Topical Delivery of a Model Phenolic Drug: Alkyloxycarbonyl Prodrugs of Acetaminophen

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Purpose. To determine whether the delivery of a phenolic parent drug by its alkyloxycarbonyl (AOC) prodrugs through hairless mouse skin would show similar dependencies on water and lipid solubilities that similar prodrugs of more polar heterocyclic amide and imide parent drugs have shown.

Methods. Flux through hairless mouse skin from suspensions in isopropyl myristate (J_{MIPM}), solubilities in IPM (S_{IPM}) and water (S_{AQ}), and partition coefficients between isopropyl myristate (IPM) and pH 4.0 buffer ($K_{IPM:4.0}$) were measured for two series of AOC derivatives of acetaminophen (APAP); their solubilities in pH 4.0 buffer ($S_{4.0}$) were estimated from $S_{IPM}/K_{IPM:\,4.0}$. Log J_{MIPM} values were calculated from the n = 43 coefficients for the parameters in the transformed Potts-Guy (Roberts-Sloan) equation, and the average error of prediction ($\Delta \log J'_{IPM}$) was calculated. The J_{MIPM}, S_{IPM}, $S_{4.0}$, and molecular weight (MW) data for this series and two other series were combined with the n = 43 database to give a n = 61 database, and new best fit coefficients were determined for the Roberts-Sloan equation: $\log J_{MIPM} = x + y \log S_{IPM} + (1 - y) \log S_{4.0} - z\,MW$.

Results. All of the 4-AOC-APAP derivatives underperformed based on their predicted log J_{MIPM} ($\Delta \log J'_{MIPM} = 0.275 \pm 0.147$ log units) and, although the two more water soluble members of this more lipid soluble series were more effective than APAP, they were only marginally so: <2 times. Addition of three new series to the n = 43 database for the Roberts-Sloan equation did not substantially change the coefficients to the parameters: x, y, z, and $r^2 = -0.322$, 0.530, 0.00337 and 0.92, respectively.

Conclusions. The topical delivery of a model phenolic drug by its AOC prodrugs through hairless mouse skin from IPM shows the same dependence on S_{IPM}, $S_{4.0}$, and MW as the delivery of polar heterocycles by their similar prodrugs.

KEY WORDS: diffusion cell experiments; flux; lipid solubility; prodrugs; Roberts-Sloan equation; transformed Potts-Guy equation; water solubility.

INTRODUCTION

Although the phenolic functional group is one of the most common functional groups found in drug molecules, only one type of promoiety has been extensively synthesized, characterized, and evaluated in diffusion cell experiments for its effect on enhancing the topical delivery of drugs containing phenolic groups: alkylcarbonyl (AC) or ester derivatives (1–4). These derivatives have mainly been of various narcotic analgetics such as morphine, buprenorphine, nalbuphine, and naltrexone because narcotic analgetics are attractive targets for topical delivery. In most instances, the ester derivatives have been successful in enhancing the delivery of the parent drug from a variety of vehicles including mineral oil and water. However, the stability of the AC type derivative of phenolic groups in water will be less than that of other types of acyl derivatives due to the facts (a) that most acyl derivatives are hydrolyzed by an addition-elimination type mechanism and (b) that the carbonyl carbon of an ester functional group will be more electrophilic than the carbonyl carbon of a carbonate or carbamate acyl derivative. The result is that, if hydrolysis takes place by an addition-elimination reaction, AC type derivatives will be more reactive in the first step.

As part of the development of broader databases for topical delivery of drugs through hairless mouse skin from isopropyl myristate (IPM) and water (AQ) (5), it was decided to use carbonate derivatives (alkyloxycarbonyl; AOC) of phenols instead of AC derivatives. Even though AC derivatives would be stable in IPM, the AOC derivatives would be more stable toward an aqueous environment, and we needed prodrugs that would be stable in both vehicles for the two databases. Acetaminophen (APAP) was chosen as the model phenolic drug because there was already some data available on the stability and solubilities of some of the targeted prodrugs (6).

In this paper, we report the synthesis, characterization, and evaluation in diffusion cell experiments, using IPM as the vehicle and hairless mouse skin, of two series of AOC derivatives of APAP. The first series is one in which the alkyl part of the promoiety is a straight alkyl chain group, and the second is one in which an oxygen is inserted in the alkyl chain. In addition, the performance of the two series in diffusion cell experiments will be compared with that predicted by the transformed Potts-Guy (Roberts-Sloan) equation (7). Finally, the results from the series, as well as results from other series of prodrugs published since the initial publication of the Roberts-Sloan equation, will be added to the Roberts-Sloan database to generate a new set of coefficients to the parameters in the equation and give a more robust Roberts-Sloan equation.

MATERIALS AND METHODS

Melting points were determined with a Meltemp capillary melting point apparatus and are uncorrected. ^{1}H-NMR spectra were obtained at 90 MHz on a Varian EM-390 spectrometer (Palo Alto, CA, USA). Ultraviolet (UV) spectra were obtained on a Shimadzu UV-265 spectrophotometer (Kyoto, Japan). The vertical, Franz type diffusion cells were from Crown Glass (Somerville, NJ, USA) (surface area 4.9 cm^2, 20 ml receptor phase volume, 15 ml donor phase volume). The diffusion cells were maintained at 32°C with a Fisher (Pittsburgh, PA, USA) circulating water bath model 25. Thin layer chromatography (TLC) analyses were run on Brinkman Polygram Sil G/UV 254 plates. Isopropyl myristate (IPM) was obtained from Givaudan (Clifton, NJ, USA). Theophylline (Th) was purchased from Sigma Chemical Co. (St. Louis, MO, USA); all other reagent chemicals were from Aldrich Chemical Co. (Milwaukee, WI, USA), and all other solvents were from Fisher. 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."

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Syntheses

To a well-stirred suspension of 4-hydroxyacetanilide (APAP, **1**, 0.01 mol) in 30 ml of CH_2Cl_2 containing pyridine (0.01 mol) was added drop-wise a 10 ml CH_2Cl_2 solution of the desired chloroformate (0.01 mol). The reaction was allowed to proceed for 2 h, then the solution was diluted to 200 ml with CH_2Cl_2, extracted with 10 ml 0.6 N HCl and 10 ml water, dried over Na_2SO_4 for 2 h, and concentrated using a rotary evaporator to give a residue. The residue was purified by recrystallization (or column chromatography in one case (**6**)) until a sharp melting point (mp) was observed, only one component was observed on TLC and the ^{1}H NMR and UV spectra were clean of any unreacted APAP. For **6**, **7,** and **8,** the chloroformate was generated *in situ* from the reaction of triphosgene (0.0033 mol) in 20 ml of CH_2Cl_2 with a 10 ml CH_2Cl_2 solution of pyridine (0.01 mol) and the corresponding alcohol (0.01 mol) added drop-wise over 10 min. In this way, the following 4-alkyloxycarbonyl (AOC) derivatives of APAP were synthesized: 4-methyloxycarbonyloxyacetanilide (**2**), 49% from diethyl ether/hexane; 4-ethyloxycarbonyloxyacetanilide (**3**), 82% from ethyl acetate/hexane; 4-propyloxycarbonyloxyacetanilide (**4**), 59% from ethyl acetate/hexane; 4-butyloxycarbonyloxyacetanilide (**5**), 63% from ethyl acetate/hexane; 4-hexyloxycarbonyloxyacetanilide (**6**), 51% from ethyl acetate/hexane; 4-(2′-methoxyethyloxycarbonyloxy) acetanilide (**7**), 44% from ethyl acetate/hexane; and 4-(1′-methyl-2′-methoxyethyloxycarbonyloxy)acetanilide (**8**), 29% from diethyl ether/hexane. Melting points (Table I) and ^{1}H NMR and UV spectra and elemental analyses (Table II) are given elsewhere.

Physicochemical Properties and Analysis

Solubilities in IPM (S_{IPM}) were determined as previously described (8) using 72 h stirring of suspensions before filtration, dilution of the filtrate with CH_3CN, and quantitation by UV spectrophotometry from the absorbances of **1** to **8** at 240 nm using their molar absorptivities (ε) in Table II. Solubilities in water (S_{AQ}) were determined as above except using only 1 h stirring of suspensions before filtration (9). A 1 h stirring time was used to match the time samples of the prodrugs were stirred in water before application in diffusion cell experiments to be reported elsewhere. Partition coefficients between IPM and pH 4.0 buffer ($K_{IPM:4.0}$) were determined as previously described (8) where the volume ratios between pH 4.0 buffer and IPM (4.0/IPM) were 2, 4, 4, 4, 20, 4, and 4 for **2**, **3**, **4**, **5**, **6**, **7** and **8**, respectively. The solubilities in pH 4.0 buffer ($S_{4.0}$) were estimated from $S_{4.0} = S_{IPM}/K_{IPM:4.0}$. All solubilities and partition coefficients were measured in triplicate. The variations in measurements are reported below.

Intact prodrugs and APAP in the diffusion cell receptor phases were quantitated by UV spectrophotometry at 240 and 280 nm using the ε values in Table II and solving the following equations simultaneously: $\varepsilon_{A240}\, C_A + \varepsilon_{P240}\, C_P = A_{240}$ and $\varepsilon_{A280}\, C_A + \varepsilon_{P280}\, C_P = A_{280}$, where C_A and C_P are the concentrations of APAP and prodrug, A is the absorbance at either 240 (A_{240}) or 280 nm (A_{280}), and ε is the molar absorptivity of either APAP (ε_A) or prodrug (ε_P) at the designated wavelengths. For the concentration of APAP, the solution is $C_A = (\varepsilon_{P280}\, A_{240} - \varepsilon_{P240}\, A_{280})/(\varepsilon_{A240}\, \varepsilon_{P280} - \varepsilon_{P240}\, \varepsilon_{A280})$ and for prodrug it is $C_P = (\varepsilon_{A240}\, A_{280} - \varepsilon_{A280}\, A_{240})/(\varepsilon_{A240}\, \varepsilon_{P280} - \varepsilon_{P240}\, \varepsilon_{A280})$. The concentrations of both species were combined to give concentrations of total APAP species.

Theophylline in the receptor phase samples was quantitated by UV spectrophotometry from its absorbance at 270 nm (ε = 10,200 L mol^{-1}) in pH 7.1 phosphate buffer containing 0.11% formaldehyde.

The donor phase compositions at the end of the first application period were determined by ^{1}H NMR spectroscopy. The donor phases were removed, filtered, and the residues analyzed by ^{1}H NMR. In DMSO-d_6, the aromatic C–H absorptions were centered at δ 7.10 and 7.57 for the prodrugs, whereas those for APAP were centered at δ 6.63 and 7.30.

Diffusion Cell Experiments

The diffusion cell experiments were run in essentially the same way as previously described (10). Briefly, female hairless mice were sacrificed by cervical dislocation. Their skins were removed by blunt dissection and placed epidermal side up in contact with pH 7.1 phosphate buffer (0.05 M, I = 0.11 M, 32°C) containing 0.11% formaldehyde (2.7 ml of 36%

Table I. Molecular Weights (MW), Melting Points (mp°C), Log Solubilities in Isopropyl Myristate (IPM) (log S_{IPM}), Log Solubilities in Water (log S_{AQ}), and Log Estimated Solubilities in pH 4.0 Buffer from Log S_{IPM} – log $K_{IPM:4.0}$ for the 4-Alkyloxycarbonyl Prodrugs of 4-Hydroxyacetanilide (APAP)

Compound[a]	MW	mp°C[d]	log S_{IPM}[e]	log S_{AQ}[e,f]	log $S_{4.0}$[g]
1, APAP	151	167–170	0.28	0.45	
2, C1	209	112–115 (115.5–116.5)	1.08	1.31 (1.46)	1.23
3, C2	223	120–122 (121–122)	0.97	0.58 (0.69)	0.65
4, C3	237	104–106 (105–108)	1.37	0.43	0.48
5, C4	251	118–120 (119–121)	1.14	–0.37 (–0.20)	–0.35
6, C6	279	108–110 (112.5–113.5)	1.22	–1.32 (–0.88)	–1.49
7,[b] MOC2	253	78–81	1.01	1.54	1.31
8,[c] MOC3i	267	120–123	0.53	0.52	0.39

[a] C1, C2 . . . refer to the chain length of the alkyl group.
[b] 2-Methoxyethyloxycarbonyl, $CH_3OCH_2CH_2OC{=}O$.
[c] 2-Methoxy-1-methylethyloxycarbonyl, $CH_3OCH_2CH(CH_3)OC{=}O$.
[d] All mp are uncorrected. Literature references are in parentheses: **2, 3, 5,** and **6** are from Ref. 6; **4** from Ref. 14.
[e] Units of mM measured at 23 ± 1°C
[f] Literature references are in parentheses from Ref. 6, measured at 37°C.
[g] Log of the solubility in pH 4.0 buffer estimated from log S_{IPM} – log $K_{IPM:4.0}$.

Table II. Molar Absorptivities (ϵ), Chemical Shifts (δ), and Elemental Analyses for **1–8**

Compound	CH_3CN^a	Buffer[a,b]		^1H NMR[c]		
	ε_{240}	ε_{240}	ε_{280}	$CH_3C=O^d$	$C_6H_4{}^c$	$O=COCH_2X^f$
1, APAP	1.36	0.98	0.191			
2	1.67	1.25	0.0560	2.15	7.06, 7.45	s, 3.95
3	1.64	1.24	0.0483	2.12	7.03, 7.42	q, 4.28
4	1.63	1.28	0.0560	2.08	7.04, 7.42	t, 4.20
5	1.75	1.24	0.0376	2.14	7.08, 7.45	t, 4.28
6	1.79	1.12	0.0623	2.14	7.04, 7.42	t, 4.20
7[g]	1.74	1.26	0.0623	2.14	7.06, 7.46	t, 4.38
8[h]	1.60	1.28	0.0520	2.08	7.04, 7.42	m, 5.00

[a] Units of 1×10^4 L/mole.
[b] Buffer: pH 7.1 phosphate buffer with 0.11% formaldehyde.
[c] Spectra run in $CDCl_3$. APAP, **1,** was not sufficiently soluble in $CDCl_3$ to obtain a spectrum in $CDCl_3$.
[d] Singlet (s).
[e] Two doublets (d) each with J = 6 Hz centered at designated positions.
[f] Four absorptions (quartet, q) with J = 5 Hz; three absorptions (triplet, t) with J = 5 Hz; a doublet of quartets or multiplet (m).
[g] Anal calcd for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53. Found: C, 56.84; H, 5.98; N, 5.53.
[h] Anal calcd for $C_{13}H_{17}NO_5$: C, 58.42; H, 6.41; N, 5.24. Found: C, 58.43; H, 6.43; N, 5.27.

aqueous formaldehyde/liter) to prevent microbial growth and to insure the integrity of the mouse skins during the course of the experiment (11). The flux of theophylline from propylene glycol (Th/PG) through hairless mouse skin after 4, 24, 48, and 120 h of contact with the receptor phase did not change significantly: 0.0061 ± 0.0006, 0.0083 ± 0.0019, 0.0094 ± 0.0012, and 0.010 ± 0.0012 μmol cm^{-2} h^{-1}, respectively. The skins were kept in contact with the buffer for at least 48 h to condition the skins and to allow UV absorbing materials to leach out of the skins. The receptor phases were changed at least three times during this time to facilitate the leaching process, and the UV spectra of the receptor phase at the of this period was clear of UV absorptions in the 220–350 nm range.

Aliquots (0.5 ml, 0.2–0.3 M) of suspensions of each prodrug in IPM were applied to the donor side of each of three diffusion cells in the first application. After the donor phase suspensions were applied, 3 ml samples of the receptor phases were removed, generally at 8, 19, 22, 25, 28, 31, 34, 44, and 48 h. The entire receptor phase was replaced with fresh receptor fluid each time a sample was removed. The amount of APAP and prodrug in each sample was determined immediately by UV spectrophotometry using the equations described above.

After the initial application period of 48 h (96 total h) and the donor phases had been removed, the donor surfaces were quickly washed with 3 × 2 ml portions of methanol to removed any residual prodrug or APAP. After the methanol wash, the skins were kept in contact with fresh receptor fluid for 23–24 h to allow any APAP or prodrugs to leach out. Samples of the receptor phases were removed and analyzed for total APAP species as above. The receptor phases were replaced with fresh receptor fluid, and 0.5 ml aliquots of a standard drug vehicle (Th/propylene glycol, PG) were applied (120 total h). Samples of the receptor phases (3 ml) from this second application were removed at 1, 2, 3, and 4 h, and the amounts of theophylline in the receptor phases were quantitated by UV spectrophotometry as above. Each time a sample was removed, the entire receptor phase was replaced with fresh receptor fluid.

In all cases, the rates of delivery of total APAP species (J_{MIPM}) or theophylline (J_{JIPM}) through skin were determined by plotting the cumulative amount (μmol) of total APAP species or Th measured in the receptor phase against time and dividing the steady-state portions of those plots by the surface area of the diffusion cells.

Solubility Parameters

The solubility parameters were obtained using the method of Fedors (12) as illustrated by Martin *et al.* (13) and Sloan *et al.* (10).

Statistical Analyses and Regression Analyses

Statistical analysis was accomplished using Student *t* test. Unless otherwise indicated, statistical significance is for $p < 0.05$. Multiple linear regression analysis was accomplished using the SPSS 7.5 statistical software package.

RESULTS AND DISCUSSION

Syntheses

The AOC derivatives of APAP were synthesized from the corresponding commercially available chloroformates using an organic base, usually pyridine, as an acid scavenger. For the hexyl-, **6**, 2-methoxyethyl-, **7**, and 2-methoxy-1-methylethyloxycarbonyl, **8**, derivatives, the chloroformates were generated *in situ* from triphosgene, pyridine, and the corresponding commercially available alcohols, then allowed to react with APAP as above. The yields of analytically pure derivatives, which were used in the diffusion cell experiments and in the solubility determinations, were in the range of 42–82% except for **8,** which was only 29%. Five of the derivatives had been previously reported, **2** to **6** (6,14), and the uncorrected melting points reported here were essentially identical with those previously reported (Table I). For the two new derivatives, **7** and **8**, elemental analyses were ±0.40% (Table II). The UV and ^1H NMR spectra were consistent with 4-AOC-APAP structures. When ^1H NMR spectra were run in DMSO-d_6, the upfield doublet in the aromatic region was shifted farther downfield than was the downfield doublet for the derivatives compared to APAP, as expected if the 4-OH

group in APAP was acylated. The molar absorptivities of the derivatives (ε values in Table II) were also higher. The ^{1}H NMR spectra of the alkyl portions of the AOC derivatives were also consistent with the proposed structures where the O = C-OC***H***$_2$X absorption was shifted downfield compared to the HOC***H***$_2$X absorption in the corresponding alcohol by the electron withdrawing effect of the carbonyl group in the AOC. The spectra were also internally consistent; the values for ε and the chemical shifts were reasonably constant from derivative to derivative.

Solubilities

The solubilities in isopropyl myristate (S_{IPM}) and water (S_{AQ}) in Table I were all determined at room temperature using UV spectroscopy and ε values in acetonitrile (Table II). The relative SD of the S_{AQ} values were all less than ±5% except for **4** (±7.1%) and **6** (±9.4%), and those values of the previously (6) characterized derivatives (**2**, **3**, **5**, and **6**) were reasonably consistent with the previous S_{AQ} values (Table I), considering that the previous values were determined at 37°C and so are all higher: 40 ± 10% for **2**, **3,** and **5**, and 280% for **6**. There are not great differences among the melting points of the derivatives (Table I), and there are not great differences among the S_{IPM} values (Table I), which were all <±5%. However, the mp for **3** (C2) and **5** (C4) are each higher than the derivatives on either side, and **3** and **5** are each less soluble in IPM than **2** and **4** or **4** and **6**, respectively. The derivative **4** exhibiting the lowest mp for the straight chain derivatives **2** to **6** also exhibited the highest S_{IPM}. Similar results can be seen for the short series where an oxygen has been inserted in the alkyl chain, **7** and **8**; **7** exhibited the lowest mp and the highest S_{IPM}. The values for S_{AQ} for both series decreased as the number of carbons in the alkyl chain increased, although the S_{AQ} value for **3** appeared to decrease disproportionately fast compared to **2** and **4**.

Regardless of the irregular behavior of the absolute solubilities, the ratios of the solubilities in IPM and AQ ($SR_{IPM:AQ}$) were reasonably well behaved. The average methylene π_{SR} in Table III was 0.57 ± 0.06 for the first series and the only methylene π_{SR} for the second series was 0.54, which was well within the SD of the average for the first series. The SD for experimentally determined partition coefficients between IPM and pH 4.0 buffer ($K_{IPM:4.0}$, Table III) were all less then ±10%. The average methylene π_K values from $K_{IPM:4.0}$ were also reasonably well behaved for the first series (π_K = 0.55 ± 0.06) and are consistent with methylene π_K values determined for other series of prodrugs (9). However the only π_K value for the second series was not consistent with that for the first series (π_K = 0.44). All K values were determined by partitioning an IPM solution against pH 4.0 buffer to be consistent with previous determinations of K and to depress ionization in the aqueous phase.

In order to be consistent with data from the transformed Potts-Guy database, which consisted primarily of log S_{AQ} values estimated from log S_{IPM} − log $K_{IPM:4.0}$, estimated log S_{AQ} values were also calculated from the data in Tables I and III, respectively, and are given as log $S_{4.0}$ values in Table III. The estimated $S_{4.0}$ values were somewhat higher than those for the directly measured values for **3**, **4**, and **5** (12 ± 7%) and somewhat lower for **2**, **6**, **7,** and **8** (29 ± 9%). For both series (**2** to **8**), the variation was 21 ± 12%.

Table III. Log Solubility Ratios Between Isopropyl Myristate (IPM) and Water (log $SR_{IPM:AQ}$), the Differences Between Log $SR_{IPM:AQ}$ (π_{SR}), the Log of Partition Coefficients Between IPM and pH 4.0 Buffer (log $K_{IPM:4.0}$), and the Differences Between Log $K_{IPM:4.0}$ (π_K) and Calculated Solubility Parameters (δ_i)

	log[a] $SR_{IPM:AQ}$	π_{SR}[b]	log[c] $K_{IPM:4.0}$	π_K[d]	δ_i[e]
2	–0.24		–0.16		12.09
3	0.39	0.63	0.32	0.48	11.80
4	0.94	0.55	0.90	0.58	11.55
5	1.52	0.58	1.50	0.60	11.35
6	2.54	0.51	2.71	0.60	10.99
7	–0.52		–0.30		11.62
8	0.01	0.53	0.13	0.43	11.31

[a] Log of the ratio of the solubilities in IPM (S_{IPM}) and water (S_{AQ}). [b] $\pi = (\log SR_{n+m} - \log SR_n)/m$, where n is the number of methylene units in the promoiety of one prodrug and m is the number of additional methylene units in the promoiety with which it is compared. [c] Log of the partition coefficient between IPM and pH 4.0 buffer. [d] The same definition as *b* above except that K is substituted for SR. [e] Calculated according to Ref. 12. Units of $(\text{cal cm}^{-3})^{1/2}$.

Analyses and Stability

The 4-AOC-APAP derivatives were stable in the IPM donor phases. When the residues from each of the donor phases was filtered from the IPM and analyzed by ^{1}H NMR spectroscopy, only intact prodrug was observed based on the absence of the aromatic C–H absorptions due to APAP and the correct ratios between C***H***$_3$(C=O)NH and O(C=O)OC***H***$_2$X absorptions. The 4-AOC-APAP derivatives should also be stable in the receptor phase. Previously determined $t_{1/2}$ values at pH 7.4 and 37°C were 150, 200, and 300 h for **2**, **3,** and **5**, respectively (6). On the other hand, the $t_{1/2}$ values for the same derivatives in pH 7.4 buffer 2% human plasma (V/V) were 180, 45, and 15 min (6), respectively, so the derivatives should hydrolyze during permeation of the skin to some degree.

Diffusion Cell Experiments

The values for the delivery of total APAP containing species from IPM suspensions of the 4-AOC-APAP derivatives (J_{MIPM}) are given in Table IV. All of the J_{MIPM} values were well within the expected ±30% variation in J values seen for *in vitro* hairless mouse skin diffusion cell experiments (16.6 ± 6.7%) except for APAP itself, which was ±37%. Only two of the derivatives performed better than APAP itself and then only marginally: **2** (1.7×) in the first series and **7** (1.4×) in the second series. Although all of the derivatives were more soluble in IPM than APAP, as seen for previous series of derivatives the derivative that gave the highest rate of delivery was not the most lipid-soluble member. Instead it was that member of each more lipid soluble series that also exhibited the higher water solubility that gave the highest rate of delivery (15–17). Compared to increases in S_{IPM} seen for the best derivatives of drugs containing polar heterocyclic NH groups (>100×), the S_{IPM} values measured for the 4-AOC derivatives were only 8× and 5× for the best performing member of each series (**2** and **7**). On the other hand, the increases in S_{AQ} values seen for the 4-AOC derivatives were greater than those measured for most of the best derivatives of drugs containing heterocyclic NH groups previously studied (<2×):

Table IV. Log Values for Experimental Fluxes of Total 4-Hydroxyacetanilide (APAP) Species from Suspensions of **1** to **8** in Isopropyl Myristate (IPM) Through Hairless Mouse Skin (log J_{MIPM}), Error in Predicting Log J_{MIPM} from Transformed Potts-Guy Equation (n = 43) (Δ log J'_{MIPM}), Error in Calculating Log J_{MIPM} from transformed Potts-Guy Equation (n = 61) (Δ log J_{MIPM}), Values for the Fluxes of Theophylline from PG as a Second Application (J_{JIPM}), and Log Permeability Coefficients (log P_{MIPM})

Compound	log J_{MIPM}[a]	Δ log J'_{MIPM}[b]	Δ log J_{MIPM}[c]	J_{JIPM}[a]	log J_{MIPM}[d]
1, APAP	–0.29	0.11	0.18	0.74	–0.57
2	–0.00	0.18	0.12	1.12	–1.08
3	–0.76	0.56	0.51	0.64	–1.73
4	–0.45	0.33	0.28	1.14	–1.82
5	–1.01	0.33	0.28	0.85	–2.15
6	–1.49	0.22	0.18	0.76	–2.71
7	–0.11	0.13	0.09	0.98	–1.12
8	–1.06	0.34	0.30	0.94	–1.59
Control[e]				1.02	
Control[f]				0.013	
Control[g]				0.015	

[a] Units of μmole cm^{-2} h^{-1}.

[b] Predicted log J_{MIPM} = –0.216 + 0.534 log S_{IPM} + 0.466 log $S_{4.0}$ – 0.00361 MW from Ref. 7 (n = 42, + C5 bis-6,9-ACOM-6-MP). Error in prediction was from experimental log J_{MIPM} – predicted log J_{MIPM}.

[c] Calculated log J_{MIPM} = –0.322 + 0.53 log S_{IPM} + 0.47 log $S_{4.0}$ – 0.00337 MW from Ref. 7 (n = 42, + C5 bis-6,9-ACOM-6-MP), Ref. 9 (Table V, n = 4), Ref. 19 (Table V, n = 6), and current data (n = 8) to give n = 61. Error in calculation was from experimental log J_{MIPM} – calculated log J_{MIPM}.

[d] Values calculated from log J_{MIPM} – log S_{IPM} = log P_{MIPM}, units of cm h^{-1}.

[e] 48 hours conditioning, 48 hours IPM treatment, methanol wash, 24 hours leaching (Ref. 10).

[f] 48 hours conditioning, 48 hours no treatment, methanol wash, 24 hours leaching (Ref. 21).

[g] 48 hours conditioning, 48 hours Th/PG treatment, methanol wash, 24 hours leaching (ref. 21).

7× for **2** and 15× for **7**. Thus, the limited increases in delivery of total APAP containing species seems to have been caused by the lower than expected S_{IPM} values. As mentioned above, **3** and **5** exhibited higher mp and lower S_{IPM} values than **2** and **4** or **4** and **6**, respectively. Derivative **3** also exhibited a relatively lower S_{AQ} value than expected. Thus it is not surprising, based on analogy to previously studied series (15–17), that **3** and to some extent **5** performed much worse (lower J_{MIPM} values) than the contiguous members of the series **2**, **4,** and **6**.

The percent of intact prodrug found in the receptor phase was in direct proportion to the flux values obtained from the steady-state region: 25–34 h. The prodrug giving the highest rate of delivery of total APAP-containing species delivered the greatest percentage of intact prodrug in each series using the data for the 31 h sample, which was representative of values obtained at the other times when samples were analyzed. For the first series, the percentages of intact C1 to C6 in the receptor phase were 64, 14, 25, 0, and 0%, respectively. The fact that the C2 derivative (**3**) gave a lower J_{MIPM} value than the C1 or C3 (**2** or **4**) derivatives was reflected in the lower amounts of intact prodrug measured in the receptor phase. Similarly, for the second series the percentages of intact prodrug were 51% and 0% for **7** and **8**, respectively. This trend in both series is probably the result of the flux being too great for the hydrolytic enzyme system to hydrolyze the prodrugs completely. A similar result was observed for the alkyloxycarbonyl derivatives of 5-fluorouracil where up to 90% of intact prodrug was obtained in the receptor phases at steady-state for the most effective prodrug (18).

The differences in the J_{MIPM} values for **1** to **8** were not dependent on different effects of the prodrugs and APAP on the permeability of the skins. The fluxes of suspensions of theophylline from propylene glycol (Th/PG) applied subsequent to the application of the prodrugs and APAP in IPM (J_{JIPM}, Table IV) were not significantly different from the flux of Th/PG applied after IPM itself: J_{JIPM} = 0.89 ± 0.18 vs. 1.02 ± 0.13, respectively. Thus, using the second application of a standard solute/vehicle (Th/PG) as a measure of changes in the resistance of the skin to permeation (10), there was no difference in the resistance of the skins to permeation caused by the application of **1** to **8** in IPM compared to application of IPM alone. Also, normalization of the J_{MIPM} values by the respective J_{JIPM} values did not change the rank order of the performances of **1** to **8** (data not shown).

When the J_{MIPM} values for **1** to **8** were normalized by dividing by their respective S_{IPM} values (log J_{MIPM} –log S_{IPM}) to give their corresponding permeability coefficients (log P_{MIPM}, Table IV), the P_{MIPM} values decreased as their respective $K_{IPM:4.0}$ values increased (Table III, Fig. 1) and their respective calculated solubility parameter values decreased (δ_i, Table III, Fig. 2) for the two series. A plot of log P_{MIPM} vs. δ_i values for the first series (**2** and **6**) gave a positive relationship (slope = 1.40, r = 0.98), which is characteristic of relationships between P_{MIPM} and δ_i previously seen for other series of more lipophilic prodrugs (19). However, in those previous examples, the more lipophilic prodrugs were of polar heterocyclic parent drugs derivatized on their amide- or imide-like NH functional groups and not of a more lipophilic phenolic parent drug. Thus, although there is no readily apparent trend in the decreasing J_{MIPM} values (and consequently decreasing P_{MIPM} values) with increasing S_{IPM} values because the S_{IPM} values are fairly flat, other measures for increasing lipophilicity, such as steadily increasing $K_{IPM:4.0}$ and decreasing δ_i with increasingly longer alkyl chain length,

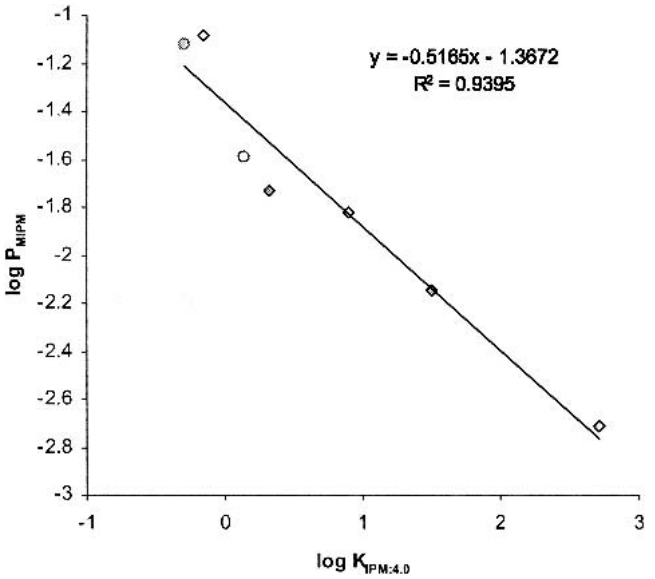

Fig. 1. Plot of log $K_{IPM:4.0}$ vs. log P_{MIPM} for the **2–6** (diamonds) and **7, 8** (circles) prodrugs of APAP.

Table V. Molecular Weights (MW), Log Solubilities in Isopropyl Myristate (IPM) (log S_{IPM}), Estimated Log Solubilities in pH 4.0 Buffer (log $S_{4.0}$), from Log S_{IPM} – Log Partition Coefficients Between IPM and pH 4.0 Buffer (log $K_{IPM:4.0}$), and Log Fluxes from Suspension in IPM Through Hairless Mouse Skin (log J_{MIPM}) for 3-Alkylcarbonyl-5-FU **(9–12)** (Ref. 9) and 3-Alkylcarbonyloxymethyl-5-FU **(13–18)** (Ref. 19)

Compound[a]	MW	log S_{IPM}[b]	log $S_{4.0}$[b,c]	log J_{MIPM}[d]
9, C1	172	0.63	2.02	0.64
10, C2	186	1.15	2.13	0.72
11, C3	200	1.34	1.36	0.34
12, C4	214	0.96	0.74	–0.26
13, C1	202	0.09	1.71	–0.22
14, C2	216	1.20	2.23	0.34
15, C3	230	1.42	1.87	0.46
16, C4	244	1.47	1.34	0.12
17, C5	258	1.63	0.89	0.00
18, C7	280	1.60	–0.29	–0.77

[a] C1, C2 . . . refer to the chain length of the alkyl group.
[b] Units of mM.
[c] Estimated from log S_{IPM} – log $K_{IPM:4.0}$.
[d] Units of μmole cm^{-2} h^{-1}.

identify the existence of such a trend. Increasing lipophilicity leads to lower J_{MIPM} and P_{MIPM}.

In order to quantify the relationship between J_{MIPM}, solubilities ($S_{4.0}$ and S_{IPM}) and molecular weight (MW), Roberts and Sloan (7) recently developed a transformation of the Potts-Guy equation that accommodated flux values from vehicles other than water and explicitly contained both lipid (S_{IPM}) and aqueous ($S_{4.0}$) parameters: log J_{MIPM} = x + y log S_{IPM} + (1 – y) log $S_{4.0}$ – z MW. The coefficients for x, y, and z had values of –0.211, 0.535, and 0.00364, respectively, for the fit of the original database minus the data for 6-mercaptopurine (n = 42) to the Roberts-Sloan equation. Data for one more member from one of the original series (bis-6,9-hexanoyloxymethyl-6-mercaptopurine) was subsequently added to the original database of seven series of prodrugs of theophylline, 5-fluorouracil, and 6-mercaptopurine to give n = 43 and slightly different coefficients for x, y, and z: –0.216, 0.534, and 0.00361, respectively, for the n = 43 coefficients. Using the latter coefficients and the values for log S_{IPM}, log $S_{4.0}$, and MW (Tables I and III), log J_{MIPM} values for the current series were predicted (data not shown), and the values for the error of prediction (experimental log J_{MIPM} – predicted log J_{MIPM} = Δ log J'_{MIPM}) were calculated (Table IV). The average Δ log J'_{MIPM} for the 4-AOC-APAP series was 0.27 ± 0.15 log units, which is quite a bit larger than the average Δ log J_{MIPM} value (see below) for any of the other series in the original database. In addition, all the derivatives performed worse than predicted using the n = 43 coefficients.

The current 4-AOC-APAP series was not the only series that uniformly performed worse than predicted using the n =

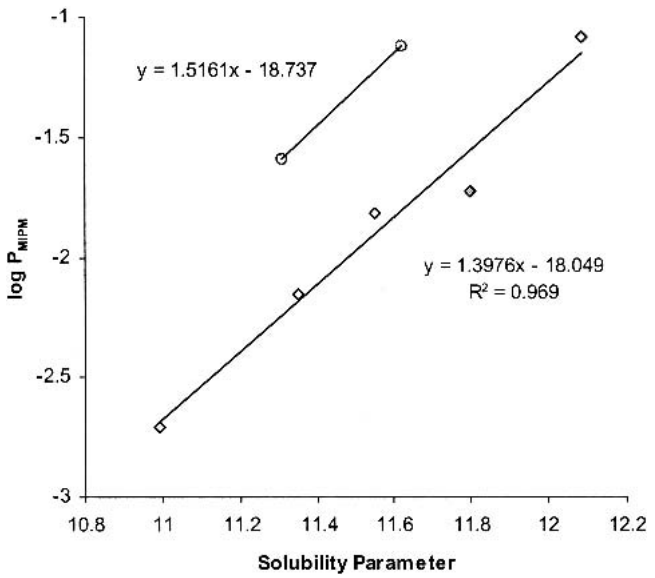

Fig. 2. Plot of solubility parameter vs. log P_{MIPM} for the **2–6** (diamonds) and **7, 8** (circles) prodrugs of APAP.

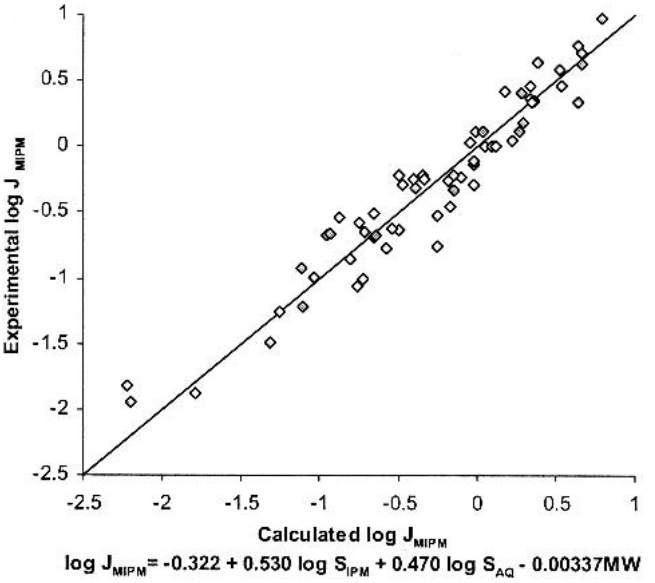

Fig. 3. Calculated vs. experimental flux through hairless mouse skin for the 61 compound date set.

43 coefficients; the 3-alkylcarbonyloxymethyl-5-fluorouracil (3-ACOM-5-FU) series did as well (19), where the average error of predicting log J_{MIPM} was $\Delta \log J'_{MIPM} = 0.19 \pm 0.09$ log units. In order to update the original database for the Roberts-Sloan equation and also to see what effect the inclusion of two new series, both of which underperformed, in the database would have on the calculated performance of the other series, the members of the 4-AOC-APAP series, the 3-ACOM-5-FU series, (19) and the 3-alkylcarbonyl-5-FU (9) series (Table V) were added to the original database to give $n = 61$, and new coefficients for the Roberts-Sloan equation were calculated. The new values for the x, y, and z coefficients were –0.322, 0.530, and 0.00337, respectively, for the $n = 61$ database (Fig. 3). The average error in calculating log J_{MIPM} (experimental log J_{MIPM} – calculated log $J_{MIPM} = \Delta$ log J_{MIPM}) was 0.15 ± 0.11 log units for $n = 61$, which was somewhat larger than the average Δ log J_{MIPM} for the $n = 43$ database: 0.13 ± 0.09 log units using the $n = 43$ coefficients. Although the average error in calculating log J_{MIPM} did not change more than 0.01 log units for most of the series, there were three series that did change substantially. The average Δ log J_{MIPM} for the 1-alkylcarbonyloxymethyl-5-FU series (1-ACOM-5-FU) worsened from 0.12 to 0.17 log units, whereas the average Δ log J_{MIPM} for the 3-ACOM-5-FU and the 4-AOC-APAP series improved from 0.19 to 0.15 and from 0.27 to 0.24 log units, respectively.

CONCLUSIONS

The delivery of the total acetaminophen (APAP) containing species (J_{MIPM}) by its alkyloxycarbonyl (AOC) derivatives from IPM, although reasonably predicted by the Roberts-Sloan equation, did not give substantially higher J_{MIPM} values than by APAP. Again, the more water soluble members of this more lipophilic series were the most effective at enhancing delivery of total APAP species through mouse skin from IPM (15–17). The addition of the 4-AOC-APAP series data and that of two other series to the database for the Roberts-Sloan equation and the subsequent fitting of all the data ($n = 61$) to the Roberts-Sloan equation resulted in the determination of new coefficients for the parameters that are not substantially different from the previous coefficients, but do account for the two underperforming series (3-ACOM-5-FU and 4-AOC-APAP) better. Although these results, which reinforce the importance of S_{AQ} on predicting flux from a lipid vehicle, are based on *in vitro* hairless mouse data, regression analysis of *in vivo* data for the delivery of nonsteroidal anti-inflammatory drugs from mineral oil through human skin also showed a significant and substantial dependence on S_{AQ} (0.28 vs. 0.47 for hairless mouse skin data) (20). Regardless of the difference in degree of hydration between *in vitro* and *in vivo*, and between hairless mouse skin and human skin, water solubility is an important parameter in predicting flux from lipid vehicles such as IPM and mineral oil.

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