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In vitro evaluation of a series of Azone analogs as dermal penetration enhancers: III. Acyclic amides

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Abstract

A series of acyclic amides was synthesized and tested for enhancement properties using excised hairless mouse skin and hydrocortisone 21-acetate as the model drug. All compounds were applied at 0.4 M (or at their respective saturation solubilities) in propylene glycol. Azone (0.4 M) was used as a standard enhancer. Enhancement ratios were calculated for flux, 24 h diffusion cell receptor concentrations (Q_{24}) and 24 h full-thickness mouse skin steroid content. Enhancer **5** showed the highest activity for flux (35.22-fold over control), 24 h receptor concentration (79.86-fold over control) and skin drug content (4.3-fold over control). These enhancement ratios were higher than those for Azone which were 19.51, 38.30 and 1.5-fold over control, respectively. Enhancers **4**, **10** and **11** showed similar Q_{24} values to Azone, and **3**, **9** and **10** increased skin steroid content to a greater extent than Azone.

Key words: Percutaneous absorption; Azone analog; Amide; Hydrocortisone 21-acetate; Hairless mouse; Flux; Skin retention

1. Introduction

The literature contains many references to structure-activity relationships of dermal penetration enhancers. For example, the effectiveness of saturated and unsaturated fatty acids and alcohols is related parabolically to alkyl chain length, with C_{10}–C_{12} being the most effective (Cooper, 1984; Aungst et al., 1986). Likewise, in a series of clofibric acid amides, the C_{10} compound was the most active (Michniak et al., 1993). Cholesterol, a component of the stratum corneum, has been reported to modify the fluidity of the phospholipids within the membrane, hence affecting its permeability (Presti, 1985; Finean, 1990). It should be noted that the length of the cholesterol steroid nucleus is practically equal to that of a C_{10}–C_{12} alkyl chain (Brain and Walters, 1993). It has therefore been suggested that a possible mode of action of these enhancers is disruption of ceramide-cholesterol or cholesterol-cholesterol interactions within the lipids of the stratum corneum. The degree of observed enhancement

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was also related to enhancer interaction with the polar head group region of the membrane lipids which was said to reversibly increase the fluidity of stratum corneum lipids (Barry, 1988; Bouwstra, 1992). Bodde (1989) and Lewis and Hadgraft (1990) suggested that enhancer activity is related to a 'spoon-shaped' conformation which has been observed for the enhancer Azone (*N*-dodecylazacycloheptan-2-one, laurocapram).

It is possible that, in addition to ring containing structures, acyclic (open ring) structures are also effective enhancers. Dodecyl-, decyl-, and octyl-*N*,*N*-dimethylaminoacetates have been shown to produce relative enhancements of 2.0, 3.8 and 2.5 (compared to Azone) using shed snake skin with indomethacin as the model drug (Wong et al., 1989). Acyclic amide containing enhancers were also prepared by Peck and Minaskanian (1989) and Minaskanian and Peck (1989). Our previous work has concerned the synthesis and evaluation of several series of novel dermal penetration enhancers all containing heterocyclic rings (five-, six- or seven-membered) (Michniak et al., 1993a–d). Most compounds showed activity in vitro and several increased transdermal delivery and/or drug skin retention to a greater degree than Azone. The present work presents in vitro hairless mouse skin permeation parameters and skin contents of hydrocortisone 21-acetate using 14 acyclic amides as enhancers.

2. Materials and methods

2.1. Materials

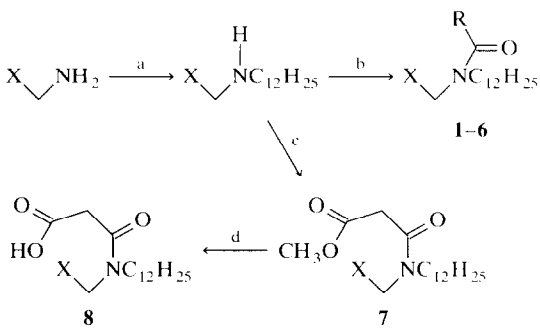
All chemicals were purchased from Aldrich Chemical Co. in the highest available purity, except hydrocortisone 21-acetate, hydrocortisone, polyoxyethylene 20 cetyl ether and propylene glycol which were obtained from Sigma Chemical Co. Baxter Diagnostics, Inc. supplied reagent grade solvents, except for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

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

2.2. Methods

2.1.2. Enhancer compounds (synthesis and spectroscopic data)

The reaction conditions for compounds **1–14** are outlined in Schemes 1–3. The melting points were determined on an Electrothermal apparatus and are uncorrected. The ^{1}H-NMR spectra were obtained on a Brüker AM 300 NMR spectrometer in $CDCl_3$ solution. Spectroscopic data agreed with assigned structures in all cases. Elemental analyses were conducted by Atlantic Microlabs, Atlanta, GA, and were within $\pm 0.4\%$ of theoretical for all compounds. All compounds were purified by MPLC with 32–63 μm 'flash' silica gel obtained from Selecto, Inc., Kennesaw, GA.



Compound	R	X
1	$CH(CH_3)_2$	CH_2OH
2	$CH(CH_3)_2$	CH_2OCH_3
3	$CH(CH_3)_2$	$CH(OCH_3)_2$
4	CH_3	CH_2OH
5	CH_3	CH_2OCH_3
6	CH_3	$CH(OCH_3)_2$
7	–	CH_2OH
8	–	CH_2OH

Scheme 1. (a) Dodecanal, Pd/C, ethanol, H_2 (50 lb/inch^2), 1 h. (b) $(RCO)_2O$ or RCOCl, dry tetrahydrofuran, reflux, 12 h. (c) Dimethyl malonate, dry methanol, reflux, 5 days. (d) Sodium hydroxide, methanol, 1 h.

N-Dodecyl-*N*-(2-hydroxyethyl)isobutyramide (**1**) was obtained as an oil in 40% yield; ^{1}H-NMR: δ 0.86 (t, 3H, terminal CH_3), 1.10 (d, 6H, CH_3 β to C = O), 1.24 (m, 18H, $(CH_2)_9$), 1.54 (m, 2H, CH_2 β to N), 2.75 (m, 1H, CH α to C = O), 3.26 (t, 2H, CH_2 α to N), 3.46 (t, 2H, CH_2 α to N and β to O), 3.47 (m, 2H, CH_2 β to N and α to O), 3.85 (br s, 1H, OH).

Anal. $C_{18}H_{37}NO_2 \cdot 1/4\ H_2O$ requires C, 71.12; H, 12.43; N, 4.61. Found: C, 71.41; H, 12.52; N, 4.65.

N-Dodecyl-*N*-(2-methoxyethyl)isobutyramide (**2**) was obtained as an oil in 27% yield; ^{1}H-NMR: δ 0.86 (t, 3H, terminal CH_3), 1.09 (d, 6H, CH_3 β to C = O), 1.24 (m, 18H, $(CH_2)_9$), 1.50 (m, 2H, CH_2 β to N), 2.76 (m, 1H, CH α to C = O), 3.29 (m, 4H, CH_2 α to N and CH_2 α to N and β to O), 3.47 (m, 5H, CH_2 β to N and α to O and OCH_3).

Anal. $C_{19}H_{39}NO_2$ requires C, 72.79; H, 12.54; N, 4.47. Found: C, 72.80; H, 12.50; N, 4.43.

N-Dodecyl-*N*-(2,2-dimethoxyethyl)isobutyramide (**3**) was obtained as an oil in 63% yield; ^{1}H-NMR: δ 0.86 (t, 3H, terminal CH_3), 1.07 (d, 6H, CH_3 β to C = O), 1.24 (m, 18H, $(CH_2)_9$), 1.55 (m, 2H, CH_2 β to N), 2.73 (m, 1H, CH α to C = O), 3.29 (m, 2H, CH_2 α to N), 3.34 (m, 2H, CH_2 α to N and β to O), 3.39 (s, 6H, $(OCH_3)_2$), 3.49 (t, 1H, CH α to O and β to N).

Anal. $C_{20}H_{41}NO_3$ requires C, 69.92; H, 12.03; N, 4.08. Found: C, 69.74; H, 11.95; N, 4.10.

N-Dodecyl-*N*-(2-hydroxyethyl)acetamide (**4**) was obtained as white crystals in 7% yield; ^{1}H-NMR: δ 0.86 (t, 3H, terminal CH_3), 1.24 (m, 18H, $(CH_2)_9$), 1.55 (m, 2H, CH_2 β to N), 2.11 (s, 3H, acetyl CH_3), 3.25 (t, 2H, CH_2 α to N), 3.50 (t, 2H, CH_2 α to N and β to O), 3.74 (m, 2H, CH_2 α to O and β to N).

Anal. $C_{16}H_{33}NO_2$ requires C, 70.80; H, 12.25; N, 5.16. Found: C, 70.71; H, 12.21; N, 5.12.

N-Dodecyl-*N*-(2-methoxyethyl)acetamide (**5**) was obtained as white crystals in 8% yield; ^{1}H-NMR: δ 0.85 (t, 3H, terminal CH_3), 1.23 (m, 18H, $(CH_2)_9$), 1.50 (m, 2H, CH_2 β to N), 2.07 (s, 3H, acetyl CH_3), 3.30 (m, 4H, CH_2 α to N), 3.47 (m, 5H, CH_2 β to N and α to O and OCH_3).

Anal. $C_{17}H_{35}NO_2$ requires C, 71.53; H, 12.36; N, 4.91. Found: C, 71.57; H, 12.32; N, 4.89.

N-Dodecyl-*N*-(2,2-dimethoxyethyl)acetamide (**6**) was obtained as an oil in 55% yield; ^{1}H-NMR: δ 0.86 (t, 3H, terminal CH_3), 1.21 (m, 18H, $(CH_2)_9$), 1.51 (m, 2H, CH_2 β to N), 2.08 (s, 3H, acetyl CH_3), 3.28 (m, 2H, CH_2 α to N), 3.33 (m, 2H, CH_2 α to N and β to O), 3.38 (s, 6H, $(OCH_3)_2$), 4.49 (t, 1H, CH α to O and β to N).

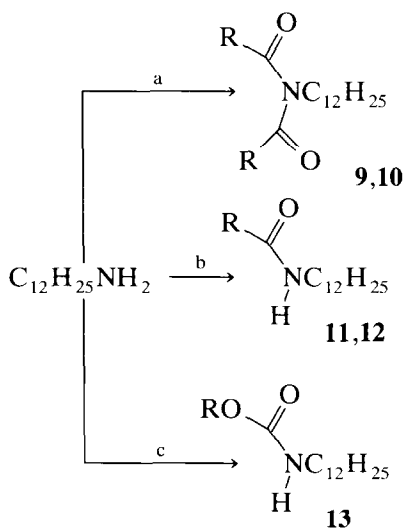
Anal. $C_{18}H_{37}NO_3$ requires C, 68.53; H, 11.82; N, 4.44. Found: C, 68.39; H, 11.87; N, 4.43.

N-Dodecyl-*N*-(2-hydroxyethyl)-2-carboxyacetamide methyl ester (**7**) was obtained as an oil in 29% yield; ^{1}H-NMR: δ 0.86 (t, 3H, terminal CH_3), 1.24 (m, 18H, $(CH_2)_9$), 1.60 (m, 2H, CH_2 β to N), 3.05 (br s, 1H, OH), 3.30 (center of AB pattern, 2H, CH_2 α to N), 3.42 (m, 2H, malonyl CH_2), 3.51 (m, 2H, CH_2 α to N and β to O), 3.78 (m, 5H, CH_2 α to O and CH_3O).

Anal. $C_{18}H_{35}NO_4$ requires C, 65.62; H, 10.71; N, 4.25. Found: C, 65.77; H, 10.76; N, 4.22.

N-Dodecyl-*N*-(2-hydroxyethyl)-2-carboxyacetamide (**8**) was obtained as white crystals in 84% yield; ^{1}H-NMR: δ 0.86 (t, 3H, terminal CH_3), 1.24 (m, 18H, $(CH_2)_9$), 1.54 (m, 2H, CH_2 β to N), 3.33 (center of AB pattern, 2H, CH_2 α to N), 3.40 (m, 2H, malonyl CH_2), 3.55 (m, 2H, CH_2 α to N and β to O), 3.79 (m, 2H, CH_2 α to O).

Anal. $C_{17}H_{33}NO_4$ requires C, 64.73; H, 10.54; N, 4.44. Found: C, 64.61; H, 10.59; N, 4.42.



Compound	R
9	$CH(CH_3)_2$
10	CH_3
11	$CH(CH_3)_2$
12	CH_3
13	CH_3

Scheme 2. (a) $(RCO)_2O$ or RCOCl, neat, reflux, 18 h. (b) $(RCO)_2O$ or RCOCl, acetonitrile, reflux, 2 h. (c) Methyl chloroformate, methylene chloride, 18 h.

N-Dodecyldiisobutyrimide (**9**) was obtained as an oil in 12% yield; ^{1}H-NMR: δ 0.85 (t, 3H, terminal CH_3), 1.14 (d, 12H, CH_3 β to C = O), 1.22 (m, 18H, $(CH_2)_9$), 1.48 (m, 2H, CH_2 β to N), 3.11 (m, 2H, CH α to C = O), 3.59 (t, 2H, CH_2 α to N).

Anal. $C_{20}H_{39}NO_2$ requires C, 73.79; H, 12.07; N, 4.30. Found: C, 73.94; H, 12.04; N, 4.18.

N-Dodecyldiacetimide (**10**) was obtained as an oil in 57% yield; ^{1}H-NMR: δ 0.86 (t, 3H, terminal CH_3), 1.24 (m, 18H, $(CH_2)_9$), 1.52 (m, 2H, CH_2 β to N), 2.38 (s, 6H, diacetyl CH_3), 3.60 (t, 2H, CH_2 α to N).

Anal. $C_{16}H_{31}NO_2$ requires C, 71.33; H, 11.60; N, 5.20. Found: C, 71.27; H, 11.63; N, 5.16.

N-Dodecylisobutyramide (**11**) was obtained as white crystals in 43% yield; ^{1}H-NMR: δ 0.86 (t, 3H, terminal CH_3), 1.13 (d, 6H, CH_3 β to C = O), 1.23 (m, 18H, $(CH_2)_9$), 1.47 (m, 2H, CH_2 β to N), 2.27 (m, 1H, CH α to C = O), 3.20 (q, 2H, CH_2 α to N), 5.40 (br s, 1H, N*H*).

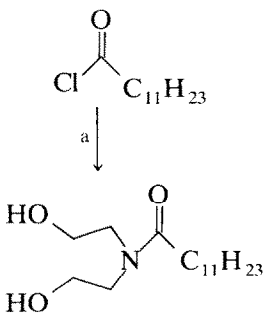
Anal. $C_{16}H_{33}NO$ requires C, 75.23; H, 13.02; N, 5.48. Found: C, 75.22; H, 12.94; N, 5.41.

N-Dodecylacetamide (**12**) was obtained as white crystals in 60% yield; ^{1}H-NMR: δ 0.86 (t, 3H, terminal CH_3), 1.23 (m, 18H, $(CH_2)_9$), 1.47 (m, 2H, CH_2 β to N), 1.95 (s, 3H, acetyl CH_3), 3.20 (q, 1H, CH_2 α to N).

Anal. $C_{14}H_{29}NO$ requires C, 73.95; H, 12.85; N, 6.16. Found: C, 73.70; H, 12.81; N, 6.14.

N-Carbomethyloxydodecylamine (**13**) was obtained as white crystals in 49% yield; ^{1}H-NMR: δ 0.86 (t, 3H, terminal CH_3), 1.23 (m, 18H, $(CH_2)_9$), 1.46 (m, 2H, CH_2 β to N), 3.14 (m, 2H, CH_2 α to N), 3.64 (s, 3H, OCH_3), 4.46 (br s, 1H, N*H*).

Anal. $C_{14}H_{29}NO_2$ requires C, 69.09; H, 12.01; N, 5.76. Found: C, 69.19; H, 12.03; N, 5.86.



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Scheme 3. (a) Diethanolamine, acetonitrile, reflux, 12 h.

N-(1-Oxododecyl)diethanolamine (**14**) was obtained as white crystals in 63% yield; ^{1}H-NMR: δ 0.86 (t, 3H, terminal CH_3), 1.24 (m, 16H, $(CH_2)_8$), 1.61 (m, 2H, CH_2 β to C = O), 2.37 (t, 2H, CH_2 α to C = O), 3.30 (br d, 2H, O*H*), 3.51 (d of t, 4H, CH_2 α to N), 3.80 (br d, 4H, CH_2 α to O).

Anal. $C_{16}H_{33}NO_3$ requires C, 66.86; H, 11.57; N, 4.87. Found: C, 66.67; H, 11.53; N, 4.86.

2.2.2. Permeability experiments

Full-thickness hairless mouse skins were mounted in unoccluded modified Franz diffusion cells with a donor area of 3.14 cm^2 and a 12 ml receptor volume. The receptor contained isotonic phosphate buffer (pH 7.2) with 0.5% w/v polyoxyethylene 20 cetyl ether as solubilizer (Chien, 1982) and 0.1% v/v of 36% aqueous formaldehyde as preservative (Sloan et al., 1991). The receptor was maintained at 37 ± 0.5°C throughout the experiment. A magnetic stirrer (600 rpm) ensured adequate mixing of the receptor phase. Following a hydration period of 1.5 h, 5 μl of enhancer solution (0.4 M enhancer in propylene glycol) was spread evenly over each skin. Several enhancers (**8**, **11** and **13**) were solids and were not soluble at 0.4 M. These were applied at their respective saturation solubilities in propylene glycol at 32 ± 0.5°C (skin surface temperature) (Michniak et al., 1993a). The enhancer solution was left on the skin and not washed off. 1 h later, 0.03 M hydrocortisone 21-acetate suspension in 500 μl propylene glycol (saturation solubility of 0.003 M) was placed on each skin (Higuchi, 1960; Davis and Hadgraft, 1991). Samples (100 μl) were withdrawn from the receptor over 24 h, each being immediately replaced by the same volume of diffusion buffer. Analysis of cumulative concentration present in the receptor chamber was corrected for any previous samples removed. Experiments were repeated at least five times. After 24 h, skins were removed and washed in methanol (100 ml) for 5 s (×3). The skins were then air-dried for 10 min, weighed and homogenized with a Kinematica GmbH tissue homogenizer (Switzerland). The average skin weight was calculated to be 0.1185 ± 0.0934 g (n = 40). After filtration and centrifugation (if required) the samples were frozen at −80°C prior to analysis. This was a method described previously by Michniak

et al. (1993a,c) and a modification of that reported by Sasaki et al. (1991). We have also described HPLC methods for analysis of skin and receptor samples and have demonstrated drug recoveries of > 95% of original donor concentrations (Michniak, et al., 1993c).

2.2.3. Data analysis

The results obtained were plotted as the cumulative amount of drug (M) against time (h). The receptor concentration consisted of the metabolite, hydrocortisone, while the skin contained a mixture of parent drug and metabolite. The latter concentrations were expressed as amount of drug (μg) per g of hydrated skin. The graphs yielded lag time (h), maximum flux at initial steady state ($\mu M\ cm^{-2}\ h^{-1}$) and receptor concentration (Q_{24}, μM), at 24 h. These parameters were recorded since plateauing of the graphs was observed closer to the 24 h time point. This could have been due to many factors, including evaporation of the donor solution (Flynn and Wiener, 1993). Enhancement ratios (ER) were calculated as the skin parameter (flux, Q_{24} or skin content) from enhancer-treated skin divided by the same parameter from control. ER for control (no enhancer present) was 1.00. Statistical treatment of parts of the data consisted of analysis of variance (ANOVA) followed by a least significant difference test (LSD) if the ANOVA indicated that a difference existed (Bolton, 1990). The level of significance (α) was selected to be 0.05. All parameters were reported as mean ± S.D.

3. Results and discussion

Table 1 shows lag times, fluxes, 24 h receptor concentrations and steroid skin contents for control, Azone and enhancers **1–14**. Enhancement ratios (ER) are presented for all parameters excluding lag times. The diffusion cell receptor contained only hydrocortisone, while the skin samples contained both hydrocortisone 21-acetate and hydrocortisone. Hydrolysis of various esters of topical corticosteroids in the skin is well established and hairless mouse skin is known to possess esterases (Taüber, 1989; Kao and Carver, 1990). It is also known that 21-esters are more susceptible to skin esterases than the 17-esters (Taüber, 1989). It is possible that hydrolysis was taking place without the action of the esterases and in addition, there is evidence that enzymes leach out into the receptor phase (Collier and Bronough, 1992). It may be concluded that skin esterases were active to some extent, since hydrocortisone was detected to a small degree in skin samples. However, the majority of the hydrolytic action (due to leached enzymes or non-enzymatic hydrolysis) was taking place in the receptor phase in our study, since only hydrocortisone (and no acetate) was found there.

Enhancers **8**, **11** and **13** were solids not soluble at 0.4 M in propylene glycol at 32 ± 0.5°C. Compounds **4**, **12** and **14**, although solids at the temperature of the skin surface, were soluble in the vehicle at concentrations exceeding 0.4 M. The highest $ER_{Q_{24}}$ and ER_{SC} were observed for **5**. For **5** the ER_{flux} was 35.22, the $ER_{Q_{24}}$ was 79.86 and the ER_{SC} was 4.3, all values significantly greater than those observed for Azone: 19.51, 38.30 and 1.5, respectively ($p < 0.05$). For Q_{24} data, only **5** showed significantly greater activity than Azone ($p < 0.05$). Skin contents of model drug were significantly higher than Azone with compounds **3**, **5**, **9** and **10** ($p < 0.05$). The highest ER_{flux} was found with compound **11**. For **11** the ER_{flux} was 57.38, approx. 3-fold greater than that observed with Azone. Compared with Azone, enhancers **4–6**, **11** and **12** all showed significantly higher ER_{flux} values ($p < 0.05$). A few enhancers exhibited significantly lower skin steroid contents than control: **1**, **7** and **8** ($p < 0.05$). In addition, the $ER_{Q_{24}}$ values for **1–3**, **6–9**, **12–14** were significantly lower than that of Azone ($p < 0.05$).

Compound **8** had the highest melting point (82–83°C) of all the solid enhancers tested, a solubility of 0.274 M (at 32 ± 0.5°C) and also exhibited the lowest activity for all parameters recorded (only **7** had a lower ER_{SC} (0.3) than **8**). However, **11**, with a melting point of 54–54.5°C and solubility of 0.226 M, showed a ER_{flux} of 57.38 which was significantly higher than that of Azone (19.51) as well as an $ER_{Q_{24}}$ (37.32) which was similar to that of Azone (38.30). Hexahydro-2-oxo-1*H*-azepine-1-acetic acid tetradecyl ester

Table 1
Effect of enhancers **1**–**14** on the percutaneous penetration and skin retention of hydrocortisone 21-acetate in hairless mouse skin

Enhancer in PG [a,f]	m.p. (°C)	L [b] (h)	Flux (μM cm^{-2} h^{-1})	ER_{flux} [e]	Q_{24} [c] (μM)	$ER_{Q_{24}}$ [c]	SC (HCA) [d] (μg g^{-1})	SC (HC) [d] (μg g^{-1})	ER_{SC} [e] (HCA and HC)
None ($n=8$)	–	1.16 ± 0.32	0.045 ± 0.016	1.00	0.751 ± 0.250	1.00	285.2 ± 21.86	ND	1.0
Azone ($n=5$)	–	0.73 ± 0.09	0.878 ± 0.251	19.51	28.760 ± 4.624	38.30	410.6 ± 34.4	9.9 ± 2.5	1.5
1 ($n=5$)	–	0.84 ± 0.21	0.613 ± 0.212	13.62	9.353 ± 2.288	12.45	185.2 ± 65.2	ND	0.7
2 ($n=5$)	–	0.30 ± 0.10	0.936 ± 0.129	20.80	9.474 ± 2.449	12.62	343.4 ± 132.1	ND	1.2
3 ($n=5$)	–	0.10 ± 0.02	0.901 ± 0.206	20.02	6.253 ± 2.268	8.33	577.2 ± 81.1	4.6 ± 3.1	2.0
4 ($n=5$)	39–41	3.03 ± 1.24	1.160 ± 0.156	25.78	27.185 ± 5.695	36.20	477.1 ± 40.7	2.9 ± 0.9	1.7
5 ($n=5$)	26.5	0.60 ± 0.29	1.585 ± 0.294	35.22	59.973 ± 6.999	79.86	1219.3 ± 281.5	4.1 ± 1.9	4.3
6 ($n=5$)	–	0.62 ± 0.15	1.104 ± 0.632	24.53	13.618 ± 5.698	18.13	508.9 ± 101.2	ND	1.8
7 ($n=5$)	–	1.74 ± 0.84	0.640 ± 0.105	14.22	5.329 ± 1.266	7.10	70.2 ± 17.2	ND	0.3
8 ($n=5$)	82–83	0.99 ± 0.19	0.255 ± 0.089	5.67	2.560 ± 0.894	3.41	126.7 ± 22.9	ND	0.4
9 ($n=5$)	–	0.84 ± 0.32	0.264 ± 0.088	5.87	3.868 + 1.944	5.15	625.3 ± 123.5	11.3 ± 2.4	2.2
10 ($n=5$)	20–25	2.96 ± 1.01	0.641 ± 0.194	14.24	28.762 ± 6.299	38.30	741.5 ± 50.6	4.8 ± 1.9	2.6
11 ($n=5$)	54–54.5	0.69 ± 0.25	2.582 ± 0.846	57.38	28.024 ± 8.397	37.32	260.2 ± 106.8	ND	0.9
12 ($n=5$)	55–55.5	0.87 ± 0.20	1.322 ± 0.255	29.38	12.293 ± 2.404	16.37	265.6 ± 58.1	ND	0.9
13 ($n=5$)	48	0.73 ± 0.14	0.293 ± 0.099	6.51	4.849 ± 1.463	6.46	293.7 ± 88.5	ND	1.0
14 ($n=5$)	42.5–43	1.50 ± 0.56	0.451 ± 0.102	10.02	7.463 ± 2.946	9.94	289.6 ± 50.4	3.3 ± 0.9	1.0

[a] PG, propylene glycol; [b] L, lag time; [c] Q_{24}, receptor concentration after 24 h; [d] SC, skin content of hydrocortisone 21-acetate (HCA) and hydrocortisone (HC) (metabolite); [e] ER, enhancement ratio compared to control (control = 1.00); [f] saturation solubilities (M) at 32 ± 0.5°C in PG of **8** = 0.274; **11** = 0.226; **13** = 0.293 (although **4**, **12** and **14** were solids, they were soluble at 0.4 M); ND, not detected.

(HAT), a solid enhancer (m.p. = 41–42°C) which we have examined previously (Michniak et al., 1993c), had a saturation solubility of only 0.136 M (at 32 ± 0.5°C) yet its ER_{SC} was 7.6, the highest we have seen for any enhancer in this model. Therefore, there is no apparent correlation between melting points, saturation solubilities and enhancement ratios for the solid enhancers.

Wong et al. (1989) reported the enhancing properties of several alkyl *N,N*-dialkyl-substituted aminoacetates in shed snake skin. The relative enhancement of model drug (indomethacin) by Azone was taken as 1.00. A piperazinyl derivative had an ER of 0.4, while its acyclic derivative had an ER of 3.8. In the present study **8**, the only compound with a free carboxylic acid moiety was the acyclic equivalent (resulting from lactone hydrolysis) of *N*-dodecyldihydro-1,4-oxazepine-5,7-dione (DDO), a compound tested previously (Michniak et al., 1993c). Both enhancers were solids, the melting point for DDO being 72–73°C. DDO exhibited significantly higher flux, Q_{24} and skin content enhancement compared with **8** ($p < 0.05$). For DDO, flux was 0.570 ± 0.114 $\mu M\ cm^{-2}\ h^{-1}$ ($ER_{flux} = 12.67$), Q_{24} was 22.475 ± 4.299 μM ($ER_{Q_{24}} = 29.93$) and skin content (HC + HCA) was 344.3 ± 26.2 $\mu g\ g^{-1}$ ($ER_{SC} = 1.2$). Conversely, compound **7** is very similar in structure to **8**, the only difference being the substitution of a methyl ester for the carboxylic acid group. This masking of the acidic group results in a compound that has a higher initial flux, ($ER_{flux} = 14.22$) and a greater Q_{24} ($ER_{Q_{24}} = 7.10$) as compared to **8**. Therefore, **7** is similar to it cyclic equivalent, with respect to flux enhancement. *N*-(1-Oxododecyl)morpholine (ODM), the cyclic ether equivalent of the acyclic diol (**14**) in this study, was also reported previously (Michniak et al., 1993d). ODM was a liquid with a flux of 1.180 ± 0.129 $\mu M\ cm^{-2}\ h^{-1}$ ($ER_{flux} = 26.22$); Q_{24} was 22.039 ± 2.066 μM ($ER_{Q_{24}} = 29.35$) and skin content (HC + HCA) was 281.4 ± 18.6 $\mu g\ g^{-1}$ ($ER_{SC} = 1.0$). The cyclic version was the same as its acyclic version for skin drug content enhancement ($ER_{SC} = 1.0$) but $ER_{Q_{24}}$ (9.94) and ER_{flux} (10.02) for **14** were both significantly lower than that seen with ODM ($p < 0.05$). These comparisons enable us to conclude that acyclic compounds with polar moieties, especially acidic ones, usually possess inferior enhancing properties than do their less polar cyclic analogs.

An important comparison may be made regarding the *N,N*-disubstituted amides. ER_{flux}, $ER_{Q_{24}}$ and ER_{SC} values for the *N,N*-disubstituted acetamides, **4** and **5**, were significantly higher than those found with the *N,N*-disubstituted isobutyramides, **1** and **2** ($p < 0.05$). ER_{flux}, $ER_{Q_{24}}$ and ER_{SC} determinations for enhancer **4** were 25.78, 36.20 and 1.7 while these same enhancement ratios were only 13.62, 12.45 and 0.7 for compound **1**. Similarly, ER_{flux}, $ER_{Q_{24}}$ and ER_{SC} values for enhancer **5** were 35.22, 79.86 and 4.3 but only 20.80, 12.62 and 1.2 for compound **2**, an even larger decline. Conversely, there was no obvious relationship between *N*-dodecyl-*N*-(2,2-dimethoxyethyl)isobutyramide (**3**) and *N*-dodecyl-*N*-(2,2-dimethoxyethyl)acetamide (**6**). A comparison between amide and imide enhancing ability may also be made. The ER_{flux} values for the *N*-dodecylisobutyramide, **11** (57.38) and *N*-dodecylacetamide, **12** (29.38) were significantly higher than those of the corresponding *N*-dodecyldiisobutyrimide, **9** (5.87) and *N*-dodecyldiacetimide, **10** (14.24) ($p < 0.05$). The above trends may be due a lipophilicity effect or to the conformation the enhancer adopts when intercalated into the membrane or both.

Consideration of skin retention or uptake of drugs is important, since in the past many topical formulations were studied using only flux values (Borsadia et al., 1992; Shah et al., 1992). Increased skin uptake of drugs may be due to binding within skin layers, effectiveness of a co-applied enhancer, co-solvent(s), partitioning, metabolism of enhancer(s) and drug(s). Unfortunately, this study only provided information as to the quantity of model drug and metabolite at 24 h in full-thickness skin. Future experiments will examine enhancer metabolism, quantity evaluation of drug and enhancer in various skin layers and partitioning studies. Such investigations may provide important information as to why **5**, **9** and **10** showed higher ER_{SC} values than other enhancers tested, including Azone.

There are several reports of enhancers which

increase skin retention and flux or increase only skin retention (Sasaki et al., 1991; Michniak et al., 1993a–d). Sasaki et al. (1991) reported high concentrations of the tested enhancers (pyrrolidinones) in the skin of albino rats and suggested that this effect correlated with high lipophilicity and affinity of the enhancers for the stratum corneum. Steroids have been known for some time to possess the capability of 'reservoir' formation within the skin (Scheuplein et al., 1969; Barry, 1976). Further, skin retention may be modified or decreased by additional metabolism and blood flow by in vivo conditions (Yu et al., 1979). The disposition of β-estradiol in skin in vitro has been shown to be dependent on experimental equipment (Pershing et al., 1993). Therefore, caution must be exercised when predicting whether increased skin retention will actually occur under human in vivo conditions.

In addition, it should be noted that these reported ER values are high since a very permeable membrane was used and would be considerably lower, for example, in an in vivo human study. It is also possible that the rank order of activity could change. The authors feel, however, that much information is being gained by testing these novel compounds using one set of experimental conditions. In conclusion, the rank order relationship of the enhancers tested in this study based on initial flux data was **11** > **5** > **12** > **4** > **6** > **2** > **3** > Azone > **10** > **7** > **1** > **14** > **13** > **9** > **8**; based on Q_{24}: **5** > Azone = **10** = **11** > **4** > **6** > **12** > **2** = **1** > **14** > **3** > **7** > **13** > **9** > **8**; and based on skin steroid contents: **5** > **10** > **9** > **3** > **6** > **4** > Azone > **2** > **13** > **14** > **12** = **11** > **1** > **8** > **7**.

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