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The effect of structure of Mannich base prodrugs on their ability to deliver theophylline and 5-fluorouracil through hairless mouse skin

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Summary

An extended series of *N*-Mannich base prodrugs of theophylline and 5-fluorouracil have been tested for their ability to deliver their parent drugs through hairless mouse skin from isopropyl myristate (IPM). There was a good correlation between the calculated solubility parameters of the parent drugs and their prodrugs, and the respective log experimental permeability coefficients. Although all of the prodrugs delivered more of the parent drug than the parent drugs did from IPM, increases in the solubility of the prodrug in the non-polar vehicle caused by synthesizing more lipid-like prodrug forms of the parent drug did not result in concomitant relative increases in delivery of the parent drug.

Introduction

Theophylline and 5-fluorouracil (5-FU) are both polar heterocyclic drugs that do not permeate skin well because of their poor solubility characteristics (Sloan et al., 1984). Because of the desirability of enhancing their delivery through skin, the effect of various vehicles on the delivery of theophylline and 5-FU through hairless mouse skin has been examined recently to determine a method for predicting the optimal vehicle to use in each case (Sloan et al., 1986; Sherertz et al., 1987a). The results from these reports showed that generally the permability coefficient for the delivery of a drug from a vehicle through skin (P), which is the rate of delivery of a drug through skin (J) divided by the solubility of the drug in the vehicle (C^v), is inversely related to C^v (Eq. 1). However, this relationship only holds if the vehicle does not significantly affect the permeability of the skin as determined by subsequent topical applications of a standard solute/vehicle combination (Sherertz et al., 1987b).

There have also been a number of prodrug approaches used to increase the delivery of theo-

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phylline (Sloan and Bodor, 1982) and 5-FU (Mollgaard et al., 1982) through skin. Generally these approaches consist of synthesizing prodrug forms that are anticipated to exhibit increased solubility because of characteristic decreased melting points and hydrogen bonding tendencies. Specifically, since the partition coefficient of a drug between the vehicle and skin (K)—which is the solubility of the drug in the skin (C^s) divided by the solubility of the drug in the vehicle (C^v)—and C^v are the primary driving forces for diffusion (Eqn. 1), increased C^v and C^s (hence K) are the ultimate goals of such synthetic manipulations. This assumes that the solvent does not effectively alter or perturb the skin. Interestingly, there has not been a method reported to predict changes in P or J caused by changes in the solubilities engendered by the syntheses of prodrug forms. This may be due to the fact that the diffusion coefficient of the prodrug in the skin as well as concentration of prodrug in the vehicle and partition coefficient of the prodrug (which are different from D, C^v and K of the parent drug) change with the use of prodrug forms that have a finite lifetime during their diffusion through skin of thickness h. However, the diffusion coefficient of a series of homologous prodrugs depends inversely on the third-root of their molar volumes so that P should not change that much for a series because of changes in the diffusion coefficient.

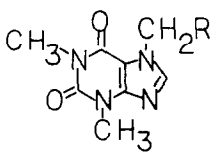
$$J = PC^v = K(D/h)C^v \quad (1)$$

Mannich base prodrugs of theophylline and 5-FU have been examined recently as a means to improve the topical delivery of the parent drugs (Sloan et al., 1984). In those cases only one prodrug each of theophylline and 5-FU was tested in diffusion cell experiments primarily because of stability problems. However, because of the fact that the Mannich base-type prodrugs hydrolyze so rapidly in water without enzymatic catalysis (Johansen and Bundgaard, 1981), it may be assumed that these prodrugs should have little effect on the apparent diffusion coefficient of the parent drug (D) in the skin. Based on the pK_a values for 5-FU (8.0 and 13.0) and theophylline (8.6) (Sloan et al., 1984), the expected $t_{1/2}$ values for hydrolysis of the Mannich bases are in the range of 10^{-5}–10^{-8} min depending on the amine used except for the Mannich bases derivatives on N-1 of 5-FU where the longest predicted $t_{1/2}$ is about 7 min for the morpholine derivative (Bundgaard and Johansen, 1981). This ensures that the effect of the prodrug form on enhanced delivery of the parent drug will be expressed through the effect of the prodrug form on C^v and K and not on D. Thus, the Mannich base prodrug series offer an excellent opportunity to examine the effect of changes in solubility engendered by a prodrug form on the delivery of the parent drug uncomplicated by concerns about effects on D.

This report describes the synthesis of several additional Mannich base prodrugs of theophylline and 5-FU and examines their abilities as well as of those previously synthesized to deliver theophylline and 5-FU through hairless mouse skin in diffusion cell experiments to determine if there is a relationship between the physicochemical properties of these prodrugs and their ability to deliver their parent drugs through skin.

Materials and Methods

The hairless mice that were used were female (18–28 g) SKH-hr-1 from Temple University Skin and Cancer Hospital. The diffusion cells were obtained from Kercso Engineering Consultants, Palo Alto, CA, and were 3 cm in diameter (7.06 cm^2 skin surface area) with a 40 ml receptor phase volume. A Labline incubator with Variomag stirrer was used to maintain a constant temperature of 32°C and to stir the receptor phases of the cells. UV spectra were recorded with a Shimadzu UV-160 spectrophotometer. MP (corrected) were taken with a Thomas-Hoover Capillary Apparatus. NMR spectra were recorded on a Varian EM390 spectrometer and IR spectra were recorded on a Beckman Accu Lab 1 spectrophotometer. Microanalyses were obtained from Atlantic Microlab. Atlanta, GA. 5-Fluorouracil and theophylline were obtained from Sigma while the amines, formaldehyde and other reagents were purchased from Aldrich. Bulk solvents were obtained from Fisher. The isopropyl myristate (IPM) was obtained from Givaudan Clifton, NJ.



I, Theophylline

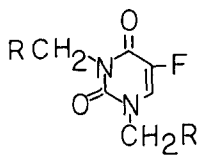
II, R = $N(CH_3)_2$

III, R = $N(CH_2CH_3)_2$

IV, R = $N(CH_2CH_2CH_3)_2$

V, R = $N(CH_2CH_2CH_2CH_3)_2$

VI, R = $N(CH_2)_5$



VII, R = $N(CH_2CH_2)_2O$

VIII, R = $N(CH_2CH_2)_2NCH_3$

IX, R = $N(CH_2)_4$

X, 5-fluorouracil

XI, R = $N(CH_2)_5$

XII, R = $N(CH_2CH_2)_2O$

XIII, R = $N(CH_2CH_2)_2NCH_3$

Syntheses

The preparation of Mannich base derivatives of theophylline

All the derivatives were prepared in exactly the same way as previously described (Sloan et al., 1984). Derivatives **II, III, IV, VII** and **IX** had been previously isolated and completely characterized (Sloan et al., 1984) and the derivatives isolated for use in this study were identical with those derivatives by ^{1}H-NMR spectroscopy and by melting point. The 3 additional derivatives (**V, VI** and **VII**) exhibited the following characteristics.

7-(Dibutylamino)methyltheophylline (V). m.p. 62–64 °C, 86% yield from petroleum ether; ^{1}H-NMR ($CDCl_3$) δ 7.70 (s, 1, N–C$\underline{H}$–N), 5.28 (s, 2, N–C$\underline{H}_2$–N), 3.6 and 3.4 (2s, 6, N–C$\underline{H}_3$), 2.6 (t, 4, J = 7 Hz, NC$\underline{H}_2$), 1.6–1.1 (m, 8, C$\underline{H}_2$), and 1.1–0.8 (m, 6, C$\underline{H}_3$). *Anal.*: Calcd. for $C_{16}H_{27}N_5O_2$: C, 59.78; H, 8.47; N, 21.80. Found: C, 60.20; H, 8.41; N, 21.43.

7-(Piperidyl)methyltheophylline (VI). m.p. 115–116 °C [lit. (Roth and Brandes, 1965) m.p. 116 °C], 85% yield from petroleum ether; ^{1}H-NMR ($CDCl_3$) δ 7.61 (s, 1 N = C$\underline{H}$–N), 5.24 (s, 2, N–C$\underline{H}_2$–N), 3.6 and 3.4 (2s, 6, N–C$\underline{H}_3$), 2.6 (t, 4, J = 6 Hz, NC$\underline{H}_2$), and 1.8–1.2 (m, 6, C$\underline{H}_2$).

7-(4′-methyl-1′-piperazinyl)methyltheophylline (VIII). m.p. 131–133 °C, 93% yield from petroleum ether; ^{1}H-NMR ($CDCl_3$) δ 7.64 (s, 1, N = C$\underline{H}$–N), 5.28 (s, 2, N–C$\underline{H}_2$), 3.6 and 3.4 (2s, 6, N–C$\underline{H}_3$), 2.27 (s, 3, N–C$\underline{H}_3$), and 2.8–2.3 (m, 8, NC$\underline{H}_2$). *Anal.*: Calcd. for $C_{13}H_{20}N_6O_2$: C, 53.42; H, 6.90; N, 28.74. Found, C. 53.30; H, 6.90; N, 28.30.

The preparation of Mannich base derivatives of 5-fluorouracil.

These derivatives were prepared by allowing two equivalents of the desired secondary amine to react with two equivalents of 37% aqueous formaldehyde at ice-bath temperature. After 5–10 min, one equivalent of 5-fluorouracil (0.02 mol) was added to the reaction mixture which was then stirred at room temperature for 4–16 h. The reaction mixture frequently solidified at this point. Any water that separated was decanted and the solid was dissolved in 150 ml of dichloromethane. The dichloromethane layer was separated, dried over Na_2SO_4 and concentrated at room temperature to give viscous oils or hard foams. These oils or foams were then triturated with various volumes of anhydrous ether overnight. The suspensions that resulted were filtered. The residues were dried in vacuo and triturated with anhydrous ether overnight again to give the desired analytically pure products.

1,3-Bis(1′-piperidinyl)methyl-5-fluorouracil (XI). 36% yield of crude **XI**, m.p. 88–92 °C, from 5 ml ether; analytical sample m.p. 96–98° (62% recovery); ^{1}H-NMR ($CDCl_3$) δ 7.48 (d, 1, J = 4 Hz, C$\underline{H}$ = CF), 497 and 4.50 (2s, 4, N–C$\underline{H}_2$–N), 2.8–2.4 (m, 8, C$\underline{H}_2$–N), and 1.8–1.3 (m, 12, C$\underline{H}_2$); UV ($CH_3C \equiv N$) max 264 nm ($\epsilon = 6.40 \times 10^3$ l/mol). *Anal.*: Calcd. for $C_{16}H_{25}FN_4O_2$: C, 59.24; H, 7.77; N, 17.26. Found: C, 59.33; H, 7.82; N, 17.22.

1,3-Bis(4′-morpholinyl)methyl-5-fluorouracil (XII). 83% yield of pure **XII**, m.p. 136–139 °C, from 50 ml ether; which was identical with an analytical sample of **XII** by ^{1}H-NMR spectroscopy [lit. (Sloan et al., 1984) m.p. 137–139 °C].

1,3-Bis(4′-methyl-1′-piperazinyl)methyl-5-fluorouracil (XIII). 37% yield of crude **XIII**, m.p. 116–120°, from 6 ml of ether; analytical sample m.p. 117–120 ° (76% recovery); ^{1}H-NMR ($CDCl_3$) δ 7.41 (d, 1, J = 4 Hz, C$\underline{H}$ = CF), 5.01 and 4.48 (2s, 4, N–C$\underline{H}_2$–N), 2.9–2.6 (m, 8, C$\underline{H}_2$–N), 2.6–2.35 (m, 8, C$\underline{H}_2$–N–CH$_3$), and 2.3 and 2.25 (2s, 6, N–C$\underline{H}_3$); UV $CH_3C \equiv N$) max 267 nm

TABLE 1

Solubilities and calculated solubility parameters for theophylline, 5-FU and their Mannich base prodrugs

Structure	Molecular weight	Solubility		Solubility parameter, δ_1 [c]
		Prodrug [a]	As parent drug [b]	
I. Theophylline	180		0.062	14.05
II. R = $N(CH_3)_2$	237	11.47	8.71	12.14
III. R = $N(CH_2CH_3)_2$	265	32.04	21.76	11.65
IV. R = $N(CH_2CH_2CH_3)_2$	293	126.3	77.59	11.27
V. R = $N(CH_2CH_2CH_2CH_3)_2$	321	153.6	86.13	10.98
VI. R = $N(CH_2)_5$	277	17.58	11.42	12.04
VII. R = $N(CH_2CH_2)_2O$	279	1.62	1.05	12.35
VIII. R = $N(CH_2CH_2)_2NCH_3$	292	4.61	2.84	11.99
IX. R = $N(CH_2)_4$	263	12.38	8.47	12.30
X. 5-FU	130		0.0051	15.0
XI. R = $N(CH_2)_5$	324	17.41	16.98	11.04
XII. R = $N(CH_2CH_2)_2O$	328	6.38	2.53	11.46
XIII. R = $N(CH_2CH_2)_2N-CH_3$	354	11.65	4.27	11.00

[a] mg of prodrug/ml of IPM.
[b] mg of parent drug/ml of IPM equivalent to mg of prodrug/ml of IPM.
[c] $(cal/cm^3)^{1/2}$.

($\epsilon = 6.99 \times 10^3$ 1/mol). *Anal.*: Calcd. for $C_{16}H_{27}FN_6O_2$: C, 54.22; H, 7.68; N, 23.71. Found: C, 54.05; H, 7.69; N, 23.60.

Determination of solubilities

The solubilities of theophylline, 5-fluorouracil and the prodrug derivatives in isopropyl myristate (Table 1) were determined by stirring an excess of the drug or derivative in isopropyl myristate (IPM) for 48 h at room temperature (23 ± 1° C), allowing the suspensions to sit at room temperature for 48 h, then filtering the suspensions through Whatman no. 1 (qualitative) filter paper. The solubility samples were carefully protected from atmospheric moisture until they were filtered by gravity. Samples of the filtrates were taken within 5 min and immediately diluted with either acetonitrile or methanol. The solubilities were determined in triplicate by UV spectroscopy and were reproducible within ±5% except of **XI** and **XIII** which were ±7%. The residues that were obtained from the filtrations were analyzed immediately by ^{1}H-NMR spectroscopy. In all cases, except for **III**, the residues from the solubility determinations of the prodrugs were composed only of intact prodrugs. In the case of **III** about 13% theophylline was present in the residue.

Determination of delivery of drugs through hairless mouse skin by the prodrugs or the parent drugs

These experiments were run essentially as previously described for the Mannich base-type prodrugs (Sloan et al., 1984) and for the parent drugs (Sloan et al., 1986; Sherertz et al., 1987a). Samples of the drugs or prodrugs as suspensions in IPM were prepared in the same way that the solubility samples were prepared, i.e. excess drug or prodrug was stirred in IPM at room temperature for 48 h and allowed to sit at room temperature for 48 h, then the mixtures were briefly stirred to resuspend the drug or prodrug before being used.

The hairless mouse skins were obtained by sacrificing the mice by cervical dislocation. The whole-thickness dorsal skins were removed using blunt dissection to separate the skins from the underlying fascia. The skins were immediately placed in diffusion cells with the dermis in contact with pH 7.3 (at 32° C) phosphate buffer (0.05 M, 0.11 ionic strength) containing 0.1 v/v 36% aqueous formaldehyde as a preservative. The skins were kept in contact with this receptor phase buffer solution for 48 h to allow UV-absorbing materials to leach from the skin. The receptor phases were changed twice during the 48 h so that

a fresh receptor phase was in place when the drug or prodrug suspensions were applied.

After 48 h, a suspension of drug or prodrug in IPM (0.5 ml) was applied to the donor side (stratum corneum) of 3 diffusion cells for each drug or prodrug being tested using an Eppendorf digital pipetter. Samples of the receptor phase (3 ml) of each cell were then taken at 0, 3, 6, 9, 12, 20, 24, 30 and 48 h after the suspensions were applied and analyzed by UV spectroscopy for either theophylline (270 nm, $\epsilon = 1.03 \times 10^4$ 1/mol) or 5-FU (265 nm, $\epsilon = 7.1 \times 10^3$ 1/mol). In those cases where the prodrugs decomposed quickly, samples were also taken at 1, 2 and 4 h. The receptor phases were replenished with fresh pH 7.3 phosphate buffer after each sample was taken. Thus, the analysis of each subsequent sample had to be corrected for all the previous samples that had been removed for analysis. This resulted in adding 7.5% of the amount of theophylline or 5-FU in each previous sample to the amount of theophylline or 5-FU in the sample being analyzed.

Values for rate of delivery of the parent drugs by the prodrugs (flux) in all cases were obtained by plotting the cumulative mg of theophylline or 5-FU measured in the receptor phase against time in hours. In all cases linear regression analysis was used to obtain the slopes for the plots of mg against h, and correlation coefficients were at least $r = 0.99$. Fluxes were obtained by dividing the above slopes by the area of the diffusion cell (7.06 cm^2), and the permeability coefficients were then obtained by dividing the fluxes ($mg/cm^2 \cdot h$) by the corresponding mg equivalent solubility of theophylline or 5-FU in IPM for each prodrug.

Calculation of solubility parameters

The calculated solubility parameters were obtained using the method of Fedors (1974) as illustrated by Martin et al. (1985) and Sloan et al. (1986).

Results and Discussion

Synthesis

The Mannich bases of theophylline were prepared in the same way as previously described (Sloan et al., 1984). The Mannich bases of 5-FU were prepared without using a solvent and with cooling during the formation of the alkylating agent. Much better yields of **XII** were obtained in this way and this was the only method that allowed for the isolation of **XI** and **XIII** as crystalline solids. Even employing this method, other Mannich bases of 5-FU resisted isolation as crystalline solids and remained as slightly impure oils which were not suitable for further study.

Solubility and stability studies

The fact that Mannich bases are not stable in the presence of water has already been discussed in numerous reports (Sloan et al., 1984; Bundgaard and Johansen, 1981). This fact is particularly important for the Mannich bases of such highly acidic imides and amides as theophylline and 5-FU since the half-lives of the Mannich bases are directly related to the pK_as of the amide or imide, i.e. the more acidic the amide or imide the faster the rate of hydrolysis of its corresponding Mannich base. However, it should be pointed out that the reaction between formaldehyde, secondary amine and amide or imide is an equilibrium reaction which quite frequently is run in the presence of water and in fact generates water during the formation of the Mannich base which must be removed to stabilize the product. Thus, the Mannich bases of amides and imides are by their very nature sensitive to adsorption or absorption of moisture in the solid state or in aprotic solvents.

The determination of the solubilities of the Mannich bases in IPM had to be carried out reasonably carefully to exclude moisture, i.e. a tight-fitting cork stopper was found to provide adequate protection. The use of sonication as previously described (Sloan et al., 1984) was found to give variable results. Simply stirring the suspensions of the prodrugs or drugs at room temperature for 48 h and allowing the suspensions to sit at room temperature for 48 h before analyzing them has given reproducible results when the prodrugs have remained intact. ^{1}H-NMR analysis of the residues from the filtration of the suspensions was used to determine that the prodrugs were intact. If there was extensive theophylline or 5-FU formation in the residues the solubility values were

variable. In those instances the result was probably due to lack of adequate exclusion of moisture from the suspensions. Also, it was found that 48 h of stirring was the least amount of time necessary to approach a reasonably constant solubility value.

In the light of the above statements, most of the differences between the solubilities previously obtained for **II, III, IV, VII**, and **IX, XII**, theophylline, and 5-FU (Sloan et al., 1984) and the present data can be readily explained. First, the differences between the solubilities previously determined by stirring suspensions of the prodrugs for 24 h instead of 48 h can be reasonably attributed to lack of sufficient stirring at least for the dimethylamino (**II**) prodrug. On the other hand, there is no significant difference between the values reported here and previously for the diethylamino (**III**) case, while in the case of the dipropylamino (**IV**) Mannich base the previous value was much lower. These results are due to the fact that in the previous and present work the Mannich base (**III**) remained essentially intact during the determination of the solubility while in the previous work the IPM insoluble material from the determination of the solubilities of **IV** was only theophylline. Excess intact Mannich base must be present during the solubility determination to give a valid solubility value. That was not the case in the previous determination of the IPM solubility of **IV**. Second, the somewhat lower solubilities of **II, VII, IX** and 5-FU previously reported are probably due to 3 h of sonication being insufficient to approach a maximum solubility value. It is not clear why the solubility of theophylline and **XII** obtained for this report are lower than those previously reported. It may be that sonication allowed different crystal modification to form which exhibited somewhat higher solubilities.

All of the Mannich base prodrugs and their parent drugs were applied to the diffusion cells as suspensions. This allowed each prodrug and parent drug to be tested at constant and maximal activity since each was in equilibrium with the solid prodrug or parent drug (Higuchi, 1960). This assumes uniform solid crystal structures for the prodrugs during the course of the experiments. The amount of prodrug that was applied in each 0.5 ml sample was variable but for most of the theophylline and theophylline prodrugs the concentration was 0.40–0.44 M, while for all of the 5-FU and 5-FU prodrugs it was 0.28–0.30 M. The concentrations of the former were fairly close to that at which theophylline and IX were previously run (0.36 M) while the concentrations of the latter were about 7 times higher than that at which 5-FU and **XII** were previously run (0.04 M). In most cases the suspensions that were applied were fluid but in the case of **IV** and **V** the concentrations of prodrug were so high that when the prodrug and IPM were mixed a semisolid was obtained. Thus, in the cases of **IV** and **V** an inexact amount of prodrug/IPM was applied that corresponded roughly to 0.5 ml.

Diffusion studies

The rate of delivery of theophylline or 5-FU by each Mannich base prodrug from IPM was evaluated and compared with the rate of delivery of the parent drugs by the parent drugs from IPM. IPM was chosen as the vehicle because the Mannich base prodrugs were stable in IPM until they were exposed to atmospheric moisture. The result was that as soon as the suspensions, or in the cases of **IV** and **V** the semisolid mixtures, of prodrug and IPM were applied to the diffusion cell, decomposition began. The appearance of the individual plots of cumulative mg of parent drug in the receptor phase versus time reflected the relative stabilities of the Mannich base prodrugs. The more stable prodrugs maintained a longer steady-state delivery of the parent drug than the less stable, more moisture-sensitive prodrugs. For example, **VII** was considered to be one of the more moisture stable theophylline Mannich base prodrugs in IPM suspension (Sloan et al., 1984) and its steady-state delivery of theophylline lasted until the 36 h sample was taken while **III** was considered to be one of the less stable prodrugs and its steady-state delivery of theophylline only lasted until after the 6 h sample was taken.

For those prodrugs that decomposed more rapidly it was difficult to obtain an accurate value for the steady-state flux since the combination of a lag time of any length and a short time until the saturated solution of the prodrug was no longer

contributing to the delivery of the parent drug meant that essentially S-shaped curves for plots of cumulative mg versus time were generated. This difficulty could be effectively overcome by using higher concentrations of prodrug/vehicle which ensured that the relative amount of intact prodrug in suspension lasted somewhat longer. The criterion that was used to determine if the concentration was high enough to ensure a steady-state delivery was that a straight line relationship between cumulative mg and time was at least maintained for 3 sample periods. This criterion was tested by using a higher applied concentration in an additional diffusion cell experiment. The observed flux remained constant at the two concentrations in all the cases tested which suggests that for these prodrugs the above criterion is valid.

However, regardless of the concentration used in each case the final result was the same. At the point where all the prodrug was decomposed to parent drug, the saturated solution of prodrug in IPM could no longer be maintained, and the plot of cumulative mg of parent drug delivered versus time leveled off and approximated the plot of cumulative mg of parent drug delivered by the parent drug versus time. Typical plots are shown in Figs. 1 and 2. The fact that only parent drug remained in the donor phase at the end of the experiment was verified by ^{1}H-NMR spectroscopic examination of the donor phase. In diffusion cell experiments with other Mannich base prodrugs, ^{1}H-NMR spectra of the donor phases were obtained during the entire course of the experiments (Kevin Siver, unpublished results). In those cases the point at which the excess prodrug in the donor phase had decomposed coincided with the point where the delivery of the parent drug leveled off.

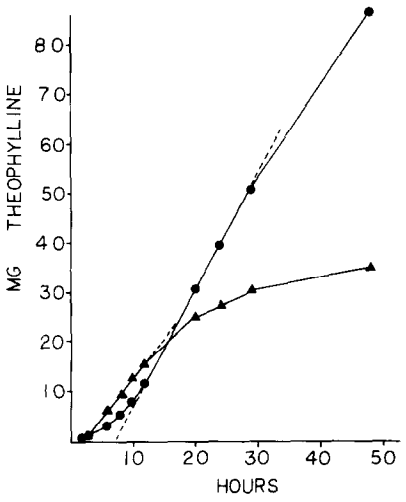


Fig. 1. Plots of average cumulative mg of theophylline delivered through hairless mouse skins from suspensions of Mannich bases of theophylline in IPM versus time: **II** (▲), **V** (●).

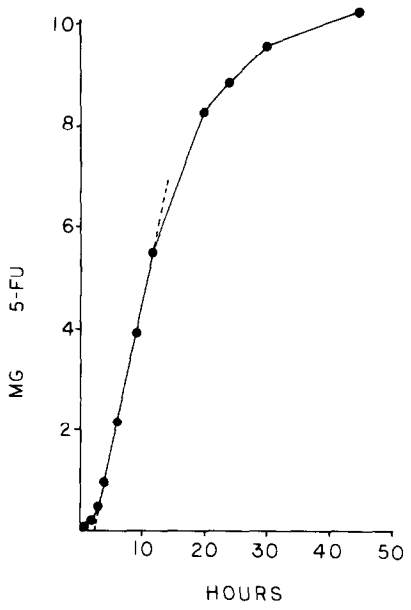


Fig. 2. A plot of average cumulative mg of 5-FU delivered through hairless mouse skins from a suspension of the Mannich base **XI** in IPM versus time.

Another point that should be made is that as the prodrugs are hydrolyzing to the parent drugs, a saturated solution of the parent drugs will develop in the donor phase that will also contribute to the apparent steady-state delivery of the parent drug by the prodrugs. However, the long lag time until steady-state delivery of the parent drugs by the parent drugs is reached, and the short lag time until steady-state delivery of the parent drugs by the prodrugs is observed, suggests that rates for the first phase of delivery of the parent drugs by the parent drugs (footnotes h and i in Table 2) are the rates that are important to consider. In that consideration the rate of delivery of the parent drug by parent drug released in the donor phase

TABLE 2

Rates of delivery and permeability coefficients of the parent drug by the parent drugs and the Mannich base prodrugs from IPM

Structure	J(±S.D.) (mg/cm^2 · h)	P (cm/h)	Lag time (h)
I [a]. Theophylline	0.041 (0.0037) [h]	0.66	11.2
II [b]. R = $N(CH_3)_2$	0.21 (0.010)	0.024	2.3
III [c]. R = $N(CH_2CH_3)_2$	0.37 (0.11)	0.017	0.7
IV [d]. R = $N(CH_2CH_2CH_3)_2$	0.36 (0.019)	0.0046	2.2
V [e]. R = $N(CH_2CH_2CH_2CH_3)_2$	0.30 (0.021)	0.0035	5.9
VI [b]. R = $N(CH_2)_5$	0.16 (0.014)	0.014	0.6
VII [b]. R = $N(CH_2CH_2)_2O$	0.11 (0.020)	0.10	2.1
VIII [b]. R = $N(CH_2CH_2)_2NCH_3$	0.090 (0.0082)	0.032	0.8
IX [b]. R = $N(CH_2)_4$	0.23 (0.014)	0.027	2.5
X [f]. 5-FU	0.022 (0.0088) [i]	4.31	15.1
XI [g]. R = $N(CH_2)_5$	0.076 (0.0030)	0.011	1.7
XII [g]. R = $N(CH_2CH_2)_2O$	0.11 (0.013)	0.043	0.7
XIII [g]. R = $N(CH_2CH_2)_2NCH_3$	0.013 (0.010)	0.0030	0.5

[a] 0.5 ml of 400 mg/5 ml suspension applied.
[b] 0.5 ml of 350 mg/3 ml suspension applied.
[c] 0.5 ml of 700 mg/3 ml suspension applied.
[d] 0.5 ml of 1500 mg/3 ml suspension applied.
[e] 0.5 ml of 2000 mg/3 ml suspension applied.
[f] 0.5 ml of 200 mg/5 ml suspension applied.
[g] 0.5 ml of 300 mg/3 ml suspension applied.
[h] Steady-state phase of diffusion process; first phase $J = 0.016 \pm 0.0044$ mg/cm^2 · h (lag time = 3.0 h); [lit. for first phase (Sloan et al., 1984) $J = 0.028 \pm 0.018$ mg/cm^2 · h, (Sloan and Bodor, 1982) $J = 0.017 \pm 0.0013$ mg/cm^2 · h].
[i] Steady-state phase of diffusion process; first phase $J = 0.0047 \pm 0.0014$ mg/cm^2 · h (lag time = 3.4 h); [lit. for first phase (Sloan et al., 1984) $J = 0.0056 \pm 0.0036$ mg/cm^2 · h].

from the prodrug contributes only a few percent (4–15%) to the total average flux in all except one of the experiments. In all the experiments, flux and permeability coefficient values corrected for such a worst case scenario of maximum contribution by the parent drugs to delivery of the parent drugs would not be significantly different from the values reported in Table 2 ($P < 0.05$). Thus, such a correction would not affect the analysis of the data.

The lag times for achieving steady-state delivery of the parent drugs with the Mannich base prodrugs are much shorter than the lag times observed for the parent drugs. The Mannich bases are much more lipid- and water-soluble than the parent drugs (Sloan et al., 1984) so that they should partition into the skin better to deliver higher concentrations of the parent drugs quickly into the first few layers of skin. This assumes rapid hydrolysis of the prodrugs to the parent drugs (Bundgaard and Johansen, 1981). These concentrations of parent drug in the skin are much higher than could have been achieved with the vehicle itself, resulting in a greater thermodynamic driving force for diffusion than normal from that vehicle and in steady-state fluxes being reached sooner.

The values for the rates of delivery of the parent drugs by the parent drugs or by the two Mannich base prodrugs from IPM that were previously reported agree well with the values obtained in this study using different diffusion cells and a different method of maintaining the cells at 32° C. In the cases of the parent drugs, the initial or first phases of the diffusion process were compared with the previous results because only the first 12 h data were used to generate those results (see Table 2); they were not significantly different in spite of the fact that 5-FU/IPM was run at a concentration 7 times higher this time. In the case of the prodrug **IX**, $J = 0.23 \pm 0.014$ mg/cm^2 · h was not significantly different from $J = 0.20 \pm 0.018$ mg/cm^2 · h previously reported (Sloan et al., 1984). On the other hand, for **XII** $J = 0.11 \pm 0.013$

$mg/cm^2 \cdot h$ was significantly different from $J = 0.030 \pm 0.0014$ $mg/cm^2 \cdot h$ previously reported (Sloan et al., 1984). That significantly lower J value was probably due to the fact that it did not approximate a steady-state value. In fact, examination of that data in the light of the present work shows that the plot of cumulative mg of 5-FU versus time could be more accurately described as an S-shaped curve.

In order to determine that the Mannich bases and/or their decomposition products were not irreversibly damaging the mouse skin more than IPM itself, second application studies were run as previously described (Koch and Sloan, 1987; Sherertz et al., 1987b; Sloan et al., 1986). The results from the application of a standard theophylline in propylene glycol suspension to skin that had been previously treated with either IPM alone or various Mannich base prodrugs in IPM showed that the fluxes of theophylline through skin pretreated with the Mannich bases in IPM were not significantly different from the flux of theophylline through skin pretreated with IPM alone (results not shown).

In order to try to find some correlation between the physicochemical properties of the parent drugs and prodrugs and their abilities to deliver the parent drugs through hairless mouse skin, a number of correlations were examined. There does not appear to be any simple relationship between J and P, and solubility nor is there any apparent relationship between calculated partition coefficient and experimental P as found for the delivery of the parent drugs by different vehicles (Sloan et al., 1986; Sherertz et al., 1987a). The one correlation that was observed was between the calculated solubility parameter of the parent drugs and prodrugs and the log experimental P. The solubility parameters were calculated as previously described (Sloan et al., 1986; Sherertz et al., 1987a). The results of plotting these calculated solubility parameters (see Table 1) against the log experimental P (see Table 2) is shown in Fig. 3. Using log P values from the steady-state J for the parent drugs and excluding the values for prodrugs containing morpholine (**VII** and **XII**) a slope of 0.73 with a correlation coefficient of $r = 0.98$ was obtained. If the log experimental P for **VII** and **XII**

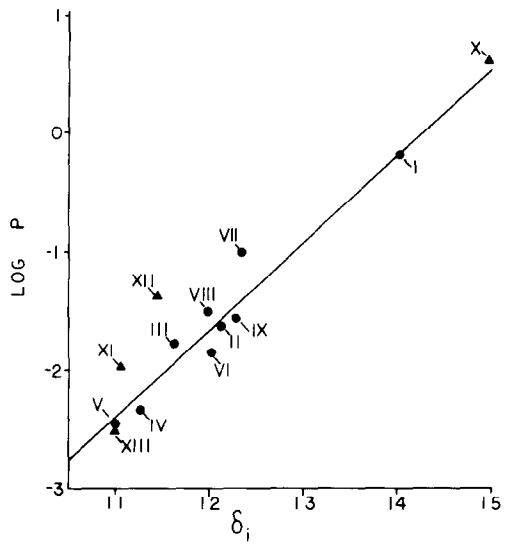


Fig. 3. A plot of log experimental permeability coefficients (log P) for the delivery of theophylline (●) or of 5-FU (▲) by suspensions of the respective Mannich base prodrugs in IPM versus the calculated solubility parameters of the prodrugs and drugs (δ_i).

are included, a slope of 0.71 and $r = 0.95$ was obtained (plot not shown).

Since solubility parameter gives a measure of the polarity of a molecule (a low value identifying a nonpolar lipid-like molecule and a high value a polar molecule), the direct correlation observed between increasing J/C^v or P and increasing solubility parameter values or polarity for the delivery of these Mannich base prodrugs and their parent drugs from a non-polar lipid-like vehicle – IPM [$\delta = 8.6(cal/cm^3)^{1/2}$] – suggests that the more similar the physicochemical properties of vehicle and solute become the more soluble the solute becomes and the lower the value for P becomes. Thus, there is a limit to gains in rate of delivery that can be realized by increasing the solubility of the parent drug in a lipid-like vehicle by making a more lipid soluble prodrug because as C^v goes up, P and partition coefficient (K) go down. For example, **XII** is the least IPM soluble of the 5-FU Mannich bases yet it is the most effective one for delivering 5-FU from IPM. Similarly, **VI** is 11 times more soluble than **VII**, yet **VII** is only marginally less effective than **VI** at delivering theophylline from IPM.

Although the Mannich base prodrugs are fairly sensitive to moisture, especially in the case where they are derivatives of fairly acidic amides and imides, they may still be useful if used in an anhydrous formulation. The fact that some of them may decompose on exposure to atmospheric moisture after only 6–8 h should not become a practical problem if the formulation is applied 3 times a day.

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References

Bundgaard, H. and Johansen, M., Hydrolysis of *N*-Mannich bases and its consequences for the biological testing of such agents. *Int. J. Pharm.*, 9 (1981) 7–16.

Fedors, R.F., A method for estimating both the solubility parameters and molar volumes of liquids. *Polymer Eng. Sci.*, 14 (1974) 147–154.

Higuchi, T., Physical chemical analysis of percutaneous absorption process from creams and ointments. *J. Soc. Cosmet. Chem.*, 11 (1960) 85–97.

Johansen, M. and Bundgaard, H., Decomposition of rolitetracycline and other *N*-Mannich bases and of *N*-hydroxymethyl derivatives in the presence of plasma. *Arch. Pharm. Chemi. Sci. Ed.*, 9 (1981) 40–42.

Martin, A., Wu, P.L. and Velasquez, T., Extended Hildebrand solubility approach: sulfonamides in binary and ternary solvents. *J. Pharm. Sci.*, 74 (1985) 277–282.

Mollgaard, B., Hoelgaard, A. and Bundgaard, H., Pro-drugs as drug delivery systems. XXIII. Improved dermal delivery of 5-fluorouracil through human skin via *N*-acyloxymethyl pro-drug derivatives. *Int. J. Pharm.*, 12 (1982) 153–162.

Roth, H.J. and Brandes, R., Synthese und Eigenschaften einiger Mannichbasen des Theophyllins, 8-Bromotheophyllins and Theobromins. *Arch. Pharmaz.*, 298 (1965) 765–770.

Sherertz, E.F., Sloan, K.B. and McTiernan, R.G., Use of theoretical partition coefficients determined from solubility parameters to predict permeability coefficients for 5-fluorouracil. *J. Invest. Dermatol.*, 89 (1987) 147–151.

Sherertz, E.F., Sloan, K.B. and McTiernan, R.G., Effect of skin pretreatment with vehicle alone or drug in vehicle on flux of a subsequently applied drug: results of hairless mouse diffusion cell studies. *J. Invest. Dermatol.*, 89 (1987) 249–252.

Sloan, K.B. and Bodor, N., Hydroxymethyl and acyloxymethyl prodrugs of theophylline: enhanced delivery of polar drugs through skin. *Int. J. Pharm.*, 12 (1982) 299–313.

Sloan, K.B., Koch, S.A.M. and Siver, K.G., Mannich base derivatives of theophylline and 5-fluorouracil: syntheses, properties and topical delivery characteristics. *Int. J. Pharm.*, 21 (1984) 251–264.

Sloan, K.B., Koch, S.A.M., Siver, K.G., Flowers, F.P., The use solubility parameters of drug and vehicles to predict flux. *J. Invest. Dermatol.*, 87 (1986) 244–252.