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Research Article

Prodrugs of Theophylline Incorporating Ethyleneoxy Groups in the Promoiety: Synthesis, Characterization, and Transdermal Delivery

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Abstract. Two different types of derivatives of theophylline (Th-H) incorporating ethyleneoxy groups into the promoiety have been synthesized. One is a soft alkyl type where *N*-methyl-*N*-methoxyethyleneoxycarbonylaminomethyl chlorides have been used to alkylate Th-H in the 7 position. The other is in an acyl type where methoxyethyleneoxycarbonyl chlorides have been used to acylate Th-H in the 7 position. All of the prodrugs were more soluble in the lipid isopropyl myristate (IPM) than Th-H, and three were more soluble in water (AQ) than Th-H. The most water-soluble prodrug gave the highest maximum delivery of total species containing Th-H through hairless mouse skin from IPM (maximum flux, J_{MMIPM})—more than seven times that of Th-H, while the other two gave more than three times that of Th-H. The acyl-type prodrugs delivered only Th-H, while the soft alkyl types delivered 60–70% Th-H plus intact prodrug. The Roberts–Sloan equation was able to predict the best performer for each type with an average of the absolute difference between the experimental $\log J_{MMIPM}$ and calculated $\log J_{MMIPM}$ ($\Delta \log J_{MMIPM}$) of 0.253 log units. The values for the present prodrugs and previously reported prodrugs that had not been previously included in the Roberts–Sloan data base (n=23) were included in the previous n=71 data base to give n=94. New coefficients for the Roberts–Sloan equation have been obtained.

KEY WORDS: ethyleneoxy groups; lipid solubility; maximum flux; Roberts–Sloan equation; theophylline; water solubility.

INTRODUCTION

Theophylline (Th-H) was the first amide/imide for which prodrugs were synthesized and characterized for their ability to enhance the topical delivery of their parent drug from a lipid vehicle isopropyl myristate (IPM) (1,2). A lipid vehicle was chosen because lipids comprise the continuous phase in many topical formulations. Although 7-alkylcarbonyloxymethyl soft alkyl prodrugs of Th-H (7-ACOM-Th) were the first prodrugs to be made, the performance of the best member of the series was not much better than Th-H itself: two times that of Th-H (1,2). Their relatively poor delivery of their parent Th-H had been attributed to their lack of adequate water solubility, S_{AQ} (3). Although they were 10–100 times more soluble in the lipid vehicle, IPM, the most water-soluble 7-ACOM prodrug, exhibited only 25% of the solubility of Th-H in water. The next most water-soluble member of their series (20% of Th-H) actually delivered the greater amount of total species containing Th-H through hairless mouse skin from their suspensions in IPM: maximum flux, J_{MMIPM}. The slightly less water-soluble member compensated for its slightly lower S_{AQ} by being 10 times more soluble in IPM than the most water-soluble member. Thus, as more series of prodrugs were synthesized, characterized, and evaluated in diffusion cell experiments using an IPM vehicle, it became obvious that the prodrug exhibiting the best balance of lipid and aqueous solubilities gave the highest flux value in the series and not necessarily the member that was most water (or lipid) soluble (4,5).

Subsequently, the solubilities in the lipid IPM, S_{IPM}, and water, S_{AQ}, and molecular weights, MW, as independent variables and the maximum flux values for the delivery of total species containing the parent drug by prodrugs through hairless mouse skin from saturated IPM, J_{MMIPM}, as the dependent variable were fitted to a solubility-based expansion of Fick's law (Eq. 1) where n=42 (6). The coefficients to the IPM and aqueous solubility parameters were approximately equal, which supported the previous observations (4,5) that a balance of improved lipid and aqueous solubilities was important to optimize flux from a lipid vehicle. Thus, to design a prodrug that gave greater delivery of total species containing

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ABBREVIATIONS: IPM, Isopropyl myristate; Th-H, Theophylline; NANMEOCAM, *N*-alkyl-*N*-methoxyethyleneoxycarbonylaminomethyl; J_{MMIPM}, Maximum delivery of species containing the parent drug through hairless mouse skin from IPM; $\Delta \log J_{MMIPM}$, the average of the absolute differences between experimental and calculated log J_{MMIPM}; ACOM, Alkylcarbonyloxymethyl; DAM, Diakylaminomethyl; MEOC, Methoxyethyleneoxycarbonyl; NANAOCAM, *N*-alkyl-*N*-alkyloxycarbonylaminomethyl.

the parent drug through hairless mouse skin from a lipid vehicle, it is necessary to improve both the lipid and the aqueous solubilities of a prodrug compared to its parent drug to obtain a significant improvement. The basis for this result is the finding that, in a series/parallel model of transdermal delivery or flux (7), the highest capacity pathway for permeants to diffuse through hairless mouse skin is through a series of alternating lipid and aqueous polar phases, which requires that the permeant exhibits adequate solubility in both phases to optimize its transdermal delivery. An alternate, lipid-only pathway in parallel with the series pathway was of much lower capacity.

$$\log J_{MMIPM} = x + y \log S_{IPM} + (1 - y)\log S_{AQ} - z\mathrm{MW} \quad (1)$$

On the other hand, the Potts–Guy (PG) (Eq. 2) [8] uses partition coefficients between octanol (OCT) and water, $K_{OCT/AQ}$, and MW as the independent variables and permeability coefficients, P, as the dependent variable in its analysis of flux databases. However, the PG equation cannot be used if the vehicle is a lipid. Only an aqueous vehicle can be used if $K_{OCT/AQ}$ is one of the independent variables. The Roberts–Sloan (RS) Eq. (1) is much more versatile. It can be used if the vehicle is water (9) or if the vehicle is a lipid such as IPM (6). In fact, when the largest collected database consisting in solubilities in the lipid octanol, S_{OCT}, and water, S_{AQ}, molecular weights, MW, and the maximum fluxes of n=184, different permeants through human skin *in vitro* from water (J_{MHAQ}) was fitted to the RS equation, the same results were obtained as when data for J_{MMIPM} for a different set of permeants was fitted to RS: The coefficients to the lipid (OCT or IPM, respectively) and aqueous solubility parameters were about the same (10).

$$\log P = x + y \log K_{OCT/AQ} - z\mathrm{MW} \quad (2)$$

Thus, it was not surprising that the much more water (and lipid) soluble 7-dialkylaminomethyl soft alkyl prodrugs of Th-H (7-DAM-Th) performed much better than Th-H: up to nine times the J_{MMIPM} of Th-H (3,11). However, the 7-DAM-Th prodrugs were not very stable and released potentially toxic or irritating secondary amines when they hydrolyzed to the parent Th-H in the skin. Besides the incorporation of an ionizable basic amine group into the promoiety to increase the water solubility and the topical delivery of its parent drug, another approach to increasing the water solubility of the prodrug *versus* its parent drug is to incorporate ethyleneoxy groups into the promoiety. This approach has been used previously to increase the water solubility and hence to increase the topical delivery of non-steroidal anti-inflammatory drugs (12) and various nucleosides (13). However, most recently, the same approach has not been very successful in increasing the topical delivery of acetaminophen (APAP) (14,15) or naltrexone (16). On the other hand, to date, there have not been any reports of the effect on topical delivery by the incorporation of ethyleneoxy groups into the promoieties of *N*-alkyl or *N*-acyl prodrugs of amide/imide type drugs such as theophylline.

Here, we report the synthesis, characterization, and evaluation in diffusion cell experiments (using saturated IPM as the vehicle) of two types of prodrugs of Th-H, which incorporate ethyleneoxy groups into their promoieties. One is a soft alkyl type. Th-H has been alkylated with *N*-methyl-*N*-methoxyethyleneoxycarbonylaminomethyl chlorides (NANMEOCAM chlorides) to give 7-NANMEOCAM-Th derivatives. The other is an acyl type. Th-H has been acylated with methoxyethyleneoxycarbonyl chlorides (MEOC chlorides) to give 7-MEOC-Th derivatives. The new derivatives have been compared with previous derivatives of the same type that did not contain ethyleneoxy groups in their promoieties. The results from both new and previous prodrugs have been added to the Roberts–Sloan database for delivery of drugs by their prodrugs from IPM through hairless mouse skin.

METHODS AND MATERIALS

Materials

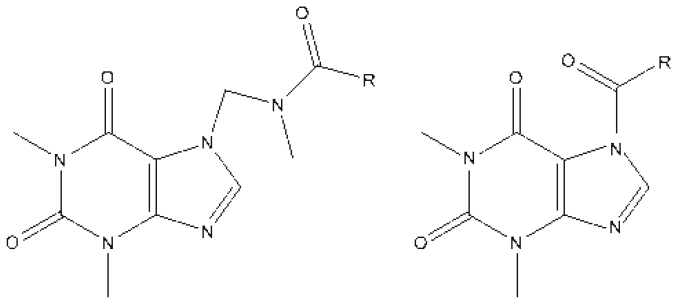
Isopropyl myristate (IPM) was obtained from Givaudan Corp. (Clifton, NJ, USA). Theophylline (Th-H) was purchased from Sigma Chemical Co. (St. Louis, MO, USA); all other reagent chemicals were from Aldrich Chemical Co. (Milwaukee, WI, USA). The water was obtained from a Millipore Milli-Q water ultrafiltration system. ^{1}H NMR spectra were obtained at 400 MHz on a Varian Unity-400 spectrometer. Thin layer chromatography analyses were run on Brinkman Polygram Sil G/UV 254 plates. Ultraviolet spectra were recorded on a Shimadzu UV-2501 PC spectrophotometer. A radiometer pH meter 26 was used to determine the pH of solutions. The vertical, Franz-type diffusion cells were from Crown Glass (Somerville, NJ, USA; 4.9 cm^2 surface area, 20 mL receptor phase volume, and 15 mL donor phase volume). The diffusion cells were maintained at 32°C with a Fisher (Pittsburgh, PA, USA) circulating water bath model 25. The female hairless mice (SKH-hr-1) were from Charles River (Boston, MA, USA). The animal research adhered to the NIH "Principles of Laboratory Animal Care." The animal experiments are conducted in full compliance with local, national, ethical, and regulatory principles and use by the Institutional Animal Care and Use Committee of the University of Florida.

Synthesis of Prodrug Derivatives

General Synthesis of Prodrugs **1–3**

1–3 were synthesized by alkylating theophylline (Th-H) with the corresponding N-methyl-N-methoxyethyleneoxycarbonylaminomethyl chlorides (NANMEOCAM chlorides) (17) in presence of mild base like triethylamine (TEA) and dichloromethane (DCM) as the solvent (Table I, Scheme 1). The alkylating agents (NANMEOCAM chlorides) were prepared *via* a three step synthesis starting from a commercially available alcohol, *i.e.*, 2-methoxyethyl alcohol (MEA), 6-methoxyethyloxyethyl alcohol (MDEA) or 2-methoxy-1-methylethyl alcohol (MPA). The alcohols were first converted into alkyloxycarbonyl imidazoles by reacting them with 1,1′-carbonyldiimidazole (CDI) in presence of DCM. The alkyloxycarbonoyl imidazoles were then reacted with methyl amine in 2-propanol to give *N*-methyl carbamic acid alkyl esters. The *N*-methyl carbamic acid alkyl esters were finally converted to NANMEOCAM chlorides based on MEA, MDEA, and MPA by reacting the *N*-methyl carbamic acid alkyl esters

Table I. Molecular Weights (MW), Melting Points (mp °C) and Molar Absoptivities of Theophylline Prodrugs in Acetonitrile (CH_3CN) and pH 7.1 Buffer with 0.11% Formaldehyde



1 – 3, 7 – 11 **4 – 6, 12 – 16**

Compound	R	MW	mp°C	UV (CH_3CN) λ_ϵ	pH 7.1 buffer[a] $\lambda_{\epsilon 260}$	$\lambda_{\epsilon 284}$
1,	$CH_2CH_2OCH_3$	325	125-127	0.760[b]	0.45	0.66
2,	$(CH_2CH_2O)_2CH_3$	369	91-93	0.710[b]	0.45	0.63
3,	$CH(CH_3)CH_2OCH_3$	339	113-115	0.726[b]	0.45	0.63
4,	$CH_2CH_2OCH_3$	282	95-96	0.692[c]		
5,	$(CH_2CH_2O)_2CH_3$	326	63-64	0.698[c]		
6,	$CH(CH_3)CH_2OCH_3$	296	102-104	0.690[c]		

[a] 1×10^4 L mol^{-1}
[b] λ_{max} at 273 nm
[c] λ_{max} at 283 nm

with trimethyl silyl chloride (TMS-Cl) and paraformaldehyde. No attempts were made to purify the corresponding alkyloxycarbonyl imidazoles, *N*-methyl carbamic acid alkyl esters, or NANMEOCAM chlorides because of their easy decomposition upon distillation or column chromatography.

The detailed synthesis is shown below.

Synthesis of alkyloxycarbonylimidazoles

Alcohol (0.01 mol) was reacted with 1.1 equivalents of 1,1′-carbonyldiimidazole (0.011 mol) in 50 mL CH_2Cl_2 overnight at room temperature. The clear solution was diluted with 50 mL CH_2Cl_2 and washed with 10 mL 1 N HCl and 2×10 mL with water. The CH_2Cl_2 layer was dried over Na_2SO_4 then concentrated to give the alkyloxycarbonylimidazole as an oil. The alkyloxycarbonylimidazoles were characterized by ^{1}H NMR.

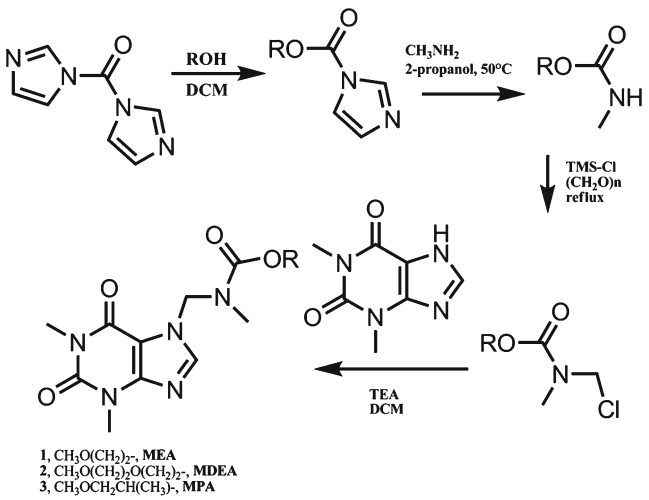
3-Oxybutyloxycarbonylimidazole from MEA: yield=98%, ^{1}H NMR ($CDCl_3$): δ 3.4 (s, 3H), δ 3.73 (t, 2H), δ 4.55 (t, 2H), δ 7.08 (d, 1H), δ 7.45 (d, 1H), δ 8.15 (s, 1H).

3,6-Dioxyheptyloxycarbonylimidazole from MDEA: yield=98%, ^{1}H NMR ($CDCl_3$): δ 3.4 (s, 3H), δ 3.55 (t, 2H), δ 3.67 (t, 2H), δ 3.84 (t, 2H), δ 4.57 (t, 2H), δ 7.07 (d, 1H), δ 7.45 (d, 1H), δ 8.16 (s, 1H).

3-Oxy-1-methylbutyloxycarbonylimidazole from MPA: yield=89%, %, ^{1}H NMR ($CDCl_3$): δ 1.4 (d, 3H), δ 3.4 (s, 3H), δ 3.6 (m, 2H), δ 5.15 (m, 1H), δ 7.08 (d, 1H), δ 7.45 (d, 1H), δ 8.15 (s, 1H).

Synthesis of N-methyl carbamic acid alkyl esters

An alkyloxycarbonylimidazole (0.01 mol) was coupled with 1.3 equivalents (0.013 mol) of aqueous methyl amine in



Scheme 1. General synthesis of prodrugs **1–3**

2-propanol (10 mL) at 50°C overnight. The reaction mixture was then concentrated under vacuum at 50°C. The oily residue obtained was dissolved in 50 mL CH_2Cl_2 and washed with 10 mL 1 N HCl, 5×3 mL water. The CH_2Cl_2 layer was dried over Na_2SO_4 and concentrated to an oil. The *N*-alkyl carbamic acid esters obtained were all oils at room temperature and were characterized by ^{1}H NMR.

N-Methyl carbamic acid 3-oxybutyloxy ester: yield=72%, ^{1}H NMR ($CDCl_3$): δ 2.8 (d, 3H), δ 3.39(s, 3H), δ 3.63 (t, 2H), δ 4.24 (t, 2H), δ 4.7(s, 1H).

N-Methyl carbamic acid 3,6-dioxyheptyloxy ester: yield=68%, ^{1}H NMR ($CDCl_3$): δ 2.8 (d, 3H), δ 3.39(s, 3H), δ 3.56 (t, 2H), δ 3.65 (t, 2H), δ 3.74 (t, 2H), δ 4.24 (t, 2H), δ 4.7 (s, 1H).

N-methyl carbamic acid 3-oxy-1-methylbutyloxy ester: yield=72%, ^{1}H NMR ($CDCl_3$): δ 1.25 (d, 3H), δ 2.8 (d, 3H), δ 3.39(s, 3H), δ 3.42 (d, 2H), δ 4.7(s, 1H), δ 5.0 (m, 1H).

Synthesis of NANMEOCAM chlorides

A suspension of an *N*-alkyl carbamic acid alkyl ester (16 mmol), 1.7 equivalents of paraformaldehyde and 13 equivalents of trimethylsilyl chloride was refluxed with a $CaCl_2$ drying tube on top of the water condenser, for 2.5 h using an oil bath. The suspension was diluted with CH_2Cl_2 and filtered to get rid of the unreacted paraformaldehyde. The clear filtrate was concentrated at 40°C under reduced pressure. The yellow oil obtained was triturated with hexane overnight. The white suspension obtained was filtered, and the filtrate was concentrated to give the desired alkylating agent as an oil. The oils were characterized by ^{1}H NMR.

N-Methyl-*N*-3-oxybutyloxycarbonylaminomethyl chloride: yield=83%, ^{1}H NMR ($CDCl_3$): δ 3.02(s, 3H), δ 3.38 (s, 3H), δ 3.62 (t, 2H), δ 4.31 (t, 2H), δ 5.33(s, 2H).

N-Methyl-*N*-3,6-dioxyheptyloxycarbonylaminomethyl chloride: yield=87%, ^{1}H NMR ($CDCl_3$): δ 3.02 (s, 3H), δ 3.39 (s, 3H), δ 3.56 (t, 2H), δ 3.66 (t, 2H), δ 3.74 (t, 2H), δ 4.32 (t, 2H), δ 5.33 (s, 2H).

N-Methyl-*N*-3-oxy-1-methylbutyloxycarbonylaminomethyl chloride: yield=90%, ^{1}H NMR ($CDCl_3$): δ 1.26 (d, 3H), δ 2.8 (s, 3H), δ 3.39(s, 3H), δ 3.45 (d, 2H), δ 5.04 (m, 1H), 5.33 (s, 2H),

Synthesis of NANMEOCAM-Th

Equimolar amounts of theophylline (Th-H) and triethylamine were allowed to react in dichloromethane (20 mL) for 20 min to give suspensions. The suspensions were then allowed to react overnight with the corresponding NANMEOCAM chlorides to give **1**, **2**, or **3** based on MEA, MDEA, or MPA, respectively. The clear solutions were diluted with dichloromethane (40 mL), and the organic layers were extracted with aqueous HCl (5 mL), aqueous $NaHCO_3$ (5 mL), and water (5 mL). The organic layers were dried over Na_2SO_4 for 30 min and concentrated to give white solids. The crude products were recrystallized from dichloromethane and hexanes (**2** and **3**) or methanol (**1**) to give the desired products.

N^7-(*N*-Methyl-*N*-3-oxybutyloxycarbonyl)-aminomethyl theophylline, **1**: 61% yield from theophylline, mp 125–127°C, ^{1}H NMR ($CDCl_3$) δ 8.04 and 7.97 (2s, 1, 8-*H*), 3.60 and 3.42 (2s, 6, NCH_3), 5.82 and 5.80 (2s, 2, NCH_2N), 4.35 and 4.28 (2m, 2, CH_2OC=O), 3.45 and 3.37 [2s, 3, $CH_2N(CH_3)C$=O], 3.65–3.57 (m, 2, CH_2OCH_3), 3.13 (s, 3, OCH_3). Elemental analysis for $C_{13}H_{19}N_5O_5$ (MW 325): C, 48.00; H, 5.89; N, 21.53. Found: C, 48.17; H, 5.92; N, 21.49.

N^7-(*N*-Methyl-*N*-3,6-dioxyheptyloxycarbonyl)-aminomethyl theophylline, **2**: 66% yield from theophylline, mp 91–93°C, ^{1}H NMR ($CDCl_3$) δ 8.04 and 7.97 (2s, 1, 8-H), 3.63 and 3.43 (2s, 6, NCH_3), 5.82 and 5.80 (2s, 2, NCH_2N), 4.35 and 4.28 (2m, 2 CH_2OC=O), 3.43 and 3.33 [2s, 3, $CH_2N(CH_3)$C=O], 3.80–3.52 (4m, 6, $CH_2CH_2OCH_2CH_2OC$=O), 3.16 (s, 3, OCH_3). Elemental analysis for $C_{15}H_{23}N_5O_6$ (MW 369): C, 48.78; H, 6.28; N, 18.96. Found: C, 48.90; H, 6.36; N, 18.96.

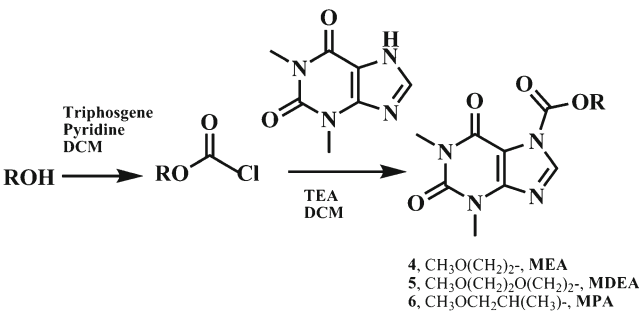
N^7-(*N*-Methyl-*N*-3-oxy-1-methylbutyloxycarbonyl)-aminomethyl theophylline, **3**: 65% yield from theophylline, mp 111–113°C, ^{1}H NMR ($CDCl_3$) δ 8.04 and 7.99 (2s, 1, 8-H), 3.64 and 3.45 (2s, 6, NCH_3), 5.83 and 5.80 (m and s, 2, NCH_2N), 3.45 and 3.35 [2s, 3, $CH_2N(CH_3)$C=O], 5.15–5.0 (2 m, 1, $CHCH_3$), 3.5–3.40 (m, 2, CH_3OCH_2), 3.10 (s, 3, OCH_3), 1.33 and 1.25 (2 d, 3, CH_3CH–). Elemental analysis for $C_{14}H_{21}N_5O_5$ (MW 339): C, 49.55; H, 6.24; N, 20.64. Found C, 49.51; H, 5.92; N, 21.49.

General Synthesis of **4–6**

The synthesis of alkyloxycarbonyl-Th derivatives (**4–6**) involves the acylation of Th-H with alkyoxycarbonyl chlorides in presence of mild base, TEA, and DCM as the solvent (Scheme 2). The synthesis of alkyloxycarbonyl chlorides from commercially available alcohols, *i.e.*, MEA, MDEA, or MPA involves the conversion of the alcohol to a chloroformate by reaction with triphosgene and pyridine in DCM as the solvent as reported by us before (14). No attempts were made to either isolate or characterize the chloroformate formed *in situ*.

Synthesis of **4–6**

To 1 equivalent of triphosgene (about 1.0 g, 0.0033 mol) dissolved in 10 mL of dichloromethane was added 0.81 g (0.01 mole, 3 equivalents) of pyridine in 10 mL dichloromethane with stirring. Then, about 3 equivalents (0.01 mol) of MEA, MDEA, or MPA were added in 5 mL dichloromethane, and the solutions were stirred at room temperature for 1 h. Theophylline (1.80 g, 0.01 mol) in 5 mL dichloromethane was added with stirring to the ice cold preformed chloroformates, followed immediately by 1.01 g (0.01 mol) of triethylamine. The reaction mixtures were stirred overnight at room temperature, diluted with 80 mL dichloromethane, extracted with a solution of 6 mL water plus 1 mL concentrated HCl, then with



Scheme 2. General synthesis of **4–6**

7 mL water. The organic layers were dried over Na_2SO_4 for 30 min, then concentrated under vacuum to give solids. The solids were recrystallized from dichloromethane and ether or petroleum ether to give the desired products **4**, **5**, or **6** based on MEA, MDEA, or MPA, respectively.

N^7-(*N*-3-oxybutyloxycarbonyl)-theophylline, **4**: 71.3% yield, mp 95–96°C, ^{1}H NMR ($CDCl_3$) δ 8.29 (s, 1, 8-H), 3.44 and 3.63 (2s, 6, NCH_3) 3.44 (s, 3, OCH_3), 4.63 (m, 2, $CH_2OC{=}O$), 3.79 (m, 2, CH_2OCH_3). Elemental analysis for $C_{11}H_{14}N_4O_5$ (MW 282): C, 46.85; H, 4.96; N, 19.86. Found: C, 46.84; H, 5.07; N, 19.91.

N^7-(*N*-3,6-dioxyheptyloxycarbonyl)-theophylline, **5**: 55.5% yield, mp 63–64°C, ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1, 8-H), 3.44 and 3.63 (2s, 6, NCH_3), 3.39 (s, 3, OCH_3), 4.63 (m, 2, $CH_2OC{=}O$), 3.90 (m, 2, $OCH_2CH_2OC{=}O$), 3.7 and 3.57 (2m, 4, $OCH_2CH_2OCH_3$). Elemental analysis for $C_{13}H_{18}N_4O_6$ (MW 326): C, 47.87; H, 5.52; N, 17.18. Found: C, 47.92; H, 5.68; N, 17.18.

N^7-(*N*-3-oxy-1-methylbutyloxycarbonyl)-theophylline, **6**: 53.3% yield, mp 102–104°C, ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1, 8-H), 3.44 and 3.63 (2s, 6, NCH_3), 3.44 (s, 3, OCH_3), 5.37 (m, 1, $CHCH_3$), 1.46 (d, 3, CH_3CH), 3.7 and 3.60 (2m, 2, CH_2OCH_3). Elemental analysis for $C_{12}H_{16}N_4O_5$ (MW 296): C, 48.66; H, 5.40; N, 18.92. Found: C, 48.60; H, 5.41; N, 18.82.

Determination of Solubilities and Partition Coefficients

Molar absorptivities were determined in triplicate for each member of the series in acetonitrile (ACN) and in pH 7.1 phosphate buffer (0.05 M, I=0.11 M, 32°C) containing 0.1% formaldehyde for **1**, **2**, and **3**. The molar absorptivities were calculated using Beer's law ($\varepsilon = A/c$).

The solubilities of the prodrugs in IPM (18,19) were determined in triplicate by stirring suspensions of the compound in 2 mL IPM with a magnetic stirrer for 24 h at room temperature (23±1°C). The test tubes containing the suspensions were sealed and thermally insulated from the stirrer. After stirring, the suspensions were filtered through a 0.45-μm nylon membrane filter. An aliquot (0.1–0.3 mL) was withdrawn from the clear filtrates of the saturated solutions and diluted to 10 mL in a volumetric flask with acetonitrile (ACN). The samples were then analyzed by UV spectroscopy. The solubilities in IPM (Table II) were calculated using molar absorptivities in ACN previously determined in triplicate at 273 nm for **1**, **2**, and **3** and 283 nm for **4**, **5**, and **6** (Table I).

Solubilities in water (S_{AQ}) (18,19) were determined by stirring suspensions of **1**, **2**, and **3** in deionized water for 1 h to limit the extent of hydrolysis (20) of the prodrugs. The samples were filtered through nylon filters, diluted with ACN and immediately analyzed by UV spectroscopy. S_{AQ} values (Table II) were determined using molar absorptivities in ACN measured at 273 nm (Table I).

For determination of partition coefficients ($K_{IPM/4.0}$), (21) between IPM and pH 4.0 acetate buffer (0.05 M, 32°C), measured volumes (0.5–1 mL) of the filtered saturated IPM solutions from the lipid solubility experiments were mixed with measured volumes of pH 4.0 acetate buffer (1–5 mL) in 10-mL test tubes. The test tubes were capped and vigorously shaken for 10 s and subsequently centrifuged for 2 min to allow the clear separation of two phases. An aliquot (0.3 mL) was withdrawn from each IPM layer and diluted to 10 mL with ACN in a volumetric flask and analyzed by UV spectroscopy as above. The $K_{IPM/4.0}$ (Table II) were calculated using the following relationship:

$$K_{IPM/4.0} = (V_{4.0}/V_{IPM})(A_F/A_1 - A_F) \quad (3)$$

where $V_{4.0}$ is the volume of pH 4.0 buffer used, V_{IPM} the volume of IPM used, A_1 the initial absorbance of the saturated IPM solution before partitioning, and A_F the absorbance of the compound remaining in the IPM after partitioning.

The solubility in pH 4.0 buffer for **4**, **5**, and **6** (Table II) can then be estimated from $K_{IPM/4.0}$ using the following equation:

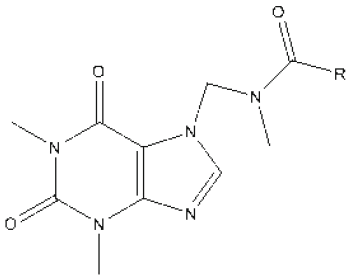
$$S_{4.0} = S_{IPM}/K_{IPM/4.0} \quad (4)$$

Determination of Flux Through Hairless Mice Skins

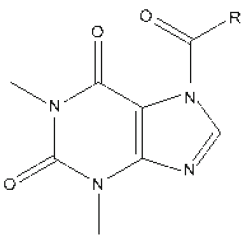
The diffusion cell experiments were run in essentially the same way as described before (18,19). The mice were rendered unconscious with CO_2 and killed by cervical dislocation. Full thickness skins were removed by blunt dissection along the length of the abdomen; the pieces were scraped to remove excess fat, cut into proper sizes, and placed dermal side down on the diffusion cells with the epidermal side in contact with the applied phase. The receptor sides of the Franz diffusion cells were filled with 20 mL of pH 7.1 buffer at 32°C containing 0.1% (*v*/*v*) formaldehyde (2.7 mL of 37% aq formaldehyde per liter) to prevent microbial growth (22). The mouse skins were stable (no change in resistance to permeation) for up to 120 h under those conditions. No air bubbles were present in the receptor sides. Magnetic stir bars were added through the side arm of the receptor compartments, and the assembled cells were suspended over stir plates to stir the contents of the receptor phases throughout the experiment. The mouse skins were kept in contact with buffer for 48 h prior to application of the donor phases to condition the membranes; the receptor phases were replaced with fresh buffer at least twice to leach out any water-soluble UV absorbing material present in the skin, which would interfere with the quantification of theophylline using UV spectroscopy.

In all cases, the prodrugs were applied as suspensions in IPM. These suspensions were prepared by stirring the test compound for 24 h in 2 mL IPM at room temperature; the final suspension concentrations exceeded the compounds solubility by at least 10-fold. Aliquots (0.5 mL) of the well-stirred IPM suspensions were evenly applied to the conditioned membrane surface. To obtain samples from the receptor compartments, 3–5 mL of buffer was removed using a Pasteur pipette from the side arm of the receptor phases and placed in a test tube for quantification using UV spectroscopy and the molar absorptivities in pH 7.1 buffer at 260 and 284 nm for **1**, **2**, and **3** and at 271 nm (λ_{max} for Th-H) for **4**, **5**, and **6**. In order to maintain sink conditions, the entire receptor contents were emptied and filled with fresh buffer after each sample was taken. Samples were collected 8, 19, 22, 25, 28, 31, and 48 h after the initial application of the donor phases. After the

Table II. Solubilities in Isopropyl mysistate (S_{IPM}) and Water (S_{AQ}), Partition Coefficients Between IPM and pH 4.0 Buffer ($K_{IPM/4.0}$) and Maximum Flux Values for the Delivery of Total Species Containing Theophylline (Th-H) Through Mouse Skin from IPM (J_{MMIPM})



1 – 3, 7 – 11



4 – 6, 12 – 16

Compound, R	log[a] S_{IPM}	log[a] S_{AQ}	log $K_{IPM:4.0}$	J_{MMIPM}[d]	% Th-H
1, $CH_2CH_2OCH_3$	0.55	1.39	-0.77	0.20	67
2, $(CH_2CH_2O)_2CH_3$	0.86	2.29	-1.05	1.61	75
3, $CH(CH_3)CH_2OCH_3$	0.94	1.22	-0.42	0.19	63
4, $CH_2CH_2OCH_3$	0.87	1.99[c]	-1.12	1.61	100
5, $(CH_2CH_2O)_2CH_3$	0.97	2.49[c]	-1.52	3.62	100
6, $CH(CH_3)CH_2OCH_3$	1.15	1.61[c]	-0.47	0.49	100
Th-H	-0.47	1.66	-2.14	0.48	100
7, CH_3	0.43	0.97	-0.51	0.16[e]	63
8, C_2H_5	1.34	1.25	-0.04	0.76[e]	74
9, C_3H_7	0.88	0.44	0.41	0.089[e]	66
10, C_4H_9	1.04	-0.08	1.07	0.071[e]	72
11, C_6H_{13}	1.27	-0.88	1.97	0.030[e]	68
12, CH_3	0.28[b]	1.45[b, c]	-1.17[b]	0.29[f]	100
13, C_2H_5	0.65[b]	1.18[b, c]	-0.53[b]	0.21[f]	100
14, C_3H_7	1.59[b]	1.43[b, c]	0.16[b]	1.06[f]	100
15, C_4H_9	1.70[b]	0.93[b, c]	0.77[b]	0.64[f]	100
16, C_6H_{13}	1.69[b]	-0.27[b, c]	1.96[b]	0.15[f]	100

[a] Units of mM or μmol cm^{-3}
[b] New experimental values determined at same time as those for **1–6**
[c] Estimated from $\log S_{IPM}$–$\log K_{IPM/4.0}$
[d] Units of μmol cm^{-2} h^{-1}
[e] Majumdar and Sloan (20)
[f] Sloan *et al.* (26)

48 h initial application periods, the remaining donor suspensions were removed by thoroughly washing the skin with methanol. Methanol wash was found to have minimal effect on the barrier properties of the skin in control studies (23). In order to quantify the amount of dermal penetration of the prodrugs, the skins were kept in contact with buffer for an additional period of 24 h. The length of the postapplication leach period was sufficient to remove 85–90% of the residual compound in the skin (24).

To evaluate the integrity of the membranes, suspensions of 35 mg/0.5 mL of Th-H/propylene glycol (PG) were applied uniformly to the membrane surface as a second application. Samples were taken after 1, 2, 3, and 4 h and placed in test

tubes for further analysis using UV spectroscopy and the molar absorptivity in pH 7.1 buffer at 271 nm (1.02×10^4 L mol^{-1}) for Th-H. The receptor phases were refilled with fresh buffer every time a sample from the receptor phase was taken. An increase in the flux of theophylline compared to controls was an indication that the barrier function of the skin had been irreversibly affected by the drug/vehicle combination (25).

Determination of Prodrugs *1*, *2*, or *3* and Th-H in pH 7.1 Buffer by UV Spectroscopy

Absorbance at any wavelength was assumed to be a combination of the absorbances of drug and any intact prodrug. Using Beer's law, the mathematical expression was

$$A_\lambda = C_P\varepsilon_{P_\lambda} + C_D\varepsilon_{D_\lambda} \quad (5)$$

where A_λ was the absorbance at a particular wavelength, C_P concentration of prodrug, C_D concentration of drug, ε_P molar absorptivity of prodrug, and ε_D molar absorptivity of drug at wavelength λ. By measuring absorbances at two wavelengths, it was possible to calculate C_D and C_P.

$$A_{\lambda 1} = C_P\varepsilon_{P\lambda 1} + C_D\varepsilon_{D\lambda 1} \quad (6)$$

$$A_{\lambda 2} = C_P\varepsilon_{P\lambda 2} + C_D\varepsilon_{D\lambda 2} \quad (7)$$

Simultaneously, solving these equations gave C_D and C_P.

$$C_P = (A_{\lambda 1}\varepsilon_{D\lambda 2} - A_{\lambda 2}\varepsilon_{D\lambda 1})/(\varepsilon_{P\lambda 1}\varepsilon_{D\lambda 2} - \varepsilon_{P\lambda 2}\varepsilon_{D\lambda 1}) \quad (8)$$

$$C_D = (A_{\lambda 1} - C_P\varepsilon_{P\lambda 1})/\varepsilon_{D\lambda 1} \quad (9)$$

where C_P and C_D were then added to give the total species of theophylline present. For Th-H prodrugs, **1**, **2**, and **3**, λ_1 was 260 nm and λ_2 was 284 nm (Table I).

Calculation of Maximum Flux

Maximum flux was calculated from the plot of the cumulative amounts of species containing theophylline species in micromoles that permeated the skin *versus* time. The slope of the best fit line passing through the steady state portion (usually 19–31 h) divided by the cross-sectional area of the diffusion cell (4.9 cm^2) gave the maximum flux of theophylline through mouse skin from IPM, J_{MMIPM}, in micromoles per square centimeter per hour or as a second application from PG, J_{JMIPM} (Tables II and III).

Statistical Analysis and Regression Analysis

Statistical analysis was accomplished using Student's *t* test. Unless otherwise indicated, statistical significance is for $p<0.05$. Linear regression analysis was accomplished using SAS 9.0.

RESULTS AND DISCUSSIONS

Syntheses and Spectral Characteristics

The soft alkyl (NANMEOCAM) derivatives of Th-H (**1–3**) were synthesized in reasonable yields (61–65%) using the same procedure that had been used to synthesize NANAOCAM derivatives of Th-H (**7–11**) (17). No attempt was made to maximize the yields of **1–3**. The ^{1}H NMR spectra of **1–3** contained absorptions for 1, 3-di-N–CH_3, N=CH–N and CH_2–N(CH_3)C=O, which exhibited similar chemical shifts as those absorptions for **7–11**, and with the CH_2–N(CH_3)C=O and N=CH–N absorptions each exhibiting two absorptions due to the contributions of two rotamers about the amide bond in solution. In one rotamer, the C=O is cis to the CH_3, and in the other, it is cis to the CH_2 in CH_2–N(CH_3)C=O. There are also two absorptions for the CH_2–OC=O groups, which are part of the first ethyleneoxy group in the promoiety. This to be expected for substituents on the carbamate-type functional group, which is similar to an amide functional group. The positions of the UV absorptions (λ_{max}) and the molar absorptivities of **1–3** in buffer were identical with those of **7–11**, while they exhibited somewhat lower molar absorptivities than **7–11** in acetonitile but at the same λ_{max}. Elemental analyses were consistent with the expected compositions (±0.4%) of the prodrugs.

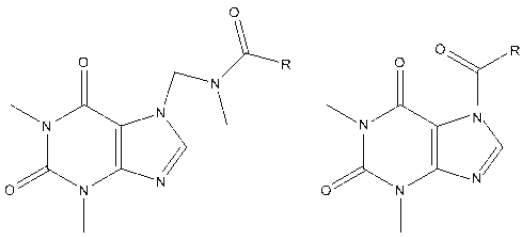
The ^{1}H NMR spectra of **4–6** contained absorptions for 1,3-di N–CH_3 and N=CH–N, which exhibited similar chemical shifts as those absorptions for **12–16** and λ_{max} and molar absorptivities in acetonitrile of the MEOC acyl-type prodrugs of Th-H (**4–6**), which were essentially identical with the alkyloxycarbonyl derivatives of Th-H (**12–16**) previously synthesized from the reactions of the corresponding alkyloxycarbonyl chlorides with Th-H (26). No attempt was made to optimize the yields of **4–6**, which were 53–71%. Elemental analyses were consistent (±0.4%) with the expected compositions of the prodrugs.

Solubilities and Partition Coefficients

The solubilities of **1–6** in IPM (S_{IPM}) were all greater than that of Th-H (10–40 times). For the soft alkylated derivatives **1–3**, only **2** was more soluble in water (S_{AQ}). These S_{IPM} and S_{AQ} values were measured directly and had SD of less than ±5%.

Although **1–3** were sufficiently stable in water to measure S_{AQ} directly, **4–6** were not. Their solubilities in water were estimated from their solubilities in IPM and their partition coefficients between IPM and pH 4.0 buffer ($K_{IPM/4.0}$): log S_{IPM}−log$K_{IPM/4.0}$. The SD for the partition coefficients were less than ±10%. They were measured by shaking a mixture of the prodrug saturated in IPM with pH 4.0 buffer for about 10 s and centrifuging the mixtures to separate the layers as previously reported for similar prodrugs that were unstable in water (21). For the acyl derivatives **4–6**, both **4** and **5** were more soluble in water than Th-H, based on their estimated solubilities. Compared to prodrugs of Th-H that had been previously evaluated in diffusion cell experiments, the incorporation of ethyleneoxy group into the promoiety has increased their estimated aqueous solubilities while adding a minimum number of ethyleneoxy groups to prevent MW values from becoming so large as to have a detrimental effect on diffusion. For example, if one compares the shortest side chain prodrugs containing the ethyleneoxy group (3C+O) with those containing methylene groups of about the same alkyl chain length (4C), the former prodrugs are all much more water soluble but less soluble in IPM: **1** is 30 times more soluble in water than **10** and only 0.32 times the solubility of

Table III. Second Application Fluxes from Suspensions of Theophylline (Th-H) in Propylene Glycol (J_{JMIPM}), Residual Amount of **1**–**6** and Th-H Leached from Skins after 24 h (C_s), Log Permeability Coefficients from $\log J_{MMIPM}$–$\log S_{IPM}$ ($\log P$), the Absolute Differences Between the Experimental (EXP) $\log J_{MMIPM}$ and Calculated (CALC) $\log J_{MMIPM}$ ($\Delta \log J_{MMIPM}$), from Eq. (1) n=71 and n=94



1 – 3, 7 – 11 4 – 6, 12 – 16

Compound	R	J_{JMIPM}[a]	Cs[b]	logP[c]	$\Delta \log J_{MMIPM}$ n = 71	$\Delta \log J_{MMIPM}$ n = 94
1,	$CH_2CH_2OCH_3$	0.53	1.10	-1.24	0.30	0.15
2,	$(CH_2CH_2O)_2CH_3$	0.70	1.38	-0.65	0.11	0.33
3,	$CH(CH_3)CH_2OCH_3$	0.46	1.74	-1.66	0.40	0.24
4,	$CH_3CH_2OCH_3$	0.69	5.59	-0.66	0.04	0.16
5,	$(CH_2CH_2O)_2CH_3$	0.72	8.78	-0.41	0.20	0.38
6,	$CH(CH_3)CH_2OCH_3$	0.76	1.02	-1.46	0.39	0.28
Th-H		0.81[d]	5.90[d]	0.15[d]		

[a] Units of μmol cm^{-2} h^{-1}
[b] Units of μmol
[c] Units of cm h^{-1}
[d] Kerr *et al.* (2)

10 in IPM; and **4** is 11 times more soluble in water than **15** and 0.15 times the solubility of **15** in IPM. The difference is even more dramatic when comparing the solubilities of prodrugs with two ethyleneoxy groups in the promoiety with those containing only methylene groups of about the same chain length: **2** (5C+2O) is 1,530 times more soluble in water than **11** (6C) and 0.39 times the solubility of **11** in IPM; and **5** is 580 times more soluble in water than **16** and 0.19 times the solubility of **16** in IPM.

Diffusion Cell Experiments

The more water-soluble members of the two series of prodrugs gave improved delivery of total species containing Th-H from IPM through hairless mouse skin, J_{MMIPM}. The most water-soluble member, **5**, gave the highest J_{MMIPM}: seven times greater than J_{MMIPM} for Th-H. The two other prodrugs that were more soluble than Th-H (**2** and **4**) also each gave higher J_{MMIPM} values than J_{MMIPM} for Th-H: three times greater (Fig. 1).

The improved water solubility of the prodrugs containing ethyleneoxy groups also resulted in improved delivery of the total species containing Th-H compared to those prodrugs not containing ethyleneoxy groups: **1** gave a 2.9 times larger J_{MMIPM} than **10** and **4** gave a 2.5 times larger J_{MMIPM} than **15** for those prodrugs containing one ethyleneoxy group while **2** gave 53 times larger J_{MMIPM} than **11** and **5** gave a 24 times

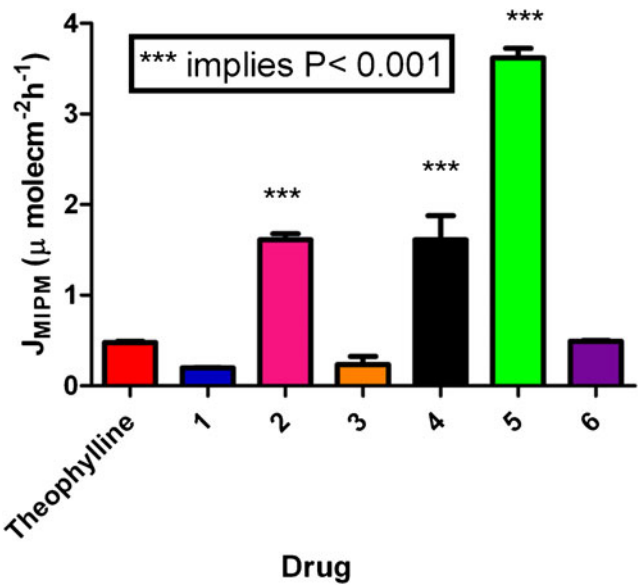


Fig. 1. Diffusion through hairless mice skins for a series of theophylline prodrugs (1–5,8)

larger J_{MMIPM} than **16** for those prodrugs containing two ethyleneoxy groups. Previously, the only APAP prodrug containing multiple ethyleneoxy groups in the promoiety that had been evaluated in diffusion cell experiments using IPM as the vehicle was a soft alkyl alkyloxycarbonyloxymethyl-type derivative containing three ethyleneoxy groups (15). Although the APAP prodrug was six times more soluble in IPM and 1.8 times more soluble in water than APAP, its J_{MMIPM} value was actually only 0.76 times that of APAP. This result was attributed to the association of two to six water molecules with each ethyleneoxy group in the prodrug during its permeation of the skin, which increased the molecular weight of the prodrug dramatically and decreased flux through the skin Eq. (1).

In each comparison of prodrugs containing an ethyleneoxy group with those that did not in the soft alkyl series (**1–3** *versus* **7–11**), the percent of Th-H delivered was about the same (63–75%). Similarly, in each of the acyl series (**4–6** *versus* **12–16**), only Th-H was delivered through the skin.

The increased J_{MMIPM} values obtained for prodrugs using promoieties containing ethyleneoxy groups was not due to increased irreversible degradation of the barrier to permeation in the skin compared to that caused by prodrugs containing only methylene groups in the promoiety. Second applications of a known permeant/solvent combination (Th-H/propylene glycol, PG) gave flux values (J_{JMIPM}) that were not higher than the average control values for J_{JMIPM} previously reported after the application of IPM alone or in combination with many other prodrugs (1.02 μmol cm^{-2} h^{-1}) (9). In fact, the present values were on average substantially lower (0.64±0.12 μmol cm^{-2} h^{-1}).

When the total amounts of species containing Th-H were allowed to leach from the membranes for 24 h, larger amounts, C_s, were obtained after the acyl-type prodrugs, **4–6**, were applied than after the alkyl-type prodrugs **1–3** were applied. Only in the case of the prodrug that gave the largest J_{MMIPM} value, **5**, was the value for C_s greater than that for Th-H itself (Table III).

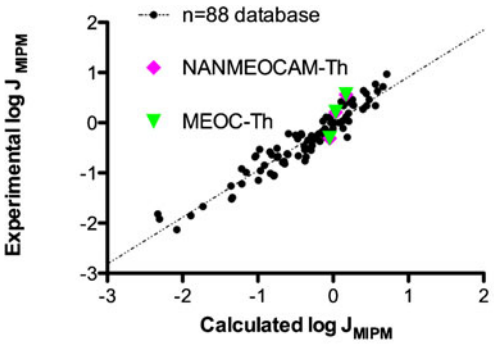
When the J_{MMIPM} values for **1–6** were divided by their respective S_{IPM} values, their permeability coefficient, P, values were obtained (Table III). The log$P$ values gave the same trend as the logJ_{MMIPM} values. A plot of log$P$ *versus* log J_{MMIPM} (not shown) gave a slope of 0.88 and r^2=0.87.

The log solubilities and molecular weights for **1–6** were used to calculate (CALC) logJ_{MMIPM} values using the most recent coefficients to Eq. (1): the RS equation (x=−0.562, y=0.501, z=0.00248) (27). The absolute differences between the experimental (EXP) logJ_{MMIPM} and CALC logJ_{MMIPM} (Δlog J_{MMIPM}) are given in Table III. The average of the absolute differences, Δ′ logJ_{MMIPM}, was 0.240 log units. This value is quite a bit larger than the Δ′ log J_{MMIPM} value of 0.153 log units obtained for the most recent fit of log solubilities, molecular weights and logJ_{MMIPM} values to the Roberts–Sloan equation, n=71 (27).

When the log solubilities, molecular weights, and log flux values for these 7-NANMEOCAM and 7-MEOC derivatives of Th-H were added to the previous J_{MMIPM}, n=71, database (27) together with similar data for the NANAOCAM derivatives of Th-H (20) and acetaminophen (28), 7-acyl derivatives of Th-H (26), and for 1,3-bisacyl derivatives of 5-fluorouracil (29), which had not been incorporated into the n=71 J_{MMIPM} database, a n=94 database was obtained. Using the log S_{IPM}, logS_{AQ} and MW values as the independent variables and log J_{MMIPM} as the dependent variable, regression analysis of the n=94 database gave the following coefficients to Eq. (1): the Roberts–Sloan equation (x=−0.377, y=0.527, z=0.00346): r^2= 0.90 (Fig. 2). The Δ′ logJ_{MMIPM} values for the prodrugs **1–6** was 0.255 log units using the n=94 coefficients to Eq. (1), which is somewhat worse than the value obtained using the n=71 coefficients to Eq. (1) and worse than the value for any other type of prodrug in the database. The Δ′ logJ_{MMIPM} value for the entire n=94 database was 0.167 log units. The Roberts–Sloan equation correctly identifies the best performing member of each prodrug series and the rank order of the performance of each prodrug in each series. The Δ logJ_{MMIPM} values calculated for **1–6** using the n=94 coefficients are given in Table III. Although there is no database of maximum fluxes of permeants through human skin *in vitro* from IPM to generate coefficients x, y, and z to the parameters in the RS equation to compare with the coefficients to the parameters generated here for the n=94 J_{MMIPM} database, a comparison is possible if the vehicle is water. For a n=184 database (10) of maximum fluxes of permeants through human skin *in vitro* from water, J_{MHAQ}, the coefficients to the parameters in the RS equation (x=−2.506, y=0.538, z=0.00402) are quite similar to those for a n=32 database of maximum fluxes through hairless mouse skin from water, J_{MMAQ} (x=−2.299, y=0.575, z=0.00160) except for the z coefficient (30). The difference in the z coefficient leads to lower predicted fluxes through human than mouse skin, but the similarities of the x and y coefficients means the effect of changes in solubilities on predicting flux will be the same regardless of the membrane and the design principles for optimizing flux will be the same (31).

CONCLUSIONS

The incorporation of ethyleneoxy groups into the promoiety of both soft alkyl and acyl-type prodrugs leads to increases in lipid solubilities and in water solubilities especially if two ethyleneoxy groups are incorporated. This has resulted in increased fluxes of total species containing theophylline, J_{MMIPM}, for the more water-soluble prodrugs in each series compared to the parent drug, theophylline, but especially if two ethyleneoxy groups are incorporated. The effect of incorporating a third ethyleneoxy group was not evaluated here, but in a previous



$$\text{Log } J_{MIPM} = -0.377 + 0.527 \log S_{IPM} + (1-0.527) \log S_{AQ} - 0.00346\ MW$$

Fig. 2. Calculated *versus* experimental flux values through hairless mouse skins from IPM using the Roberts–Sloan equation (n=94)

paper where the promoiety of a soft alkyl prodrug of acetaminophen contained three ethyleneoxy groups, a decrease in J_{MMIPM} was obtained regardless that the prodrug was much more lipid and water soluble than its parent. This result was attributed to the adverse effect of increased molecular weight compounded by the association of two to six water molecules per ethyleneoxy group in the promoiety. Thus, incorporation of two ethyleneoxy groups in the promoiety may be optimal for increasing J_{MMIPM} with this type of promoiety.

The fit of lipid and aqueous solubilities, molecular weights and flux values from recent publications of the maximum flux of prodrugs from IPM to the Roberts–Sloan equation was excellent with $r^2=0.90$ and a residual of only 0.167 log units for the entire $n=94$ database. The coefficients to the y and z parameters of the Roberts–Sloan equation for this database are very similar to those obtained for the fit of a database comprised of lipid and aqueous solubilities, molecular weights, and flux values of molecules from water through human skin *in vitro*. The differences in the x coefficients for the fit of the two databases to the Roberts–Sloan equation can be attributed to difference in skin thickness and vehicle effects. Thus, hairless mouse skin is a reasonable surrogate for human skin because the effect of solubilities and molecular weight are the same.

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