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In vitro evaluation of a series of Azone analogs as dermal penetration enhancers: IV. Amines

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Abstract

Dermal enhancement properties of 12 novel amine enhancers (Azone analogs) were studied using in vitro diffusion cell techniques. Standard enhancers tested were Azone, didodecylamine, dodecylamine, and stearylamine. The synthesis of these novel compounds is presented. Hydrocortisone 21-acetate was used as the model drug and its transdermal permeation and skin retention were examined using hairless mouse skin. Enhancement ratios (ER) were determined for flux, 24 h diffusion cell receptor concentrations (Q_{24}), and 24 h full-thickness skin steroid content. ER for all parameters for control was 1.00. Control was no pretreatment of the skin. All enhancers were applied at 0.4 M in propylene glycol 1 h prior to steroid application. *N*-dodecyldiethanolamine showed the greatest Q_{24} value (ER 56.16) while *N*-(2-methoxyethyl)dodecylamine showed the greatest skin retention (ER 2.0). Azone ER values were Q_{24} 38.30 and skin retention 1.5, and those for didodecylamine were 13.06 and 1.1, respectively. In general, tertiary cyclic amine and secondary amine enhancers showed less activity for flux than the tertiary acyclic amine compounds.

Keywords: Azone analog; Enhancer; Amine; Percutaneous absorption; Hairless mouse; Skin retention; Steroid

1. Introduction

This is a continuation of a study examining structure-activity relationships in a series of Azone analogs (Michniak et al., 1993a,b, 1994a,b). Correlations between chemical structure and activity give insight into possible mechanisms of action as well as aid in the design of future compounds (Brain and Walters, 1993). Previous related work from other sources included the use of 1-alkyl- and 1-alkenylazacycloalkanone derivatives, terpenes, and alkyl *N*,*N*-dialkyl-substituted aminoacetates (Wong et al., 1989; Okamoto et al., 1991; Williams and Barry, 1991).

This investigation examined 12 novel amines, four cyclic and eight acyclic structures (Scheme 1 and Table 1). Previously, we have reported data using acyclic and cyclic amides with *N*-dodecyl-*N*-(2-methoxyethyl) acetamide being the most active acyclic enhancer (Michniak et al., 1993c, 1994a). Despite published opinions to the contrary, the

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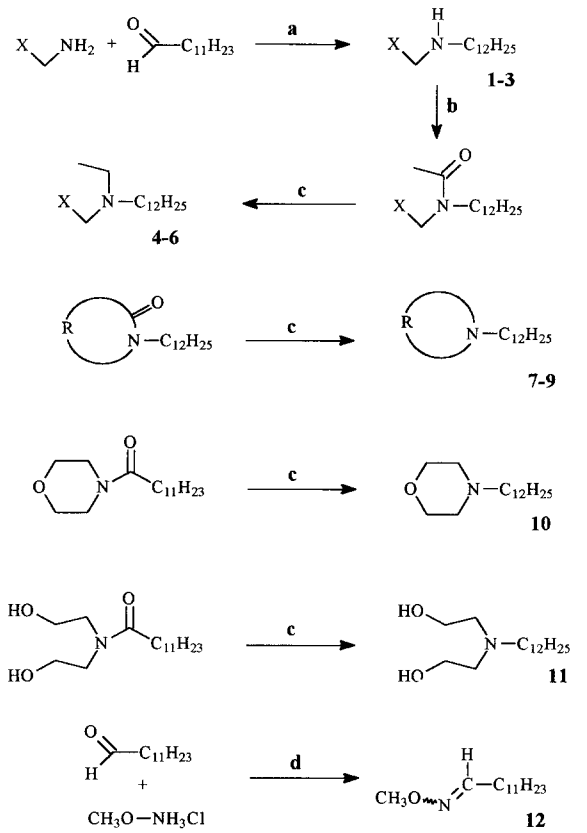

Scheme 1. (a) Pd/C (10%), H_2, C_2H_5OH, 25°C, 1 h. (b) Ac_2O, pyridine, $MeCl_2$, 25°C, 12 h. (c) LAH, THF, 67°C, 3 h. (d) Pyridine, CH_3OH, 65°C, 1 h.

flux-enhancing activity of the latter compound conclusively showed that a cyclic structure was not an absolute requirement for activity in compounds structurally related to Azone (Ghosh and Banga, 1993). Other acyclic amides were also prepared by Miniskanian and Peck (1988), and Peck and Miniskanian (1988). A few acyclic amines have been investigated in the past, stearylamine, didodecylamine, and dodecylamine being the three chosen as standards for our study (Aungst et al., 1986, 1990; Ghosh and Banga, 1993). Dodecylamine and stearylamine (10% w/v or v/v in propylene glycol) were found to enhance naloxone flux through human cadaver skin. Dodecylamine was the most active, producing a flux (J) of 25.1 $\mu g\ cm^{-2}\ h^{-1}$, while stearylamine flux was 19.4 $\mu g\ cm^{-2}\ h^{-1}$. Control flux was 1.6 $\mu g\ cm^{-2}\ h^{-1}$ (Aungst et al., 1986). These authors then examined other model drugs with dodecylamine/propylene glycol under the same conditions: testosterone, benzoic acid, indomethacin, fluorouracil, and methotrexate, and in every case, obtained a marked increase in flux values (Aungst et al., 1990). For example, fluorouracil flux increased from control 1.4 $\mu g\ cm^{-2}\ h^{-1}$ with propylene glycol alone, to 527.6 $\mu g\ cm^{-2}\ h^{-1}$ with dodecylamine. However, more lipophilic drugs such as testosterone showed much less enhancement. Stearylamine, however, enhanced indomethacin flux only slightly increasing the value from control 0.5 to 0.7 $\mu g\ cm^{-2}\ h^{-1}$. With fluorouracil, the flux enhancement was greater, from control 1.9 to 21.7 $\mu g\ cm^{-2}\ h^{-1}$.

Table 1

Compound	R	X
1,4	–	$HOCH_2$-
2,5	–	CH_3OCH_2-
3,6	–	$(CH_3O)_2CH$-
7	-$(CH_2)_3$-	–
8	-$(CH_2)_4$-	–
9	-$(CH_2)_5$-	–

The amines in the present work were in most cases, directly analogous to cyclic and acyclic amides which we have previously found to be active. The use of cyclic amines as transdermal penetration enhancers is not well-reported in the literature.

Enhancement of drug delivery can result in three possible outcomes: (a) enhanced transdermal penetration, applicable particularly for systemic drug delivery; (b) enhanced skin concentrations of drug, applicable for topical/localized skin tissue targeting; and (c) a combination of (a) and (b). The importance of examining skin retention of drugs has been stressed by several authors including Reifenrath et al. (1991), Borsadia et al. (1992), and Sasaki et al. (1991).

The experimental protocol for this study remained unchanged in order that direct comparison may be made to our previous publications in this series. All novel and standard enhancers were applied at 0.4 M in propylene glycol 1 h prior to application of hydrocortisone 21-acetate. Hairless mouse skin was used in all experiments.

2. 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. Didodecylamine (Pract.) was obtained from Eastman Kodak Co., Rochester, NY. 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.

3. Methods

3.1. Enhancer compounds

Reaction conditions for compounds **1–12** are outlined in Scheme 1. 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.

N-Dodecylethanolamine (**1**) was obtained as white crystals in 65% yield, m.p. 40–41°C; ^{1}H-NMR ($CDCl_3$) δ 0.85 (t, 3H, terminal CH_3), 1.23 (m, 18H, $(CH_2)_9$), 1.45 (m, 2H, CH_2 β to N), 2.47 (br s, 1H, OH), 2.58 (t, 2H, CH_2 α to N), 2.74 (t, 2H, CH_2 α to N and β to O), 3.62 (t, 2H, CH_2 α to O and β to N).

Anal. ($C_{14}H_{31}NO$) Calc.: C, 73.30; H, 13.62; N, 6.11. Found: C, 73.36; H, 13.58; N, 6.11.

N-(2-Methoxyethyl)dodecylamine (**2**) was obtained as a clear oil in 54% yield; ^{1}H-NMR ($CDCl_3$) δ 0.81 (t, 3H, terminal CH_3), 1.21 (m, 18H, $(CH_2)_9$), 1.42 (m, 2H, CH_2 β to N), 2.39 (t, 2H, CH_2 α to N), 2.73 (t, 2H, CH_2 α to N and β to O), 3.32 (s, 3H, OCH_3), 3.44 (t, 2H, CH_2 α to O and β to N).

Anal. ($C_{15}H_{33}NO$) Calc.: C, 74.01; H, 13.66; N, 5.75. Found: C, 73.77; H, 13.64; N, 5.64.

N-(2,2-Dimethoxyethyl)dodecylamine (**3**) was obtained as a clear oil in 44% yield; ^{1}H-NMR ($CDCl_3$) δ 0.83 (t, 3H, terminal CH_3), 1.23 (m, 18H, $(CH_2)_9$), 1.43 (m, 2H, CH_2 β to N), 2.57 (t, 2H, CH_2 α to N), 2.70 (d, 2H, CH_2 α to N and β to O), 3.34 (s, 6H, $(OCH_3)_2$), 4.45 (t, 1H, CH α to O and β to N).

Anal. ($C_{16}H_{35}NO_2$) Calc.: C, 70.28; H, 12.90; N, 5.12. Found: C, 70.11; H, 12.87; N, 5.05.

N-Ethyl-*N*-(dodecyl)ethanolamine (**4**) was obtained as a clear oil in 4% yield; ^{1}H-NMR ($CDCl_3$) δ 0.86 (t, 3H, terminal dodecyl CH_3), 0.99 (t, 3H, terminal ethyl CH_3), 1.24 (m, 18H, $(CH_2)_9$), 1.41 (m, 2H, CH_2 β to N), 2.42 (t, 2H, dodecyl CH_2 α to N), 2.53 (m, 4H, ethyl and 2-hydroxyethyl CH_2 α to N), 3.04 (br s, 1H, OH), 3.50 (t, 2H, CH_2 α to O and β to N).

Anal. ($C_{16}H_{35}NO \cdot 1/8\ H_2O$) Calc.: C, 74.00; H, 13.68; N, 5.39. Found: C, 73.76; H, 13.64; N, 5.17.

N-Ethyl-*N*-(2-methoxyethyl)dodecylamine (**5**) was obtained as a clear oil in 3% yield; ^{1}H-NMR: δ 0.85 (t, 3H, terminal dodecyl CH_3), 1.00 (t, 3H, terminal ethyl CH_3), 1.23 (m, 18H, $(CH_2)_9$), 1.40 (m, 2H, CH_2 β to N), 2.41 (t, 2H, dodecyl CH_2 α to N), 2.51 (t, 2H, ethyl CH_2 α to N), 2.59 (t, 2H, 2-methoxyethyl CH_2 α to N), 3.32 (s, 3H, OCH_3), 3.42 (t, 2H, CH_2 α to O and β to N).

Anal. ($C_{17}H_{37}NO$)Calc.: C, 75.21; H, 13.74; N, 5.16. Found: C, 75.25; H, 13.73; N, 5.06.

N-Ethyl-*N*-(2,2-dimethoxyethyl)dodecylamine (**6**) was obtained as a clear oil in 20% yield; ^{1}H-NMR ($CDCl_3$) δ 0.83 (t, 3H, terminal dodecyl CH_3), 0.87 (t, 3H, terminal ethyl CH_3), 1.22 (m, 18H, $(CH_2)_9$), 1.40 (m, 2H, CH_2 β to N), 2.43 (t, 2H, dodecyl CH_2 α to N), 2.54 (m, 4H, ethyl and 2,2-dimethoxyethyl CH_2 α to N), 3.33 (s, 6H, $(OCH_3)_2$), 4.40 (t, 1H, CH α to O and β to N).

Anal. ($C_{18}H_{39}NO_2$) Calc.: C, 71.70; H, 13.04; N, 4.64. Found: C, 71.81; H, 13.09; N, 4.68.

N-Dodecylpyrrolidine (**7**) was obtained as a clear oil in 85% yield; ^{1}H-NMR ($CDCl_3$) δ 0.86 (t, 3H, terminal CH_3), 1.24 (m, 18H, $(CH_2)_9$), 1.48 (m, 2H, CH_2 β to N), 1.73 (m, 4H, 3 and 4-CH_2), 2.38 (t, 2H, CH_2 α to N), 2.46 (m, 4H, 2 and 5-CH_2).

Anal. ($C_{16}H_{33}N \cdot 1/8\ H_2O$) Calc.: C, 79.51; H, 13.86; N, 5.79. Found: C, 79.39; H, 13.76; N, 5.54.

N-Dodecylpiperidine (**8**) was obtained as a clear oil in 81% yield; ^{1}H-NMR ($CDCl_3$) δ 0.85

(t, 3H, terminal CH_3), 1.23 (m, 18H, $(CH_2)_9$), 1.40 (m, 2H, CH_2 β to N), 1.40–1.55 (m, 6H, 3, 4 and 5-CH_2), 2.23 (t, 2H, CH_2 α to N), 2.33 (m, 4H, 2 and 6-CH_2).

Anal. ($C_{17}H_{35}N$) Calc.: C, 80.56; H, 13.92; N, 5.53. Found: C, 80.47; H, 13.86; N, 5.41.

N-Dodecylhomopiperidine (**9**) was obtained as a clear oil in 70% yield; ^{1}H-NMR ($CDCl_3$) δ 0.85 (t, 3H, terminal CH_3), 1.23 (m, 18H, $(CH_2)_9$), 1.41 (m, 2H, CH_2 β to N), 1.41–1.60 (m, 8H, 3, 4, 5 and 6-CH_2), 2.41 (t, 2H, CH_2 α to N), 2.59 (m, 4H, 2 and 7-CH_2).

Anal. ($C_{18}H_{37}N$) Calc.: C, 80.82; H, 13.94; N, 5.24. Found: C, 80.88; H, 13.90; N, 5.15.

N-Dodecylmorpholine (**10**) was obtained as a clear oil in 85% yield; ^{1}H-NMR ($CDCl_3$) δ 0.85 (t, 3H, terminal CH_3), 1.23 (m, 18H, $(CH_2)_9$), 1.38 (m, 2H, CH_2 β to N), 2.26 (t, 2H, CH_2 α to N), 2.39 (t, 4H, 3 and 5-CH_2), 3.66 (t, 4H, 2 and 6-CH_2).

Anal. ($C_{16}H_{33}NO$) Calc.: C, 75.23; H, 13.02; N, 5.48; O, 6.26. Found: C, 75.14; H, 13.00; N, 5.40.

N-Dodecyldiethanolamine (**11**) was obtained as a clear oil in 31% yield; ^{1}H-NMR ($CDCl_3$) δ 0.85 (t, 3H, terminal CH_3), 1.23 (m, 18H, $(CH_2)_9$), 1.43 (m, 2H, CH_2 β to N), 2.49 (t, 2H, CH_2 α to N), 2.62 (t, 4H, CH_2 α to N and β to O), 3.59 (t, 4H, CH_2 α to O). Anal. ($C_{16}H_{35}NO_2$) Calc.: C, 70.28; H, 12.90; N, 5.12. Found: C, 70.03; H, 12.89; N, 5.09.

(*E,Z*)-*O*-Methyl-1-dodecyloxime (**12**) was obtained as a clear oil in 97% yield; TLC (hexanes/dichloromethane/ethyl acetate, 2:1:1) $R_f = 0.85$ and 0.9; ^{1}H-NMR ($CDCl_3$) δ 0.86 (t, 3H, terminal CH_3), 1.23 (m, 16H, $(CH_2)_8$), 1.41 (m, 2H, CH_2 β to C = N), 2.21 (m, 2H, CH_2 α to C = N, center of *E* and *Z* signals), 3.82 (s, 3H, OCH_3, center of *E* and *Z* signals), 6.82 (t, 1H, $(C_{11}H_{23})H$-C = N, center of *E* and *Z* signals).

Anal. ($C_{13}H_{27}NO$) requires C, 73.18; H, 12.75; N, 6.57. Found: C, 73.28; H, 12.71; N, 6.51.

3.2. Skin penetration and retention study

In this study, unoccluded modified Franz cells (donor area 3.14 cm^2, receptor volume 12 ml) maintained at 37 ± 0.5°C were used. Excised full-thickness hairless mouse skins were placed over the receptor compartments and were allowed to hydrate for 1.5 h ($n = 5$). The receptors contained isotonic phosphate buffer (pH 7.2), 0.1% v/v of 36% aqueous formaldehyde as preservative (Sloan et al., 1991) and 0.5% w/v polyoxyethylene 20 cetyl ether as solubilizer (Chien, 1982). The receptor solutions were continuously stirred at 600 rpm.

All enhancers tested except for **1**, didodecylamine, and stearylamine were liquids at 32 ± 0.5°C, the temperature of the skin surface. Enhancer **1** (m.p. 40–41°C) was found to be soluble in propylene glycol at 0.4 M, the concentration used for the study. Dodecylamine was a solid at room temperature but melted at 30–32°C. Stearylamine (m.p. 55–57°C) and didodecylamine (m.p. 45°C) were applied at their respective saturation solubilities in propylene glycol at 32 ± 0.5°C (3.71 and 2.72 mM).

Following hydration, each skin was covered with 5 μl of enhancer in propylene glycol (all 0.4 M except stearylamine and didodecylamine). This solution was left on the skins for 1 h and was not washed off. A saturated suspension (500 μl) of hydrocortisone 21-acetate in propylene glycol was then applied to each cell. Saturation solubility of the steroid in propylene glycol has been reported to be 0.003 M (Davis and Hadgraft, 1991). The receptor phase was then sampled over 24 h. Each 100 μl sample was replaced immediately by an equal volume of diffusion buffer. Analysis of each subsequent sample was corrected for any previous samples removed. At 24 h skins were removed, washed in methanol (100 ml) for 5 s (×3). Following room temperature drying for 10 min, each skin was weighed, cut up, and finally homogenized using a Kinematica GmbH tissue homogenizer (Switzerland). Average skin weight was 0.1129 ± 0.0995 g ($n = 50$). The cleaned (filtered) extracts were then frozen (−80°C) with other receptor samples. All samples were analyzed using HPLC methodology. Methods and drug recoveries have been described previously (Sasaki et al., 1991; Michniak et al., 1993a,b,c, 1994a,b).

3.3. Data analysis

Cumulative amounts of drug (M) corrected for sample removal were plotted against time (h).

The permeation profiles yielded the following: lag time (h), maximum flux at initial steady state (μM cm^{-2} h^{-1}), and receptor concentrations at 24 h (Q_{24}, μM). The receptor was found to contain only hydrocortisone, while the skin extracts contained mainly the unchanged acetate with small amounts of hydrocortisone. Skin steroid contents were expressed as μg steroid per g of hydrated full-thickness mouse skin. Enhancement ratios (ER) were calculated as skin parameter (flux, Q_{24}, skin content) from enhancer treated skin divided by the same parameter from control (no enhancer present). Controls were assigned as 1.00. All parameters reported as mean ± S.D. Data treatment involved analysis of variance (ANOVA) followed by a least significant difference test (LSD) if the ANOVA indicated that a difference existed. The level of significance was selected as 0.05 (Bolton, 1990).

4. Results and discussion

All enhancers tested improved transdermal penetration of hydrocortisone 21-acetate (Table 2). Skin samples contained mainly the parent compound (hydrocortisone 21-acetate) and some hydrocortisone. Receptor samples, however, contained only the metabolite (hydrocortisone). Esterase activity in the skin was responsible for part of the hydrolysis as well as hydrolysis which took place in the diffusion cell receptor medium

Table 2
Effect of enhancers on steroid skin retention and skin permeation parameters

Enhancer in PG [a,b]	L [c] (h)	Flux_{max} (μM cm^{-2} h^{-1})	ER_{flux} [g]	Q_{24} [d] (μM)	$\text{ER}_{Q_{24}}$ [g]	SC(HCA) [e] (μg g^{-1})	SC(HC) (μg g^{-1})	ER_{SC} (HCA + HC)
None (n = 8)	1.16 ± 0.32	0.045 ± 0.016	1.00	0.751 ± 0.250	1.00	285.2 ± 21.6	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
Didodecylamine (n = 5)	0.75 ± 0.12	1.560 ± 0.196	34.66	9.812 ± 1.845	13.06	307.7 ± 109.5	5.7 ± 2.6	1.1
Dodecylamine (n = 5)	2.72 ± 1.05	0.733 ± 0.219	16.29	7.819 ± 1.773	10.41	376.8 ± 69.8	94.0 ± 42.5	1.7
Stearylamine (n = 5)	3.76 ± 1.09	0.829 ± 0.158	18.42	6.124 ± 0.592	8.15	172.7 ± 50.7	2.7 ± 0.6	0.6
1 (n = 5)	0.38 ± 0.12	0.531 ± 0.205	11.80	6.162 ± 0.195	8.21	346.3 ± 47.0	ND	1.2
2 (n = 5)	0.17 ± 0.08	0.720 ± 0.253	16.00	8.133 ± 1.721	10.83	561.0 ± 96.9	ND	2.0
3 (n = 5)	0.55 ± 0.19	0.593 ± 0.219	13.18	5.751 ± 0.598	7.66	436.7 ± 186.7	ND	1.5
4 (n = 5)	0.59 ± 0.19	0.412 ± 0.109	9.16	4.148 ± 0.750	5.52	163.7 ± 64.7	10.0 ± 1.1	0.6
5 (n = 5)	0.25 ± 0.08	1.390 ± 0.405	30.89	15.770 ± 4.300	21.00	119.7 ± 32.2	36.1 ± 13.9	0.6
6 (n = 4)	0.37 ± 0.13	0.362 ± 0.100	8.04	7.965 ± 3.860	10.61	409.4 ± 147.3	16.8 ± 4.3	1.5
7 (n = 5)	0.73 ± 0.19	0.801 ± 0.210	17.80	4.540 ± 0.593	6.05	252.0 ± 66.5	7.5 ± 3.7	0.9
8 (n = 5)	0.76 ± 0.10	0.444 ± 0.126	9.87	6.381 ± 2.854	8.50	227.3 ± 23.3	21.7 ± 10.9	0.9
9 (n = 5)	0.64 ± 0.10	0.690 ± 0.205	15.33	7.523 ± 2.324	10.02	239.3 ± 45.3	7.6 ± 2.3	0.9
10 (n = 5)	0.49 ± 0.09	1.150 ± 0.099	25.56	9.581 ± 2.649	12.76	434.1 ± 75.8	11.4 ± 5.3	1.6
11 (n = 5)	3.61 ± 0.36	0.725 ± 0.291	16.11	42.176 ± 15.822	56.16	122.4 ± 30.3	6.9 ± 2.3	0.5
12 (n = 5)	0.45 ± 0.12	0.623 ± 0.174	13.84	4.495 ± 0.960	5.90	204.3 ± 56.3	ND	0.7

[a] PG, propylene glycol.
[b] Melting point of solid enhancer **1**: 40 °C.
[c] L, lag time.
[d] Q_{24}, receptor concentration after 24 h.
[e] SC, skin content of hydrocortisone 21-acetate (HCA) and hydrocortisone (HC) (metabolite).
[f] ND, not detected.
[g] ER, enhancement ratio calculated as permeation parameter after enhancer treatment divided by the corresponding parameter from control (control = 1.00).

(Taüber, 1989; Kao and Carver, 1990; Collier and Bronaugh, 1992, Mukhtar et al., 1992; Michniak et al., 1994a).

The highest enhancement ratio (ER) for 24 h receptor concentrations was observed for compound **11** (ER 56.16). Azone ER was 38.30, and control was taken as 1.00. Initial flux values were also reported since in some cases, plateauing of the permeation profile graphs was observed. This particularly occurred when more active enhancers were being tested. Possible causes may have been evaporation of the donor phase in the unoccluded cells, some unknown effect on the skin membrane itself, or loss of sink conditions in the receptor. The highest initial fluxes were obtained for didodecylamine (ER 34.6) and **5** (ER 30.89). It is important to note that didodecylamine was effective at very low saturation solubility in propylene glycol (2.62 mM).

Steroid contents were very variable with the enhancers tested. Compound **2** produced the highest steroid skin retention (ER_{SC} 2.0) while Azone produced a 1.5-fold increase. Dodecylamine produced an ER_{SC} of 1.7. Compounds **4**, **5**, **7–9**, **11**, **12**, and stearylamine all produced ER_{SC} values of less than 1.00. All compounds produced significantly higher flux values and Q_{24} compared with controls ($p < 0.05$). Skin contents were significantly higher with Azone, dodecylamine, **2**, **3**, **6**, and **10** ($p < 0.05$). Compared with Azone, compound **11** produced a higher Q_{24} value ($p < 0.05$).

It is possible that in the case of high Q_{24} but low skin levels, the enhancer pool in the donor and upper skin levels was low, but the compound had adequately affected the skin lipid layers to enhance transdermal penetration (Barry, 1988). This would result in higher Q_{24} but low steroid retention in the skin. In the latter case, the compounds were ineffective or very mild enhancers and may have, in addition, affected partitioning of the drug from the donor. The result was a low skin steroid retention and low transdermal penetration (Christensen et al., 1993).

Didodecylamine produced a higher $ER_{Q_{24}}$ value (13.06) and a lower skin retention (ER_{SC} 1.1) compared with dodecylamine ($ER_{Q_{24}}$ 10.41) and skin retention (ER_{SC} 1.7). Stearylamine, with a longer side chain, produced the least activity ($ER_{Q_{24}}$ 8.15, and ER_{SC} 0.6). Azone with its cyclic structure and dodecyl side chain was the best of the standard enhancers tested producing an $ER_{Q_{24}}$ of 38.30 and ER_{SC} of 1.5.

Aungst et al. (1986) reported the effects of dodecylamine and stearylamine on naloxone flux. Dodecylamine was a more effective enhancer compared to stearylamine. These authors did not report any data on didodecylamine. The same rank order of enhancement was also reported for indomethacin and 5-fluorouracil (Aungst et al., 1990). These experiments were performed on human cadaver skin which is known to show less enhancement than hairless mouse skin, which is one of the most permeable skin type models. These authors also concluded that the best enhancements were seen with compounds containing C_{12} hydrophobic chains.

For the secondary amines **1–3**, $ER_{Q_{24}}$ values were 8.21, 10.83, and 7.66, the highest being observed for the compound containing a 2-methoxyethyl side chain (**2**). The highest skin steroid retention was also produced by **2** (ER_{SC} 2.0). The ER_{SC} for skin retention for **1** was 1.2 and for **3** was 1.5.

Tertiary amines **4–6** were also prepared and again, for $ER_{Q_{24}}$ the most active compound was **5**, the structure possessing the 2-methoxyethyl side chain. $ER_{Q_{24}}$ for **4** was 5.52, for **5** was 21.00, and for **6** was 10.61. However, for skin retention, **6** showed highest activity (ER_{SC} 1.5) while **4** and **5** were below control (ER_{SC} 0.6 for both). The most active tertiary amine for skin retention possessed a 2,2-dimethoxyethyl side chain. It should also be noted that **5**, a tertiary amine had a $ER_{Q_{24}}$ value twice that of the corresponding secondary amine, **2**. However, skin retention ER_{SC} values decreased from 2.0 (for **2**) to 0.6 (for **5**).

These tertiary amines (**4–6**) may also be compared with *N*-dodecyl-*N*-(2-hydroxyethyl) acetamide, *N*-dodecyl-*N*-(2-methoxyethyl) acetamide, and *N*-dodecyl-*N*-(2,2-dimethoxyethyl) acetamide, enhancers which we have published previously (Michniak et al., 1994a). In both cases, the structure containing the 2-methoxyethyl side chain exhibited the highest activity for $ER_{Q_{24}}$. *N*-Dodecyl-*N*-(2-methoxyethyl) acetamide exhib-

ited the highest skin retention (ER_{SC} 4.3), with lower ER values for *N*-dodecyl-*N*-(2-hydroxyethyl) acetamide (1.7), and *N*-dodecyl-*N*-(2,2-dimethoxyethyl) acetamide (1.8).

Compounds **7–9** were five-, six-, and seven-membered ring analogs with similar Q_{24} and skin retention values (Q_{24}: $ER_{Q_{24}}$ 6.05 for **7**; 8.50 for **8**; 10.02 for **9**, and skin steroid retention: ER_{SC} 0.9 for **7–9**). There was no correlation between the ER values for **7–9** and those for *N*-dodecyl-2-pyrrolidinone, *N*-dodecyl-2-piperidinone, and *N*-dodecylpyrrolidine-2-thione which also have been published previously (Michniak et al., 1993b). In all three cases, reduction from an amide to an amine decreased ER_{SC} values for skin steroid retention. The ER_{SC} for **7–9** was 0.9, for *N*-dodecyl-2-pyrrolidinone 2.2, for *N*-dodecyl-2-piperidinone 1.6, and for *N*-dodecylpyrrolidine-2-thione 2.1. Also, Q_{24} values for **7–9** were lower than those for the three previously published enhancers. All three compounds (**7–9**) produced similar skin retentions (0.9), however, $ER_{Q_{24}}$ values were lowest for the pyrrolidine ring-containing enhancer (**7**, $ER_{Q_{24}}$: 6.05) and highest for the homopiperidine ring-containing compound (**9**, $ER_{Q_{24}}$: 10.02). For the piperidine containing compound **8**, $ER_{Q_{24}}$ was 8.50.

Compound **10** was the reduced form of *N*-(1-oxododecyl)morpholine, again published previously (Michniak et al., 1993b). Reduction again resulted in an overall loss of flux-enhancing activity. Q_{24} and skin retention ER values for **10** were 12.76 and 1.6, respectively, and for *N*-(1-oxododecyl)morpholine were 29.35 and 1.0, respectively.

Surprisingly, reduction of *N*-(1-oxododecyl)diethanolamine to compound **11**, which was the most polar of this series, greatly enhanced transdermal flux. $ER_{Q_{24}}$ for **11** was 56.16, the highest for all the compounds tested including Azone. Studies using *N*-(1-oxododecyl)diethanolamine and published previously, produced an $ER_{Q_{24}}$ of 9.94. The corresponding ER_{SC} for skin retention for this compound was 1.0. Skin retention for **11**, however, was much lower (ER_{SC} 0.5).

Compound **12** (*E,Z*)-*O*-methyl-1-dodecyloxime, a chemical class not previously examined for flux-enhancing properties, gave disappointing ER values for both Q_{24} (5.90) and skin steroid content (0.7).

This comparison of the data for the enhancers in the present work to their corresponding amides enables us to make several conclusions. First, with the exception of compound **11**, the alkyl amines are poorer flux enhancers than their amide counterparts, whether cyclic or acyclic. Second, it is possible that the amine counterpart of a weakly active amide flux enhancer, e.g., compound **11**, may show enhanced activity. The only structure-activity relationship that may be noted from this short series of compounds is that tertiary cyclic amine and secondary amine enhancers were less active for increasing flux than tertiary acyclic amine enhancers. It should be noted that the overall geometry of an enhancer may be less important for flux enhancement than its lipophilicity.

It has been suggested that enhancers such as these long chain alkyl amine enhancers exert their action by one or more of the following mechanisms: (a) interaction with stratum corneum lipids; (b) solvent action which directly solubilizes tissue components; (c) interaction with intracellular protein layers within the stratum corneum; and (d) increasing the partitioning of the drug into the skin (Barry, 1988, 1991). Work is presently in progress to evaluate partitioning and possible mode(s) of action of these compounds using such techniques such as differential scanning calorimetry (DSC), FTIR spectroscopy, and small angle X-ray scattering (SAXS) techniques. These have been used successfully by others (Bouwstra et al., 1989; Mak et al., 1990; Brain et al., 1993).

It should be noted that all these compounds have the potential of inducing skin damage and studies will be performed to examine the irritation potential, particularly of the free amines (Lashmar et al., 1988; Hadgraft et al., 1993).

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