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Hydroxymethyl and acyloxymethyl prodrugs of theophylline: enhanced delivery of polar drugs through skin

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Summary

A series of 7-acyloxymethyl prodrugs of theophylline has been prepared by the acylation of 7-(hydroxymethyl)theophylline and by the alkylation of theophylline with an acyloxymethyl halide. The lipid solubilities of all the prodrugs were markedly improved over that of theophylline but, in addition, the succinamate and the glycinate derivatives exhibited increased water solubilities. As a result, prodrugs which exhibited partition coefficients between 0.03 and 16.7 were obtained. Selected acyloxymethyl prodrugs as well as 7-(hydroxymethyl)theophylline were effective in increasing the delivery of theophylline through hairless mouse skin by 3.5–5 times that of theophylline. Several of the prodrugs, when applied topically to normal and hairless mice, inhibited DNA synthesis in the skins of the mice after they had undergone UV irradiation.

Introduction

The hypotheses (Bourne et al., 1974) that adenosine 3′,5′-monophosphate (cAMP) and cAMP-modifying agents mediate inflammatory responses and the suggestion (Voorhees and Duell, 1971) that low levels of cAMP were somehow responsible for psoriasis have led to numerous attempts to treat various inflammatory conditions and psoriasis with agents known to affect cAMP levels (Stawiski et al., 1975; Laugier

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et al., 1973; Anonymous, 1974). For the most part these efforts have met with limited success. Systemic doses result in relatively high systemic levels of the agents before their local concentrations reach effective levels. On the other hand, local administration of most of these agents is not a viable alternative because the agents are simply too polar to efficiently penetrate the biphasic barrier of the skin.

Theophylline is typical of many such agents. Theophylline has been shown to increase cAMP levels in vitro because of its phosphodiesterase activity (Beavo et al., 1970). This observation, in turn, led to the suggestion that it should be useful in the treatment of psoriasis (Voorhees et al., 1971). Indeed, theophylline has been shown to be at least partially effective (50%) in the treatment of psoriasis when given orally (Iancu et al., 1979) but only marginally so when it was used topically (Berenbein et al., 1979).

On the other hand, theophylline has only a narrow therapeutic range (Piafsky and Ogilvie, 1975) in the oral treatment of asthma. Since cAMP is also implicated in the regulation of asthma (Bourne et al., 1974), the same narrow therapeutic range probably holds for the treatment of psoriasis with oral theophylline that holds for the treatment of asthma with oral theophylline. Thus, doses of theophylline that would be high enough to regulate cAMP also would result in blood levels that are very close to systemic toxic levels. This explains why (Iancu et al., 1979) even higher oral doses of theophylline were not used in the treatment of psoriasis when oral doses comparable to those used to control asthma gave only 50% control of psoriasis; the dosage level was probably already close to giving toxic blood levels.

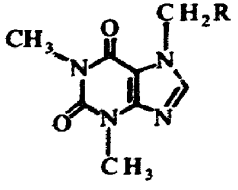
The ideal solution to the problem of the narrow systemic therapeutic range would be to deliver theophylline topically for the treatment of psoriasis. This would eliminate the necessity of high systemic levels of theophylline and would result in local therapeutically effective levels. However, theophylline is a high melting, polar lipophobic molecule (Table 1) which hinders its facile absorption through skin (Higuchi, 1960). Therefore, a program was undertaken to improve the topical delivery of theophylline (Bodor and Sloan, 1977).

There are 3 ways to improve the delivery of drugs, and those are development of: (1) an analogue which has better physical properties; (2) a prodrug which exhibits better physical properties than its parent; and (3) a better formulation. The second approach has been taken in this work. N-Acyl derivatives of theophylline, a conventional prodrug approach which had already been used to give a controlled oral delivery form of theophylline (Bodor et al., 1978), was not considered to be an attractive approach here because of the lability of such derivatives. On the other hand, although N-alkyl derivatives exhibit some of the desired physical properties for a prodrug of theophylline such as shelf stability and increased water solubility (for example see caffeine in Table 1), they are not prodrugs; that is, they do not completely revert to theophylline in vivo. Such 7-alkyltheophyllines require an oxidative metabolic activation step before they become chemically labile and capable of disassociation into an aldehyde and theophylline, e.g.,

$$\geq N\text{-}CH_3 \xrightarrow{\text{cytochrome P-450}} \geq N\text{-}CH_2OH \rightleftharpoons \geq N\text{-}H + CH_2{=}O$$

TABLE 1

M.P. AND SOLUBILITY OF DERIVATIVES OF THEOPHYLLINE



Compound, R =	M.P. (°C)	Solubility (g/l)			Partition coefficient (heptane/water)
		H_2O	IPM [a]	Heptane	
I theophylline	270–274	8.3	0.04	–	–
II HO–	260–262	–	0.74	0.008	–
III CH_3CO_2–	163–166	3.74		0.03	0.14
IV $C_2H_5CO_2$–	142–144	–		–	–
V $C_3H_7CO_2$–	102–105	3.89	8.40	0.65	0.16
VI $C_5H_{11}CO_2$–	79– 82	0.71		2.26	2.4
VII $C_7H_{15}CO_2$–	65– 68	0.12		0.52	16.7
VIII $(CH_3)_3CCO_2$–	108–109.5	2.01	6.59	1.55	0.90
IX $(C_2H_5)_2NCOCH_2CH_2CO$–	100–103	26.3		0.17	0.04
X $C_2H_5OCO_2$–	126–127	3.87		0.18	0.034
XI $(CH_3)_2NCH_2CO_2$–	112–113	>1 g/ml		< 10 mg/500 ml [b]	–
XII C_2H_5O–	111–113	18.56		0.44	0.031
XIII caffeine	238	21.8		–	–

[a] IPM is isopropyl myristate.

[b] Solubility of the free base.

The α-hydroxyalkyl derivatives that result from the oxidation step are themselves prodrugs. However, their stability in solution is minimal so that they usually are not considered as practical solutions to drug delivery problems unless it is possible to use excess aldehyde in the formulation to force the above equilibrium to the left. In spite of this drawback, α-hydroxyalkyl derivatives are still an appealing approach precisely because they are unstable; all that is necessary is to stabilize the α-hydroxyalkyl group in a transient manner so that the derivatives (in essence prodrugs of prodrugs) will have a practical shelf life. Therefore, O-acylated derivatives of the hydroxymethyl derivatives of theophylline have been prepared and investigated as a general means of stabilizing N-α-hydroxyalkyl derivatives and improving their delivery and that of their parent drugs through skin. The preliminary results of that investigation with theophylline are reported here.

Methods and Materials

The hairless mice that were used were SKH-hr-1 from Temple University Skin and Cancer Hospital. The diffusion cells were obtained from Kercso Engineering Consultants, Palo Alto, CA. The [^{3}H]thymidine was obtained from New England Nuclear, the hydroxyapatite (DNA grade) from Bio-Rad and the scintillation fluid (Insta-Gel) from Packard. TLC were run on Brinkman Polygram Sil G/UV 254; ether or ether–acetone mixtures. MP (uncorrected) were taken with a Thomas-Hoover Capillary apparatus. NMR spectra were recorded on a Varian T-60, IR spectra on a Beckman Accu Lab 4 spectrophotometer and UV on a Beckman model 25 spectrophotometer. Microanalyses were obtained by Midwest Microlabs, Indianapolis, IN. Theophylline was obtained from Sigma, the acid chlorides and chloromethyl pivalate from Aldrich and the bulk solvents from Mallinckrodt.

Syntheses

The preparation of 7-(hydroxymethyl)theophylline

To 14.69 g (0.145 mol) of triethylamine was added 26.0 g (0.145 mol) of theophylline and 26 g of 36% aqueous formaldehyde. The reaction mixture soon became homogeneous upon vigorous stirring (2 min) whereupon 30 ml of THF was added and the stirring was stopped. The reaction was allowed to crystallize over the weekend to give 24.9 g (82% yield) of the desired product as a white solid: ^{1}H NMR ($CDCl_3$) δ 7.55 (s, 1, N=C$\underline{H}$–N), 5.6 (broad s, 3 C$\underline{H}_2$–O$\underline{H}$) and 3.63 and 3.45 (two s, 6, C$\underline{H}_3$N); IR (KBr) 3400 cm^{-1} (m) (OH) and 1710, 1670 and 1650 cm^{-1} (s) (C=O); TLC (silica gel, ether) R_f 0.09.

The preparation of 7-(butyryloxymethyl)theophylline

To an ice-cold suspension of 7-(hydroxymethyl)theophylline (7.52 g, 0.036 mol) and butyryl chloride (3.81 g, 0.036 mol) in 100 ml of CH_2Cl_2 was added, with stirring, triethylamine (3.98 g, 0.039 mol). The reaction was removed from the ice bath and allowed to warm to room temperature for 1 h. The resulting clear solution

was concentrated in vacuo to a solid white residue which was triturated overnight with Et_2O. The trituration was filtered and the filtrate was concentrated in vacuo to a white solid which was dissolved in 40 ml of CH_2Cl_2. The CH_2Cl_2 solution was extracted with 25 ml of 0.1 N NaOH, dried with Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give 3.86 g (39% yield, m.p. 102–105°C) of white powder which was the desired product: NMR ($CDCl_3$) δ 7.92 (s, 1, N=C<u>H</u>), 6.27 (s, 2, OC<u>H</u>$_2$–N), 3.59 (s, 3, NC<u>H</u>$_3$), 3.42 (s, 3, NC<u>H</u>$_3$), 2.70 (t, 2, O=CC<u>H</u>$_2$C<u>H</u>$_2$–, J = 7 Hz), 1.65 (m, 2, O=CCH$_2$C<u>H</u>$_2$CH$_3$, J = 7 Hz), 0.92 (t, 3, O = CCH$_2$C<u>H</u>$_2$C<u>H</u>$_3$, J = 7 Hz); IR (KBr) 1750 cm^{-1} (s) (O–C=O), 1705, 1665 cm^{-1} (s) (N–C=O); TLC (silica gel, ether) R_f = 0.20.

Anal. Calcd. for $C_{12}H_{16}N_4O_4$: C, 51.42; H, 5.75; N, 19.99. Found: C, 51.60; H, 5.80; N, 20.11.

The following 7-(acyloxymethyl)theophyllines were prepared in a similar manner.

7-(Propionyloxymethyl)theophylline: m.p. 142–144°C, 50% yield; ^{1}H NMR ($CDCl_3$) δ 7.93 (s, 1, N=C<u>H</u>–N), 6.24 (s, 2, N–C<u>H</u>$_2$–O), 3.56 (s, 3, N–C<u>H</u>$_3$), 3.38 (s, 3, N–C<u>H</u>$_3$), 2.40 (q, 2, J = 7 Hz, O=C–C<u>H</u>$_2$) and 1.13 (t, 3, J = 7 Hz, O=C–CH$_2$C<u>H</u>$_3$); IR (KBr) 1755 cm^{-1} (s) (O–C=O), 1710 and 1660 cm^{-1} (s) (N–C=O).

Anal. Calcd. for $C_{11}H_{14}N_4O_4$: C, 49.62; H, 5.30; N, 21.05. Found: C, 49.59; H, 5.31; N, 21.15.

7-(Hexanoyloxymethyl)theophylline: m.p. 65–68°C; 52% yield; ^{1}H NMR ($CDCl_3$) δ 7.91 (s, 1, N=C<u>H</u>–N), 6.27 (s, 2, N–C<u>H</u>$_2$O), 3.6 (s, 3, N–C<u>H</u>$_3$), 3.4 (s, 3, N–C<u>H</u>$_3$) and 2.37 (t, 2, O=CH–C<u>H</u>$_2$); IR (KBr) 1755 cm^{-1} (s) (O–C=O), 1705 and 1670 cm^{-1} (s) (N–C=O).

Anal. Calcd. for $C_{14}H_{20}N_4O_4$: C, 54.52; H, 6.54; N, 18.17. Found: C, 54.40; H, 6.54; N, 18.10.

7-(Pivalyloxymethyl)theophylline: m.p. 106–108°C, 38% yield; TLC (silica gel, ether) R_f 0.26; UV (pH 4.5) λ_{max} 276 nm (log ϵ = 3.85), no other absorptions especially in region from 220 to 250 nm; identical with product obtained by alkylation with chloromethyl pivalate by NMR spectroscopy.

7-(Octanoyloxymethyl)theophylline: m.p. 79–82°C, 46% yield; TLC (silica gel, ether–acetone, 10:5) R_f 0.54; ^{1}H NMR ($CDCl_3$) δ 7.87 (s, 1, N=C<u>H</u>–N), 6.23 (s, 2, N–C<u>H</u>$_2$–O), 3.61 (s, 3, C<u>H</u>$_3$–N), 3.45 (s, 3, C<u>H</u>$_3$–N) and 2.37 (t, J = 7 Hz, 2, C<u>H</u>$_2$C=O).

Anal: Calcd. for $C_{16}H_{24}N_4O_4$: C, 57.13; H, 7.19; N, 16.66. Found: C, 56.91; H, 7.23; N, 1703.

7-(Ethoxycarbonyloxymethyl)theophylline: m.p. 126.5–127.5°C 33% yield; TLC (silica gel, ether–acetone, 10:5) R_f 0.47; ^{1}H NMR ($CDCl_3$) δ 7.88 (s, 1, N=C<u>H</u>–N), 6.25 (s, 2, N–C<u>H</u>$_2$O), 3.60 (s, 3, C<u>H</u>$_3$–N), 3.50 (s, 3, C<u>H</u>$_3$–N), 4.17 (q, J = 7 Hz, 2, OC<u>H</u>$_2$CH$_3$) and 1.3 (t, J = 7 Hz, 3, OCH$_2$C<u>H</u>$_3$).

Anal: Calcd. for $C_{11}H_{14}N_4O_5$: C, 46.81; H, 5.00; N, 19.85. Found: C, 46.53; H, 4.99; N, 20.07.

7-(Acetyloxymethyl)theophylline: m.p. 163–166°C, lit. m.p. 165°C (Roth and Brandes, 1965).

Preparation of 7-(pivalyloxymethyl)theophylline: a suspension of 3.60 g (0.02 mol) of theophylline and 1.38 g (0.01 mol) of potassium carbonate in 75 ml of acetone was

refluxed overnight then 3.00 g (0.02 mol) of chloromethyl pivalate was added and the mixture was refluxed for an additional 2 days. The suspension was filtered. The residue was washed with acetone (200 ml). The combined acetone solutions were concentrated in vacuo. The residue from the acetone was extracted with boiling heptane (200 ml). The heptane solution was filtered while hot then cooled to give 1.50 g (m.p. 108–109.5°C, 26% yield) of the desired product: TLC (silica gel, ether) R_f 0.26; ^{1}H NMR ($CDCl_3$) δ 7.87 (s, 1, N=C$\underline{H}$–N), 6.23 (s, 2, N–C$\underline{H}_2$–O), 3.61 (s, 3, C$\underline{H}_3$–N), 3.43 (s, 3, C$\underline{H}_3$–N) and 1.2 (s, 9, (C$\underline{H}_3$)$_3$C).

Anal: Calcd. for $C_{13}H_{18}N_4O_4$: C, 53.05; H, 6.16; N, 19.04. Found: C, 53.06; H, 6.20; N, 19.32.

The preparation of 7-(ethoxymethyl)theophylline: a suspension of 7.20 g (0.04 g mol) of theophylline and 2.76 g (0.02 mol) of potassium carbonate in 200 ml of acetone was refluxed for 2 days then 4.40 g (0.046 mol) of ethoxymethyl chloride was added and the suspension was refluxed for an additional 2 days. The suspension was then filtered and the filtrate was concentrated. The concentrate was subsequently chromatographed on Silic AR CC-7 using ether–acetone (50:1) as the eluent to give the desired product: 3.43 g, m.p. 110–112°C, 35% yield; ^{1}H NMR ($CDCl_3$) δ 7.78 (s, 1, N=C$\underline{H}$–N), 5.83 (s, 2, N–C$\underline{H}_2$–O), 3.63 (s, 3, C$\underline{H}_3$–N), 3.43 (s, 3, C$\underline{H}_3$–N), 3.65 (q, $\underline{J}$ = 7 Hz, 2, OC$\underline{H}_2$CH$_3$) and 1.2 (t, $\underline{J}$ = 7 Hz, 3, C$\underline{H}_3$CH$_2$–O); TLC (silica gel, ether–acetone, 10:5) R_f 0.35.

Anal: Calcd. for $C_{10}H_{14}N_4O_3$: C, 50.41; H, 5.92; N, 23.52. Found: C, 50.54; H, 5.99; N, 23.58.

The preparation of 7-(N,N-diethylsuccinamyloxymethyl)theophylline: to a mixture of 10.3 g (0.05 mol) of dicyclohexylcarbodiimide, 10.50 g (0.05 mol) of hydroxymethyltheophylline and 8.65 g (0.05 mol) of N,N-diethylsuccinamic acid (Pressman et al., 1948) was added 100 ml of dichloromethane. The suspension that resulted was stirred overnight then filtered. The filtrate was extracted with 20 ml of 5% NaOH, dried over Na_2SO_4 and concentrated to give 14.1 g of a clear gum. The gum was crystallized twice from dichloromethane–ether (10:100 ml) to give 7.55 g (m.p. 105–106°C, 41% yield) of the desired product as white crystals: ^{1}H NMR ($CDCl_3$) δ 7.9 (s, 1, N=C$\underline{H}$–N), 6.27 (s, 2, N–C$\underline{H}_2$–O), 3.58 (s, 3, C$\underline{H}_3$–N), 3.40 (s, 3, C$\underline{H}_3$–N), 3.33 (q, $\underline{J}$ = 7 Hz, 4, N–C$\underline{H}_2$CH$_3$), 2.67 (s, 4, O=C$\underline{H}_2$C$\underline{H}_2$C=O), 1.2 (t, $\underline{J}$ = 7 Hz, 3, N–CH$_2$C$\underline{H}_3$) and 1.08 (t, $\underline{J}$ = 7 Hz, 3, N–C$\underline{H}_2$CH$_3$).

Anal: Calcd. for $C_{16}H_{23}N_5O_5$: C, 52.59; H, 6.34; N, 19.44. Found: C, 52.28; H, 6.22; N, 19.35.

Preparation of 7-(N,N-dimethylglycyloxmethyl)theophylline methane sulfonate: to a mixture of 6.30 g (0.03 mol) of hydroxymethyltheophylline, 6.20 g (0.03 mol) of dicyclohexylcarbodiimide and 3.13 g (0.03 mol) of N,N-dimethylglycine was added 30 ml of pyridine. The suspension was stirred at room temperature for 24 h; it was diluted with 200 ml of dichloromethane and filtered. The filtrate was concentrated at room temperature to give a yellow oil which was redissolved in dichloromethane (30 ml) and ether (50 ml). After 2 h, the solution was decanted from the gum that had formed and was further diluted to 400 ml with ether, then cooled with an ice bath. After 2 h the precipitate that had formed was filtered to give 4.92 g (55% yield) of the dimethylglycinate of hydroxymethyltheophylline: ^{1}H NMR ($CDCl_3$) δ 7.88 (s, 1,

N–CH=N), 6.28 (s, 2, N–CH_2–O), 3.6 (s, 3, N–CH_3), 3.46 (s, 3, CH_3–N), 3.25 (s, 2, CH_2–N) and 2.37 (s, 6, N–CH_3). The entire 4.92 g of the glycinate ester was immediately dissolved in 20 ml of CH_2Cl_2, cooled with an ice bath and allowed to react overnight with 1.66 g (0.0173 mol) of methane sulfonic acid. The mixture was diluted with 25 ml of ether and filtered to give 6.40 g (m.p. 192–193°C, 97% yield) of the glycinate ester salt: ^{1}H NMR (D_2O) δ 8.33 (s, 1, 1'=CH–N), 6.47 (s, 2, OCH_2–N), 4.30 (s, 2, $\oplus$N–CH_2), 3.50 (s 3, CH_3–N), 3.30 (s, 3, CH_3–N), 3.07 (s, 6, $(CH_3)_2$–N$\oplus$) and 2.83 (s, 3, $CH_3SO_3\ominus$).

Anal. Calcd. for $C_{13}H_{20}N_5O_7S$: C, 39.99; H, 5.16, N, 17.68. Found: C, 39.67; H, 5.28; N, 17.70.

Determination of penetration of hairless mouse skin by theophylline and its prodrugs

Full thickness dorsal skin of 12–14-week-old hairless mice was used. The mice were sacrificed by snapping their spinal cords. The excised skin was gently scraped to remove fat and visceral debris then gently secured over the diffusion cells with a rubber gasket. The diffusion cells have been described previously (Loftsson and Bodor, 1981). The receptor side of the cell (39 ml) was filled with pH 7.4 isotonic phosphate buffer containing 0.1% formaldehyde and was stirred magnetically. A total of 300 μl of suspensions of the compounds in isopropyl myristate were applied to the donor side of the membrane (area = 4.9 cm^2). All the compounds were 0.355 M in isopropyl myristate so that 10.6×10^{-5} mole of each compound was applied to the donor side of the membrane. The suspensions were prepared by sonicating 7.14×10^{-4} mole of each compound in 2 ml of isopropyl myristate for 15 min. The solubility of each compound in isopropyl myristate was determined by sonicating 50 mg of each compound in 5 ml of isopropyl myristate for 15 min then allowing the suspensions to settle completely. Samples (50 μl) of each supernatant were diluted with methanol and the concentrations determined by UV.

Samples (3 ml) were taken of the receptor phase at 3, 6, 9 and 12 h and replaced with 3 ml of buffer each time. The samples were analyzed immediately by UV; i.e. within 1 h. V had a $T_{1/2}$ decomposition to theophylline of 5 days at pH 7.4 and room temperature while VIII was apparently stable under those conditions for 3 days.

In order to determine the amount of diffused material present as theophylline or theophylline prodrug in the receptor phase, the extent of partitioning of theophylline, pivalyloxymethyl and butyryloxymethyltheophylline between dichloromethane (100 ml) and either water or aqueous sodium hydroxide (10 ml of 2 N) was determined. It was found that the aqueous sodium hydroxide effectively separated theophylline from the prodrugs without causing decomposition of the prodrugs. Then, 2 ml aliquots of each diffusion cell sample were diluted to 10 ml with water and the water layers were extracted with 100 ml of dichloromethane. The dichloromethane solutions were extracted with 10 ml of 2 N sodium hydroxide, allowed to settle, separated and concentrated. The residues were then dissolved in 5 ml of methanol and analyzed by UV. In all the cases except the pivalyloxymethyl derivative, only theophylline was found in the receptor phase. In the case of the pivalyloxymethyl derivative, 19%, 9.5% and 15% of theophylline was present as the

intact prodrug in the individual cells after 12 h. The hydroxymethyl derivative of theophylline completely reverted to theophylline in buffer or water within seconds.

The diffusion cells were followed for 12 h. After 12 h the skin began to smell and the rate of appearance of theophylline in the receptor phase decreased significantly at the next sample time (24 h).

Determination of inhibition of DNA synthesis by prodrugs of theophylline

The method of Du Vivier et al. (1975) was used with the following modifications. Initial experiments were done using ddY-strain mice whose dorsal skin had been clipped and depilated. Later, hairless mice were used.

In the initial experiments, 3 days after depilation, the mice were irradiated with an 8 W germicidal lamp (254 nm) for 5 min at 40 cm. A 0.1 ml solution or suspension of the drug or the vehicle alone (control) was then applied topically to the skin at 3, 8, 24, 32 and 48 h after the skin was irradiated. After 53 h 30 μCi of [^{3}H]thymidine (21 Ci/mM) was injected intraperitoneally. Three hours later (56 h) the mice were sacrificed by ether inhalation. The depilated skin was removed from the carcass and the epidermis was separated from the dermis by heating the skin on a hot plate at 55°C for 2 min. The epidermis was immediately frozen and kept at −20°C until assayed.

The epidermis was homogenized with 5 ml of 0.24 M phosphate buffer, pH 6.8, containing 8 M urea, 1 mM ethyldiaminetetraacetic acid and 1% sodium dodecyl sulfate. Hydroxyapatite (0.3 g) was added to the homogenate. The mixture was stirred and centrifuged at 1000 rpm for 10 s. The precipitate was washed 3 times with 3 ml of 0.24 M phosphate buffer, pH 6.8. The precipitate was then mixed with 4 ml of 0.48 M phosphate buffer, pH 6.8, and the mixture was centrifuged at 1000 rpm for 10 s. The supernatant was centrifuged at 3000 rpm for an additional 10 s, then analyzed by UV spectroscopy at 260 nm; one optical density unit was approximately equal to 50 mg/ml of DNA (Felsenfeld and Hischman, 1965). The ratio of the absorbance 260 nm:280 nm should be > 1.8; otherwise, there is protein contamination and it is impossible to obtain consistent results. A 1 ml sample of the DNA supernatant was then added to 3 ml of 11 M urea and 8 ml of scintillation fluid, and the radioactivity was counted to give cpm.

In the experiment using hairless mice, isopropyl myristate was used as the vehicle in which to apply the drugs.

Determination of partition coefficients and solubilities

Partition coefficients were determined in triplicate according to the methods described by Hansch (Hansch, 1973) except that heptane was used instead of octanol as the lipid phase. Solubilities were determined in triplicate ($\pm 3\%$) also by adding an excess of the derivative to the solvent and sonicating the suspensions for 20 min. The suspensions were then centrifuged and the supernatants were analyzed by UV spectroscopy.

Results and discussion

The acyloxyalkyl derivatives were prepared by two methods. The first method involved the use of 7-(hydroxymethyl)theophylline (II) as an intermediate. II was conveniently prepared in excellent yield from the alkylation of theophylline (I) with excess formaldehyde. II could then be stored under desiccation without decomposition for several months and used as needed. Subsequent acylation of II provided the acyloxymethyl derivatives in Table 1. In most cases the corresponding acid chloride was allowed to react with II in the presence of an acid scavenger. This gave the simple aliphatic esters of II. On the other hand, the more complicated esters required neutral conditions and dicyclohexylcarbodiimide (DCI) to effect reaction. Thus, II was condensed with N,N-dimethylglycine and N,N-diethylsuccinamic acid to give XI and IX; respectively. In the latter case, the acid chloride of N,N-diethylsuccinamic acid apparently underwent an intramolecular reaction between the acid chloride and the amide groups (Hall, 1956) to give polymeric products while in the former case the acid chloride gave negligible yields of XI when allowed to react with II in the presence of base.

The only serious drawback to the first method—the acylation method—is that it is practically limited to derivatives of II. Few aldehydes other than formaldehyde (Zaugg and Martin, 1965) form stable α-hydroxyalkyl derivatives of such relatively acidic amides as theophylline (pK_a 7.4). Most of those that do are like chloral and have their own pharmacological activity which limits their use.

The second method that was used was the alkylation of I with α-acyloxyalkyl halides. A wide variety of α-acyloxyalkyl halides are available from the reaction of aldehydes with acid halides (Bodor and Kaminski, 1980; Bodor et al., 1980; Adams and Vollweiler, 1981) so that both the acyl portion and the alkyl portion of the acyloxyalkyl derivative could, in theory, be varied systematically. However, the ready availability of II from I and the commercial availability of the acid chlorides made the first method of preparing the prodrugs more attractive for this preliminary investigation. Therefore, only one example of an acyloxyalkyl theophylline (VIII) was prepared by the alkylation route. It was identical with the VIII prepared from the acylation of II with pivalyl chloride. 7-(Ethoxymethyl)theophylline (XII) was also prepared by the alkylation of theophylline. XII did not revert to theophylline in plasma and was not further studied. All of the remaining derivatives hydrolyzed completely in plasma after 12 h (Sloan and Bodor, unpublished results).

It was possible that the alkylation of theophylline either by formaldehyde or by an acyloxyalkyl halide could give the 9- rather than the 7-alkylated derivatives. Since VIII, which was obtained by the acylation of II, was identical with VIII obtained from the reaction of I with chloromethyl pivalate, both alkylation reactions apparently take place at the same position on theophylline. The UV spectrum of VIII and the other acyloxymethyl derivatives exhibited only one pH-independent absorption at 276 nm. Since 1,3,7-trialkylxanthines exhibit only one pH-independent absorption at 272 ± 1 nm while 1,3,9-trialkylxanthines exhibit an additional absorption at about 235 nm (Gulland et al., 1934), the acyloxyalkyl derivatives have been assigned the 1,3,7-trisubstituted structures as shown.

Table 1 contains the solubilities that were determined for the theophylline derivatives for the simple aliphatic esters of II. Maximum heptane solubility was obtained for the hexanoyloxymethyl derivative VI, while maximum water solubility was obtained for the C_2–C_4 acyl derivatives. Although VII exhibited a lower melting point than VI, it was much less soluble in both water and heptane than VI. As expected, the incorporation of the polar amide and amine groups into the acyl portion of the derivatives to give IX and XI, respectively, resulted in considerably greater water solubility for those derivatives. It is interesting that II is almost 20 times more soluble than I in isopropyl myristate. The water solubility of II was not determined because of its very fast decomposition [1] analogous to the reported facile decomposition of hydroxymethyl derivatives of amides and imides (Bundgaard and Johansen, 1980). However, there are numerous studies (Bansal et al., 1981; Bundgaard and Johansen, 1980) showing the enhanced water solubilities of such derivatives so that it is reasonable to assume that II is also more water-soluble than I. It is precisely this increase in water solubility that has resulted in the reported lower partition coefficients of N-hydroxymethyl derivatives and the conclusion that hydroxymethyl derivatives are less lipophilic than their parent drugs. This is obviously not the case for the theophylline hydroxymethyl derivative and its importance becomes immediately obvious when percutaneous absorption is considered because of the importance of obtaining a balanced increase in lipid and water solubility to optimize absorption.

Table 2 and Fig. 1 contain the results of the diffusion experiments. VIII and V were 3.5 and 4.4 times more effective than theophylline at delivering theophylline through skin from an isopropyl myristate vehicle. 7-(Hydroxymethyl)theophylline was also quite effective at delivering theophylline topically; almost 5 times as effective as theophylline. 7-(Hydroxymethyl)theophylline (II) and 7-(butyryloxymethyl)theophylline (V) were completely converted to theophylline during their diffusion through the skin. The ease with which II and V dissociates and hydrolyzes, respectively, to I makes it almost impossible to say exactly where and when they are converted [2]. On the other hand, the 7-(pivalyloxymethyl)theophylline (VIII) derivative should not be considered entirely as a prodrug since $14.5 \pm 5\%$ of the intact prodrug was found in the receptor phase at the end of the experiments.

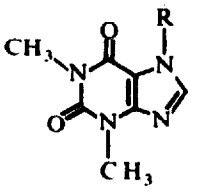
At this point it should be mentioned that, since the exact composition of the species that are diffusing through the skin at each point and time during their transit is unknown, it is not useful to discuss the diffusivity of the prodrugs. Instead, only the amount of theophylline or its prodrug delivered to the receptor phase has been considered.

[1] The results of NMR studies in D_2O showed that the hydrolysis of XI gave only theophylline and no detectable hydroxymethyltheophylline. Assuming that the hydrolysis occurs by a $B_{AC}2$ cleavage mechanism, this suggests a very short half-life of hydroxymethyltheophylline in aqueous media. Sloan, unpublished results.

[2] However, the II in the applied phase, both suspended and in solution, was intact as measured by NMR for up to 72 h. Sloan, unpublished results.

TABLE 2

DIFFUSION OF THEOPHYLLINE AND ITS PRODRUGS THROUGH HAIRLESS MOUSE SKIN



Compound		% Concentration [a]	Mole of applied drug in solution ($\times 10^6$) [b]	% of applied drug as theophylline diffused after 12 h	Mole of applied drug as theophylline diffused after 12 h ($\times 10^6$, mean ± S.D.) [c]
I	R=H	6.5	0.067	4.5	4.81 ± 0.35
II	R=CH_2OH	7.5	1.05	22.3	23.59 ± 5.96 [d]
V	R=$CH_2O_2CC_3H_7$	10.0	9.0	19.9	21.41 ± 2.19 [d]
VIII	R=$CH_2O_2CC(CH_3)_3$	10.5	6.75	16.0 [e]	16.97 ± 2.68 [d,e]

[a] Total drug in suspension and solution.

[b] Average of 3 determinations ± < 1% in isopropyl myristate.

[c] n = 3.

[d] The rate of delivery of I by II ($6.16\times10^{-7} \pm 1.66\times10^{-7}$ mol/cm^2·h) was significantly different ($P<0.05$) from the rate of delivery of I by V ($4.03\times10^{-7} \pm 3.54\times10^{-9}$ mol/cm^2·h) and the rate of delivery of I by VIII ($3.3\times10^{-7} \pm 5.1\times10^{-9}$ mol/cm^2·h) was significantly different ($P<0.05$) from the rate of delivery of I by V based on the mean ± S.D. of the slopes of lines in Fig. 1.

[e] Contains 14.5 ± 5% of the theophylline as intact VIII.

From Fig. 1 we see that the lag time (1.7 h) for theophylline, V and VIII are all the same but that the lag time for II is apparently much longer (4.3 h) and that it exhibits an apparently greater rate of delivery of I, just the opposite of what would normally be expected from the relative lag time. The greater rate of delivery of I by II appears to be primarily a consequence of II being 16 times more soluble than I in isopropyl myristate (Table 1) (Higuchi, 1960; Idson, 1975). Based on the solubility data, the performance of II as a prodrug is not surprising; the relative (to II) lack of performance by V and VIII, however, is. There does not appear to be an adequate explanation for the concomitant increased lag time and increased delivery of theophylline exhibited by II compared to V and VIII. On the other hand, the reason the more lipophilic prodrugs apparently are less effective at delivering theophylline than a more hydrophilic prodrug (V and VIII vs II) may simply be that the optimum partition coefficient in this series is less than one (Treherne, 1956).

The inhibition of DNA synthesis in mice has been used as a measure of the antiproliferative activity of the prodrugs (Du Vivier et al., 1975). The results of the effect of the prodrugs on inhibition of DNA synthesis are presented in Table 3. Within the limits of the experiment, there does not appear to be a solvent effect due

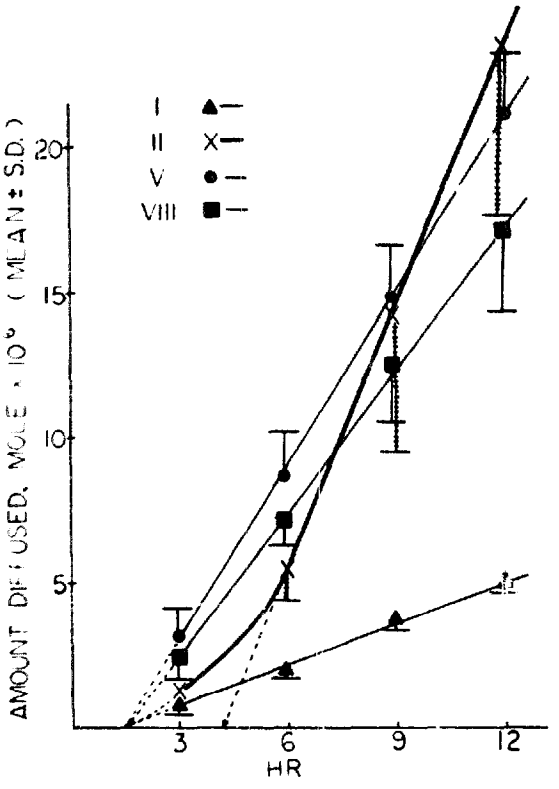


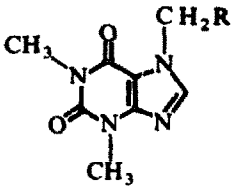
Fig. 1. Diffusion of theophylline and its derivatives through hairless mouse skin.

to the applied phase on the % inhibition but there is an apparent effect based on the type of animal used. The results show that theophylline prodrug V is almost as effective as the potent fluorinated steroid fluocinoline acetonide in inhibiting DNA synthesis, albeit at a 10-fold higher concentration. Concentrations of the prodrugs that were comparable (1%) to that of the steroid were completely ineffective while the steroid was still quite potent at 0.1% in this test. The greater activity of V compared to VIII may be a consequence of its more complete hydrolysis to I observed in the diffusion cell experiments.

Thus, although the acyloxymethyl prodrug approach has not been optimized, it has been shown that 7-acyloxymethyl derivatives of theophylline are, indeed, prodrugs which effectively increase the delivery of theophylline through the skin, and that once the theophylline has been delivered it is an effective antiproliferative agent. In addition, it has been shown that a N-hydroxymethyl derivative can effectively increase the delivery of its parent drug across a biological membrane. Thus, the suggestion that such hydroxymethyl derivatives would make good prodrugs (Pitman, 1981) has been substantiated. Furthermore, the present results suggest that hydroxymethyl derivatives of other amide and imide drugs (which are not topically effective), may enhance the percutaneous delivery of those drugs also. However, the long lag time for delivery of the parent drug to the receptor phase, in view of the short time

TABLE 3

EFFECT OF THEOPHYLLINE PRODRUGS ON INCORPORATION OF [^{3}H]THYMIDINE INTO DNA OF UV-IRRADIATED MOUSE EPIDERMIS



Drug	% Concentration	Mice	CPM/10 μg DNA (mean ± S.E.)	% Inhibition
Propylene glycol	–	12	623 ± 66	
Non-irradiated	–	12	230 ± 44	
R = $-O_2CC(CH_3)_3$	10	10	414 ± 77	53
R = $-O_2CC_3H_7$	10	7	254 ± 88	94
	1	8	641 ± 86	0
R = $-O_2C(CH_2)_2CON(C_2H_5)_2$	10	10	569 ± 90	14
FA [a]	1	12	210 ± 79	105
	0.1	10	389 ± 81	60
Isopropyl myristate		3	647 ± 95	
Non-irradiated		6	270 ± 93	
R = $-O_2CC(CH_3)_3$	10	6	462 ± 365	49
R = $-O_2CC_3H_7$	10	4	311 ± 155	89
R = $-O_2C(CH_2)_2CON(C_2H_5)_2$	10	6	653 ± 262	0
Isopropyl myristate		5 [b]	938 ± 58	
Non-irradiated		5 [b]	165 ± 12	
FA [a]	1	4 [b]	337 ± 17	77
R = $-O_2CC_3H_7$	10	5 [b]	548 ± 139	50

[a] Fluocinolone acetonide.
[b] Hairless mice.

over which most topicals are applied, may become a significant factor in deciding in favor of the acyloxymethyl prodrug approach. In addition, the lack of chemical stability of hydroxymethyl derivatives mitigates against their use in aqueous formulations.

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