine liver esterase all derivatives displayed first-order kinetics in human plasma. Generally the rates of hydrolysis were slower in human plasma than when using porcine liver esterase, the exceptions being the acetate, **11**, and valerate, **10**, derivatives. As was seen with porcine liver esterase the short chain valerate, **10**, and nonanoate, **9**, carboxylic acid derivatives were hydrolyzed much faster than the longer chain fatty acid derivatives. Moreover, the polyunsaturated derivatives, Theo-m-GLA, Theo-m-ALA and Theo-m-EPA were all hydrolyzed significantly faster than the less unsaturated derivatives. Although there are numerous esterases present in plasma, as with the porcine liver esterase data, differences in the bulkiness and/or linear parameter of the unsaturated fatty acid moieties is also a reasonable explanation.

Plotting log k obtained in human plasma versus the lipophilicity index, log K, a similar parabolic relationship was obtained (Fig. 2) with maximum rate of cleavage occurring between the C5 and C9 derivatives. For homologous series of straight-chain saturated carboxylic acid derivatives the maximum rate of cleavage generally occurs with C3–C9 carboxylic acid derivatives (Silverman, 1992). Hence, it is generally assumed that saturated carboxylic acid moieties greater than C9 would be hydrolyzed at even slower rates. Certainly this was the case in this study for the SA acid derivative, **1**, where we did not see any hydrolysis using porcine esterase and only very slow hydrolysis in human plasma. However, comparing the C18 polyunsaturated derivatives, Theo-m-GLA and Theo-m-ALA with Theo-m-SA there is nearly two orders of magnitude difference in their rates of hydrolysis. Clearly, this data demonstrates that the nature of the fatty acid moiety (unsaturation versus saturation as well as number, position and configuration of double bonds) must be recognized with regards to esterase hydrolysis and ultimately prodrug development.

It is possible that the descending portion of the curves comprising the fatty acid derivatives in Fig. 2 could be due to physical reasons affecting the enzymatically decreased rate of hydrolysis. These would include micelle formation, decreased solubility or protein binding (Sinkula and Yalkowsky, 1975). We did not measure critical micelle concentrations (CMC), however, similar related compounds such as the acyloxymethyl quaternary ammonium salts of myristic acid (14 carbons) were found to have CMCs in the 10^{-4} to 10^{-3} range (Bodor et al., 1980). Therefore, our initial

concentration of 4×10^{-5} suggests that we are below the CMC. Although, generally, fatty acids are insoluble in water, the fact that the lipophilicity indices, log K, of these theophylline derivatives, which are proportional to the octanol/water partition coefficient, log P, increase as the degree of saturation increases suggests that the solubility of these derivatives, while likely to be small, is decreasing as the degree of saturation increases. This decrease in solubility must be recognized when interpreting the data in Fig. 2. Additionally we cannot exclude protein binding of the derivatives which may affect the rate of hydrolysis as a possibility for the observed data in Fig. 2.

In summary, *N*-acyloxymethyl theophylline carboxylic acid esters were prepared and evaluated with regards to the in vitro rates of hydrolysis using porcine liver esterase and human plasma. All derivatives were found to undergo hydrolysis but with varying rates, presumably, depending on the molecular bulkiness and linear parameter of derivative. The polyunsaturated derivatives containing the three or more double bonds were hydrolyzed faster than their more saturated fatty acid counterparts with the same number of carbons.

Although the short chain valeric and NA acid derivatives were hydrolyzed the fastest it should be noted that in vivo studies must be conducted to determine the optimum derivative for a specific oral or topical application. At any rate, the results presented here lead one to speculate that these unsaturated fatty acid moieties may be useful as derivatizing agents in prodrug design for two reasons. Firstly, although the unsaturated fatty acid derivatives with three or more double bonds are not hydrolyzed at the maximum rate they are hydrolyzed at markedly faster rates than the corresponding saturated or monounsaturated derivatives and, hence, may still serve as a delivery vehicle for other drugs. Secondly, since the PUFAs have beneficial medicinal properties of their own, there may be an added advantage in delivering two bioactives (drug and PUFA) to treat a disease condition.

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