Synthesis and *in Vitro* Transdermal Penetration Enhancing Activity of Lactam *N*-Acetic Acid Esters

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Abstract □ A homologous series of *N*-acetic acid esters of 2-pyrrolidinone and 2-piperidinone has been prepared and evaluated for its ability to enhance the skin content and flux of hydrocortisone 21-acetate in hairless mouse skin *in vitro*. Enhancement ratios (ER) were determined for flux (J), 24-hour diffusion cell receptor cell concentrations (Q_{24}), and 24-h full-thickness mouse skin steroid content (SC) and compared to control values (no enhancer present). In addition, in an attempt to abrogate toxicity, these dermal penetration enhancers were designed to have the potential for biodegradation by dermal esterases. 2-Oxopyrrolidine-α-acetic acid dodecyl ester (**5**) showed the highest enhancement ratios for J (ER 67.33) and Q_{24} (ER 180.66). 2-Oxopiperidine-α-acetic acid decyl ester (**10**) showed a high Q_{24} (ER 162.07) but a lower J (ER 12.67). 2-Oxopyrrolidine-α-acetic acid decyl ester (**3**) showed the highest enhancement ratio for SC (ER 8.7). The ER Q_{24} for **3**, **5** and **10**, as well as other lactam *N*-acetic acid esters in this work, were significantly higher than the ER found using Azone as enhancer.[†]

Penetration through the skin of topically administered agents designed to have systemic activity has been a goal of pharmaceutical scientists for many years. Numerous advantages to topical drug administration exist, including ease of access, improved patient compliance, absence of first-pass metabolism, the ability to continuously administer potent drugs or those with long half-lives, and the ability to deliver agents which are unsuitable for oral administration.[1]

The major barrier to drug penetration is presented by the uppermost layer of the skin, the stratum corneum. Many methods have been used to decrease skin resistance to the passage of drugs. These include the prodrug approach,[2] iontophoresis,[3] phonophoresis,[4] and dermal penetration enhancers.[5] This last approach is most convenient; however, these compounds must be highly effective, show no irritancy or toxicity, and be stable and pharmacologically inert.[6] Many groups of chemicals have shown enhancer activity, including pyrrolidinones,[7] urea and its derivatives,[8] many surfactants,[9] dimethyl sulfoxide and its derivatives,[10] and Azone[1] (laurocapram, 1-dodecylazacycloheptan-2-one). Azone has been the most widely studied chemical penetration enhancer of the 1980s although a considerable number of analogues with improved activity have been described.[12−18]

Several studies suggest that Azone acts by solubilizing or increasing the entropy of highly ordered stratum corneum lipids.[19] Skin conductance experiments suggest that Azone also increases skin water content.[20] This is supported by the fact that Azone is an effective enhancer for both hydrophilic and lipophilic drugs.[21]

Azone analogues with the potential for biodegradability have also been actively pursued.[22,23] In an attempt to acheive high flux-enhancing activity and potential biodegradibility, we have incorporated an ester moiety into an Azone-like structure.

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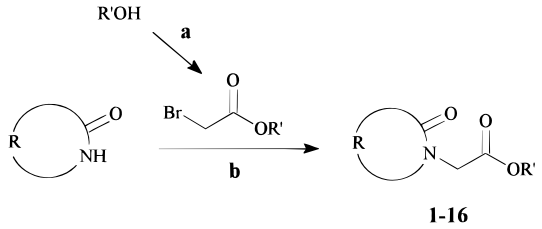

1-16

Scheme 1—(a) $BrCH_2COBr$, hexanes, 69 °C, 4 h. (b) (i) NaH, THF, room temperature, 4 h, (ii) THF, room temperature, 20 h.

Experimental Section

All chemicals were purchased from Aldrich Chemical Co. in the highest available purity, except hydrocortisone 21-acetate, polyoxyethylene 20 cetyl ether, and propylene glycol, which were obtained from Sigma Chemical Co. Baxter Diagnostics, Inc., supplied reagent grade solvents, except for methanol and acetonitrile, which were HPLC grade. Azone was synthesized as previously described.[24]

Thin layer chromatography (TLC) was performed with Merck precoated silica gel plates, type 60-F_{254}, and visualization was accomplished with iodine vapor. The melting points were determined on an Electrothermal apparatus and are uncorrected. Infrared spectra were recorded on a Beckman Acculab 4 spectrophotometer either neat or using the potassium bromide technique when the sample was a liquid or a solid at room temperature, respectively. ^{1}H-NMR spectra were obtained on a Brüker AM 300 NMR spectrometer. UV spectra were recorded on a Beckman DU-6 spectrophotometer. Elemental analyses were conducted by Atlantic Microlabs, Atlanta, GA, and were within ±0.4% of theoretical for all compounds. All compounds were purified by flash chromatography with 32−63 μm silica gel obtained from Selecto, Inc., Kennesaw, GA.

Male hairless mice strain SKHl (hr/hr), 8 weeks old, were obtained from Charles River Laboratories, Inc., Wilmington, MA.

Synthesis of Lactam *N*-Acetic Acid Esters—The 5- and 6-membered lactams (**1**−**14**) were prepared with acetate esters at the 1-position (Table 1). The bromoacetate esters were prepared by reaction of the appropriate alcohol with bromoacetyl bromide, and this crude bromoacetate ester was used to alkylate the sodium salt of the various lactams. This is a similar method to that used previously to prepare hexahydro-2-oxo-1*H*-azepine-1-acetic acid alkyl esters for evaluation as penetration enhancers[25]. Compounds **15** and **16**, azepine homologues of the compounds prepared in the present work, have been previously found to be essentially inactive as flux enhancers.[25] Empirical formulas, melting points (mp), and synthetic yields are listed in Table 2.

General Procedure for Synthesis of the Lactam *N*-Acetic Acid Esters—*2-Pyrrolidinone-1-acetic Acid Dodecyl Ester (**5**)*—Bromoacetyl bromide (21.19 g, 105 mmol) (**CAUTION**: lachrymator and strong alkylating agent)[26] was added to a solution of 1-dodecanol (19.56 g, 105 mmol) in hexanes (250 mL). The solution was refluxed for 4 h, after which time the solvent was removed *in vacuo* to yield crude dodecyl bromoacetate. 2-Pyrrolidinone (0.85 g, 10 mmol) was dissolved in Na-dried THF (100 mL) and NaH (0.24 g, 10 mmol) (**CAUTION**: highly caustic, pyrophoric)[26] was added. This mixture was stirred for 2 h under a drying tube, during which time a cloudy suspension formed. The crude dodecyl bromoacetate (3.07 g, 10 mmol) in Na-dried THF (50 mL) was added dropwise over 10 min and the mixture was stirred at room temperature for 20 h. The NaBr was filtered off and the solvent removed *in vacuo*. The crude product was

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Table 1—Ring Size and Alkyl Side Chain Length of Compounds 1–16

Compd	R	R′
1	$-(CH_2)_3-$	*n*-C_8H_{17}
2	$-(CH_2)_3-$	*n*-C_9H_{19}
3	$-(CH_2)_3-$	*n*-$C_{10}H_{21}$
4	$-(CH_2)_3-$	*n*-$C_{11}H_{23}$
5	$-(CH_2)_3-$	*n*-$C_{12}H_{25}$
6	$-(CH_2)_3-$	*n*-$C_{13}H_{27}$
7	$-(CH_2)_3-$	*n*-$C_{14}H_{29}$
8	$-(CH_2)_4-$	*n*-C_8H_{17}
9	$-(CH_2)_4-$	*n*-C_9H_{19}
10	$-(CH_2)_4-$	*n*-$C_{10}H_{21}$
11	$-(CH_2)_4-$	*n*-$C_{11}H_{23}$
12	$-(CH_2)_4-$	*n*-$C_{12}H_{25}$
13	$-(CH_2)_4-$	*n*-$C_{13}H_{27}$
14	$-(CH_2)_4-$	*n*-$C_{14}H_{29}$
15[a]	$-(CH_2)_5-$	*n*-$C_{12}H_{25}$
16[a]	$-(CH_2)_5-$	*n*-$C_{14}H_{29}$

[a] See ref 25.

Table 2—Melting Point (if applicable), Yield, and Empirical Formulas of Compounds 1–14

Compd	Mp (°C)	Yield (%)	Formula[a]
Azone		52	$C_{18}H_{35}NO$
1		24	$C_{14}H_{25}NO_3$
2		21	$C_{15}H_{27}NO_3$
3		45	$C_{16}H_{29}NO_3$
4		35	$C_{17}H_{31}NO_3$
5		51	$C_{18}H_{33}NO_3$
6		31	$C_{19}H_{35}NO_3$
7	42.5–43.5	47	$C_{20}H_{37}NO_3$
8		50	$C_{15}H_{27}NO_3 \bullet {}^1/_4H_2O$
9		52	$C_{16}H_{29}NO_3 \bullet {}^1/_4H_2O$
10		44	$C_{17}H_{31}NO_3$
11		26	$C_{18}H_{33}NO_3 \bullet {}^1/_4H_2O$
12	28–29	47	$C_{19}H_{35}NO_3$
13	39–40	33	$C_{20}H_{37}NO_3$
14	38–39	37	$C_{21}H_{39}NO_3$

[a] All compounds were analyzed for C, H, and N and the results agreed to ±0.4% of the theoretical values.

purified via flash chromatography with a hexanes/ethyl acetate (2:1) to (1:2) eluotropic series to yield a clear oil (**5**) (1.58 g, 51%): TLC (hexanes/ethyl acetate, 2:1) $R_f = 0.28$; IR (neat) ν C=O 1720, 1622 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.83 (t, 3H, terminal CH_3), 1.21 (m, 18H, $(CH_2)_9$), 1.58 (m, 2H, CH_2 β to O), 2.05 (m, 2H, 4-CH_2), 2.32 (t, 2H, 3-CH_2), 3.44 (t, 2H, 5-CH_2), 4.00 (s, 2H, acetyl CH_2), 4.07 (t, 2H, CH_2 α to O) ppm. Anal. ($C_{18}H_{33}NO_3$) Calcd: C, 69.41; H, 10.68; N, 4.50. Found: C, 69.31; H, 10.65; N, 4.53.

Permeability Experiments—*In Vitro Diffusion Cell Experiments*—Hydrocortisone 21-acetate was chosen as the penetrant (model drug). Full-thickness hairless mouse skins were placed in unoccluded, modified Franz diffusion cells. The diffusional area was 3.14 cm^2. The surface of the skin was maintained at a constant temperature of 32 ± 0.5 °C. The receptor phase (volume 12 mL) was continuously stirred at 600 rpm and consisted of isotonic phosphate buffer (pH 7.4) with 0.1% v/v aqueous formaldehyde as preservative.[27] The aqueous solubility of hydrocortisone 21-acetate is 0.28 mg/mL.[28] In order to maintain sink conditions polyoxyethylene 20 cetyl ether was added to the receptor phase as a solubilizer.[29,30] Dorsal mouse skin samples were placed in diffusion cells and were allowed to equilibrate for 1.5 h. At this time the enhancer solution (0.4 M) in 5 μL propylene glycol was spread on the diffusional area. Although solid enhancers are normally applied at their saturation solubilities if they are under 0.4 M, all of the solid enhancers in the present work (**12**−**14**) were soluble at 0.4 M and 32 ± 0.5 °C. One hour after enhancer pretreatment, hydrocortisone 21-acetate (0.03 M) suspended in 500 μL of propylene glycol was spread on the skins. Samples were withdrawn from the receptor phase over 24 h. The receptor volume was replenished with 100 μL of phosphate buffer after each sample. Analysis of each sample was corrected for each previous sample which had been removed. Each experiment was repeated five or more times.

Table 3—Initial Flux (J), 24-h Receptor Cell Concentration (Q_{24}), and Skin Content (SC) of Hydrocortisone and Hydrocortisone 21-Acetate (HC + HCA) for Compounds 1–16

Compd ($n = 5$)	J (μM/cm^2 h)	Q_{24} (μM)	SC (μg/g)
Control ($n = 8$)	0.045 ± 0.016	0.75 ± 0.25	285.2 ± 21.6
Azone	0.88 ± 0.25	28.76 ± 4.62	420.5 ± 36.9
1	0.85 ± 0.27	14.88 ± 1.89	1054.3 ± 40.9
2	0.79 ± 0.19	16.10 ± 1.22	1008.3 ± 312.5
3	1.72 ± 0.22	54.20 ± 18.26	2471.5 ± 816.2
4	0.69 ± 0.15	23.49 ± 12.11	1351.5 ± 100.2
5	3.03 ± 0.84	135.50 ± 20.52	474.9 ± 108.4
6	0.81 ± 0.30	26.21 ± 2.53	463.1 ± 110.0
7	1.16 ± 0.21	25.22 ± 3.46	673.1 ± 100.0
8	0.83 ± 0.20	16.93 ± 4.91	331.5 ± 125.5
9	0.60 ± 0.11	44.66 ± 8.61	560.2 ± 163.2
10	0.57 ± 0.22	121.55 ± 30.99	212.9 ± 90.9
11	1.64 ± 0.15	30.72 ± 4.69	331.3 ± 112.1
12	1.07 ± 0.03	29.35 ± 5.11	1277.7 ± 454.9
13	0.54 ± 0.15	42.73 ± 6.22	1098.3 ± 275.1
14	1.70 ± 0.19	64.11 ± 10.35	1755.0 ± 563.4
15	0.05 ± 0.02	2.91 ± 1.09	449.2 ± 39.8
16	0.09 ± 0.01	2.41 ± 1.05	2167.4 ± 85.9

Skin Retention Studies—After 24 h the skins were removed from the diffusion cell and immersed in methanol several times for a total of 5 s. The skins were then dried for 10 min, weighed, and cut up with scissors, and 4 mL of methanol was added. The skin/methanol was homogenized with a Kinematica, GmbH tissue homogenizer. The samples were then filtered to remove debris, centrifuged if turbid, and frozen at −80 °C until analysis. Recovery experiments showed that skin steroid content and total corrected receptor concentrations accounted for >95% of steroid administered.[31]

HPLC Analysis of Samples—Samples were analyzed by HPLC utilizing a Waters 6000A solvent delivery system Model U6K injector (injection volume 50 μL), a Waters differential UV detector Model ALC 202, an Alltech C_{18} Versapack reverse-phase column (4.1 mm X 30 cm; 10 μm) at ambient temperature with a Hewlett Packard integrator. Hydrocortisone and hydrocortisone 21-acetate were detected at 254 nm and were eluted using a acetonitrile/water (4:6) mobile phase at 1 mL/min.

Data Analysis—Penetration profiles for hydrocortisone were constructed by plotting the receptor cell concentration of steroid (μM) vs time (h). Profile analysis gave initial flux values, J (μM/cm^2 h), which were calculated from the slope of the linear region of the profile (Figure 1, Table 3). In each case the flux graph, after a short lag time, gave a linear portion consistent with steady-state Fickean diffusion. Sink conditions were assumed to exist during the experiments. Receptor cells contained only hydrocortisone (HC) whereas skin samples contained a mixture of hydrocortisone 21-acetate (HCA) and HC. Skin contents were calculated as micrograms of HCA + HC per gram of hydrated full-thickness skin.

The effectiveness of each compound was evaluated using enhancement ratios (ER), where ER = skin parameter from enhancer treated skin/skin parameter from control (Table 4). Skin parameters used were flux (J), 24 h receptor concentrations of steroid (Q_{24}), or total steroid skin content (SC). Statistical treatment of the data consisted of an analysis of variance (ANOVA) followed by a least significant difference test (LSD) if the ANOVA indicated that a difference existed. The level of significance (α) was selected to be 0.05.

Results and Discussion

Control experiments were run with only propylene glycol and penetrant with no enhancer present. Permeation profiles for controls, Azone, **5**, and **10** are presented in Figure 1. Control values were as follows: $J = 0.045 \pm 0.016$ μM/cm^2 h, $Q_{24} = 0.75 \pm 0.25$ μM, and SC = 285.2 ± 21.6 μg/g for total HC and HCA.

Azone produced a $J = 0.88 \pm 0.25$ μM/cm^2 h, $Q_{24} = 28.76 \pm 4.62$ μM, and SC = 420.5 ± 36.9 μg/g for total HC and HCA.

Table 4—Enhancement Ratios for Flux (ER_J), 24-h Receptor Cell Concentration ($ER_{Q_{24}}$), and Skin Content (ER_{SC}) of Hydrocortisone and Hydrocortisone 21-Acetate (HC + HCA) for Compounds 1–16

Compd	ER_J	$ER_{Q_{24}}$	ER_{SC}
Control	1	1	1
Azone	19.55	38.35	1.5
1	18.89	19.84	3.7
2	17.55	21.47	3.5
3	38.22	72.27	8.7
4	15.33	31.32	4.7
5	67.33	180.66	1.7
6	18.00	34.94	1.6
7	25.78	33.63	2.4
8	18.44	22.57	1.2
9	13.33	59.55	2.0
10	12.67	162.07	0.8
11	36.44	41.02	1.2
12	23.78	39.13	4.5
13	12.00	56.97	3.9
14	37.77	85.48	6.2
15	1.13	3.87	1.6
16	1.96	3.26	7.6

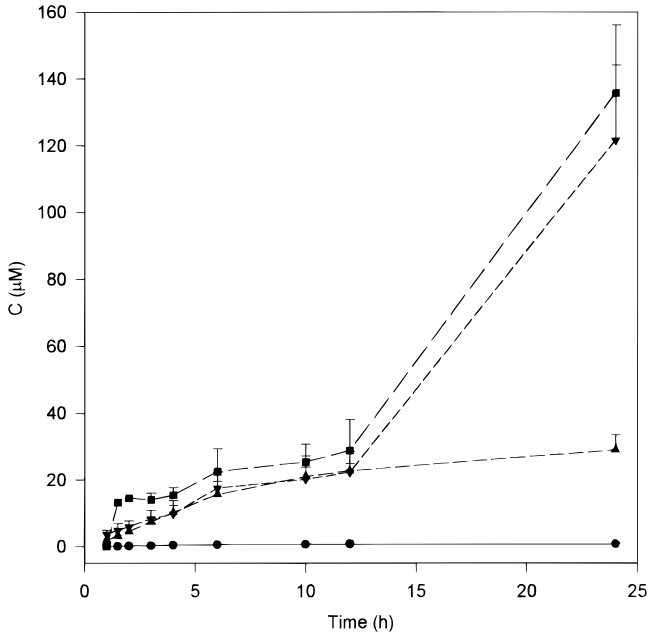

Figure 1—Receptor phase steroid concentration (*C*) for control (●), and with Azone (▲), **5** (■) and **10** (▼) as permeation enhancer.

The receptor phase contained only HC, whereas skin samples contained primarily HCA with some HC.

The lactam acetic acid esters (**1**−**16**) were developed as novel enhancers which were not previously reported to show transdermal or topical penetration enhancing activities. Initially, the acetic acid dodecyl and tetradecyl esters of ϵ-caprolactam[25] (**15**, **16**), 2-piperidinone (**12**, **14**), and 2-pyrrolidinone (**5**, **7**) were prepared.

With regard to the skin content enhancement of these compounds a clear trend is noted. When the side chain consists of an acetic acid tetradecyl ester, larger rings tend to result in increased topical delivery of HCA; ER_{SC} for **7** is 2.4, for **14**, 6.2 and for **16**, 7.6 ($p < 0.05$ compared with control). This was despite the fact that the saturation solubility for **16** was only 0.14 M, a concentration three times lower than that at which other lactam acetic acid esters were applied. Compound **3**, an exception to the above trend, gave the highest ER_{SC} of any enhancer in this work, 8.7 times over the control ($p < 0.05$). As such, **3** or **16** may be useful in enhancing the delivery of drugs which act locally within the skin, e.g. topical methotrexate or 5-fluorouracil treatment for psoriasis.

Another trend may be observed from consideration of $ER_{Q_{24}}$ values for **5**, **7**, **12**, **14**−**16**. That is, at least in this series, smaller rings tend to cause transdermal enhancement to a greater degree. For instance, when the side chain is an acetic acid dodecyl ester (**5**), this results in an $ER_{Q_{24}}$ of 180.66; **12** gives an $ER_{Q_{24}}$ of 39.13 and **15** gives an $ER_{Q_{24}}$ of only 3.87. Also, compounds which give high ER_{SC}'s give low $ER_{Q_{24}}$'s and vice versa. For instance, **5** gives the highest $ER_{Q_{24}}$, 180.66, but only a low ER_{SC}, 1.7. Conversely, **16** gives a high ER_{SC} of 7.6 but an $ER_{Q_{24}}$ of only 3.26. Compound **14** is something of an exception, as it has a high ER_{SC}, 6.2, and $ER_{Q_{24}}$, 85.48 ($p < 0.05$ compared with control).

Since we were most interested in the possibility of transdermal enhancement, we chose to prepare a homologous series around the 5- and 6-membered ring lactams in hopes of finding homologues with even higher $ER_{Q_{24}}$'s. To this end, **1**-**7** (2-oxopyrrolidine-α-acetic acid octyl to tetradecyl esters) and **8**-**14** (2-oxopiperidine-α-acetic acid octyl to tetradecyl esters) were synthesized.

The alkyl chain lengths for which $ER_{Q_{24}}$ was greatest were observed to be different for the 2-oxopyrrolidine-α-acetic acid ester group (**1**−**7**) and the 2-oxopiperidine-α-acetic acid ester group (**8**−**14**). Q_{24} maxima were reached with acetic acid decyl and tetradecyl ester moieties for 2-piperidinone (**10** and **14**); in contrast, the 2-pyrrolidinone nucleus exhibited Q_{24} maxima with acetic acid decyl and dodecyl ester substitutents (**3** and **5**).

It has been reported in the literature that even though some activity may be observed with enhancers with very short alkyl side chains or no side chain at all, the best enhancement occurred when the length was about C_8−C_{12}. For example, Sasaki *et al*. tested three pyrrolidinones, *N*-methyl-, *N*-hexyl-, and *N*-lauryl-2-pyrrolidinone (LP), with 5-fluorouracil, triamcinolone acetonide, indomethacin, and flurbiprofen *in vitro* using rat skin[7]. The *N*-hexyl derivative and LP were more effective at enhancing the permeation of the hydrophilic drugs, however LP was the most active of all three compounds tested. We have also found that LP was an effective enhancer using hairless mouse skin *in vitro* with hydrocortisone 21-acetate as a model drug.[24]

Barry and Bennett have reported relatively high activity using 2-pyrrolidinone and *N*-methyl-2-pyrrolidinone using mannitol, progesterone, and hydrocortisone *in vitro* in human skin.[33] However, it must be borne in mind that each study utilized a different animal model, vehicles, and experimental protocols which may have contributed to the variation in data. Our results with several pyrrolidinones, including 2-pyrrolidinone and *N*-methyl-2-pyrrolidinone, showed no activity in hairless mouse skin *in vitro* using HCA and HC in propylene glycol.[24,34]

Several efforts have been made to synthesize and investigate biodegradable enhancers, such as dodecyl 2-(*N*,*N*-dimethylamino)propionate and clofibric acid esters.[31,35] The degradation of the compounds would occur due to the presence of esterases in mouse and human skins.[32] This same rationale was utilized in the present study; however, no attempts were made to evaluate the extent of biodegradability of the enhancer tested.

The proposed mechanisms by which these compounds exert their action have been investigated by several authors using liposomal models, differential scanning calorimetry, small angle X-ray diffraction, FT-infrared spectroscopy and other techniques.[36−39] Barry has proposed that the mechanism of action may be explained using the lipid-protein-partitioning (LPP) theory.[40,41] This states that enhancers act by one of three primary means. Enhancers may alter the lipid domain

of the stratum corneum, may interact with the protein components, or may increase the partitioning of the model drug, the coadministered vehicles, or water into the skin. The alteration of the lipid domain occurs by fluidization of the stratum corneum lipids.[42−45]

Few investigators have studied the possible mechanism(s) of action of pyrrolidinones and piperidinones; however, Azone has been extensively studied. Recently, Bouwstra and Bodde reviewed literature flux studies and subsequent differential thermal analysis, small and wide angle X-ray diffraction, freeze−fracture electron microscopy (FFEM), freeze-substitution electron microscopy (FSEM), and nuclear magnetic resonance data and concluded that the mechanism of action of *N*-alkyl-Azones on human stratum corneum is more complex than previously thought.[46] After evaluating 0.15 M *N*-hexyl- through *N*-hexadecyl-Azones in propylene glycol, it was determined that changes in stratum corneum barrier properties depended on alkyl chain length. The influence on stratum corneum of *N*-hexyl-Azone in propylene glycol was similar to the effect produced by propylene glycol alone. However, when the stratum corneum was pretreated with *N*-octyl-Azone, small-angle X-ray diffraction studies revealed lipid fluidization. Both FFEM and FSEM confirmed these findings and showed that the fluidization was occurring to a greater extent in the center of the intercellular space rather than close to the cell boundaries. ^{1}H-NMR experiments also revealed that *N*-dodecyl-Azone induced a "liquid microenvironment" within the stratum corneum.

These findings may be different in other animal skins such as the hairless mouse and will depend on the vehicle and the model drug used. The effect of model drug changes and skin type is well-illustrated by Hadgraft *et al*.[47] Schuckler and Lee in a recent study found that the extent on the *N*-dodecyl-Azone accumulation in human stratum corneum could be correlated with its effect on the diffusion coefficient of diazepam. This coefficient was highest when the skin content of the enhancer was 12% w/w. This high quantity was surprising and implied that on a molar basis there was more Azone present in the stratum corneum than lipid.[48]

Studies on model systems such as skin lipids and phospholipids have shown that *N*-dodecyl-Azone has an effect on the hydrocarbon chains inside the bilayer structures.[49,50] However, when lamellar phases were prepared from brain ceramides, free fatty acids, and cholesterol or from hydrated phosphatidylcholine, no fluidization of lipids was observed.[51,52]

It has been shown in the literature that there is a parabolic effect of alkyl chain lengths of enhancers, with C_{10}−C_{12} being the most active.[53] Pyrrolidinone enhancers have also been shown to have higher activity with a *N*-dodecyl side chain, compared to *N*-methyl or *N*-hexyl.[7,54] It may be of interest to note that this chain length corresponds to the length of the steroid nucleus of cholesterol, suggesting that the mechanism of action of these agents involves the disruption of ceramide−cholesterol or cholesterol−cholesterol interactions.[55]

In this study the highest Q_{24} values were observed with **5**, the dodecyl ester in the pyrrolidinone series, and in the piperidinone series, the decyl ester (**10**) produced the highest Q_{24}. Our data are in agreement with literature predictions for effective enhancer alkyl chain lengths. Considering ring size, activity was higher with 2-oxopyrrolidine-α-acetic acid dodecyl ester (**5**) than with the homologous 2-oxopiperidine-α-acetic acid ester (**12**), which was better than **15** or Azone. This is supported by Mirejovsky and Takruri, who compared hexamethylene lauramide and its piperidine and pyrrolidinone analogs using an *in vitro* technique, hairless mouse skin, and 1% hydrocortisone. The enhancer solutions contained 5% enhancer, 20% propylene glycol adjusted to 100% with alcohol. Percent diffusion reported (mean ± SD) was 67 ± 6 for hexamethylene lauramide, 67 ± 7 for the piperidine analog, 83 ± 11 for the pyrrolidinone analog, and 64 ± 6 for Azone.[18] Taken together this indicates that enhancer length as well as overall lipophilicity would be necessary descriptors for predicting enhancer activity.

Molecular modeling studies have sugested a spoon shape for Azone.[56,57] The electrostatic fields around the ceramide head groups have both electronegative and electropositive regions on opposite sides of the molecules. The interaction between these two areas within the molecules binds the head groups together. Azone shows an electronegative site, at the carbonyl function, but no positive site and it has been proposed that intercalation of Azone into the ceramides will result in an unbalanced electronegative site on the latter. This may be the mechanism by which Azone exerts it permeation-enhancing effects.[55] This is supported by Williams, who compared series of Azone analogs with either two electronegative sites or both a positive and negative site. The former compounds were enhancers whereas the latter actually slowed drug permeation possibly due to the hypothesized interaction with the ceramides.[58] Some agents, however, increase skin permeation rates by other mechanisms or their combination, such as an influence on stratum corneum proteins, cosolvency, or an alteration of the thermodynamic activity of the model drug within the skin.[5]

In summary, the present study examined a homologous series of *N*-acetic acid esters of 2-pyrrolidinone and 2-piperidinone. The highest flux-enhancing activity was noted for 2-oxopyrrolidine-α-acetic acid dodecyl ester (**5**), which produced a 70-fold increase in flux of HCA over control values (J = 3.03 ± 0.84 μM/cm^2 h with **5**; J = 0.045 ± 0.016 μM/cm^2 h with control); Q_{24} values were also high, 135.50 ± 20.52 μM for **5**, and 0.75 ± 0.25 μM for control ($p < 0.05$). Azone gave values of 0.88 ± 0.25 μM/cm^2 h for flux and 28.76 ± 4.62 μM for Q_{24} ($p < 0.05$). Skin content of steroid for **5** was similar to that of Azone (474.9 ± 108.4 μg/g for **5**; 420.5 ± 36.9 μg/g for Azone). The highest skin steroid content was observed for 2-oxopyrrolidine-α-acetic acid decyl ester (**3**): 2471.5 ± 816.2 μg/g ($p < 0.05$).

The highest Q_{24} for the 2-oxopiperidinone-α-acetic acid esters was observed for the decyl ester (**10**), 121.55 ± 30.99 μM, while the highest skin content was recorded for the tetradecyl ester (**14**), 1755.0 ± 563.4 μg/g ($p < 0.05$). As a number of these compounds are significantly more active than Azone, they have potential for further development as effective dermal penetration enhancers.

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