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The enhancement index concept applied to terpene penetration enhancers for human skin and model lipophilic (oestradiol) and hydrophilic (5-fluorouracil) drugs

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Summary

A series of cyclic monoterpenes has been assessed as skin penetration enhancers towards a model lipophilic drug, oestradiol. In vitro permeation experiments on human epidermal membranes showed that the terpenes varied in their activities; hydrocarbon (e.g., limonene) and cyclic ether (e.g., 1,8-cineole) terpenes were effective accelerants providing approximately 4-fold increases in the permeability coefficient of aqueous oestradiol, whereas alcohols (e.g., carveol), ketones (e.g., menthone) and epoxides (e.g., pinene oxide) were ineffective. The results of this study are compared with terpene activities towards a model hydrophilic drug, 5-fluorouracil. A novel concept, the enhancement index (EI), is introduced to compare differences in terpene activities towards the two permeants; EI provides information as to the partition coefficient and maximum achievable permeation enhancement for a drug, together with a measure of a penetration enhancer's activity towards that drug expressed as a percentage of the maximum effect. This approach permits useful comparisons between the activities of various enhancers towards different drugs.

Introduction

The rate determining step for transdermal delivery of most drugs is provided by the stratum corneum (Scheuplein, 1965). Its structure has been depicted in the brick and mortar model (Michaels et al., 1975; Elias, 1981) in which anucleate keratinised cells are embedded in a lipid mortar. The stratum corneum lipids are arranged in multiple bilayers providing alternate hydrophobic and hydrophilic barriers. Drugs must diffuse through the intercellular lipid matrix, and to reduce reversibly the resistance of this pathway researchers employ penetration enhancers (or accelerants). These materials interact reversibly with stratum corneum constituents to disrupt the highly ordered structure and hence facilitate drug diffusion. Many established penetration enhancers are synthetic chemicals which are not yet approved by regulatory authorities for use with drugs. Recently, a novel series of penetration enhancers, classed as terpenes or terpenoids, has been described (Williams and Barry, 1989, 1990). These

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chemicals may provide a series of safe, naturally occurring penetration enhancers whose toxicities are well documented (e.g., Opdyke, 1974–1976). Several terpenes were shown to be effective accelerants for the hydrophilic cytotoxic drug, 5-fluorouracil (Williams and Barry, 1991). The present study extends the investigation to the effects of some terpenes on transdermal permeation of a model lipophilic drug, oestradiol (ES).

Topical oestrogens are employed when endogenous hormones are lacking, such as in postmenopausal women. A transdermal oestradiol patch, Estraderm TTS, has recently been developed to treat menopausal symptoms. Clinical trials have indicated that transdermal delivery holds many advantages over oral oestrogen administration, including reduced variation in serum hormone concentrations, a more normal oestrone : oestradiol ratio and minimal pharmacological effects on hepatic proteins (Powers et al., 1985; Crust et al., 1989; Yum, 1989).

Our report also compares the activities of terpene penetration enhancers towards the model hydrophobic drug (oestradiol) and the model hydrophilic drug (5-fluorouracil). A novel concept, the enhancement index (EI), is used to compare accelerant actions for the two drugs; it is hoped that such an approach may be of value with a wide variety of drugs and enhancers. A significant advantage of the method is that it allows an assessment of the maximum benefit which can be expected in chemically enhancing the skin permeation of a particular drug.

Materials and Methods

The terpenes used as received were α-pinene, 3-carene, terpinen-4-ol, carveol, carvone, pulegone, menthone, α-pinene oxide, limonene oxide, cyclohexene oxide, cyclopentene oxide and 7-oxabicyclo[2.2.1]heptane supplied by Aldrich Chemical Company, *d*-limonene and 1,8-cineole provided by Sigma Chemical Company, α-terpineol obtained from BDH Chemicals Limited and piperitone from Field and Co. Ascaridole, the main constituent of oil of chenopodium was isolated from the oil (supplied by Field and Co.) by fractional distillation in vacuo (Pinder, 1960). The chemical formulae of these terpenes are given in Fig. 1.

An assessment of the terpene purities has been published (Williams and Barry, 1991); no single impurity was present in each terpene at greater than 2%, and such traces were considered to be at sufficiently low thermodynamic activities that their effects on skin permeability would be negligible compared with that of the main terpene. As an initial assessment of accelerant activity, all terpenes were employed as neat liquids.

The model lipophilic permeant was [2,4,6,7-^{3}H(N)]oestradiol (NEN Research Products), radiochemical purity 99%. Unlabelled oestradiol (Sigma Chemical Company) was used to prepare a saturated aqueous drug solution (0.003 mg/ml at 30°C, Michaels et al., 1975).

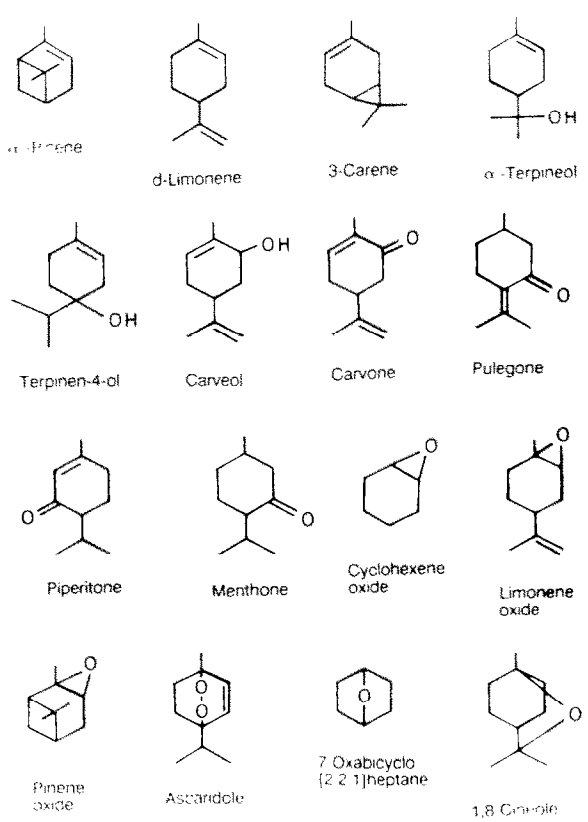

Fig. 1. The structural formulae of terpenes used in this study.

Preparation of human skin membranes

Caucasian abdominal skin (male and female, age 17–89) was obtained postmortem. Excess fatty and connective tissues were removed and the samples stored at −20°C (Harrison et al., 1984).

Full thickness membranes. Skin samples were trimmed of fatty material to provide tissue approximately 1 cm thick and essentially flat. The samples were clamped between stainless steel plates with a polythene sheet covering the stratum corneum and the membrane refrozen to adhere the fatty layer to the metal. The upper plate and polythene sheet were removed and the stratum corneum surface of the tissue was thawed slightly before a membrane, approximately 430 μm, was cut using a Duplex Electro Dermatome 7, providing a sample of full thickness skin comprising stratum corneum, nucleate epidermis and some dermal tissue.

Stripped full thickness membranes. Samples of full thickness skin with the stratum corneum removed were prepared by tape stripping (Clipper tape). Typically 25–30 strippings were required to remove the stratum corneum from full thickness skin membranes. Fully hydrated stratum corneum comprises approximately 30 μm of the membrane, hence the resulting stripped full thickness tissue provided a sample approximately 400 μm thick.

Epidermal membranes. Epidermal membranes, incorporating the anucleate stratum corneum and nucleate epidermal tissue, were prepared by a heat separation technique (Kligman and Christophers, 1963). Skin samples trimmed of fatty tissue were immersed in water at 60°C for 45 s, after which the epidermal membranes were teased off the underlying dermis. The membranes were floated on an aqueous solution of 0.002% sodium azide for 36 h to ensure full hydration of the stratum corneum.

Stratum corneum membranes. Stratum corneum samples were prepared from epidermal membranes (Kligman and Christophers, 1963); epidermal membranes were floated overnight on an aqueous solution of trypsin (0.0001% w/v) and sodium hydrogen carbonate (0.5% w/v) at 37°C. The enzyme digests the nucleate epidermal tissue allowing the remnants to be removed by swabbing. The stratum corneum membranes were floated on water before use to ensure full tissue hydration.

A diagrammatical representation of the skin membranes used in this study is shown in Fig. 2.

Permeation experiments

Experiments at 32 ± 1°C used an automated diffusion apparatus with 24 stainless-steel diffusion cells (diffusional area 0.126 cm^2) and 0.002% aqueous sodium azide as flow-through receptor solution (Akhter et al., 1984).

Fully hydrated epidermal membrane samples were mounted in the cells and treated with 150 μl aliquots of saturated radiolabelled ES. To ensure saturation of the donor solution a crystal of ES, with the same radioactivity as the drug solution, was placed in each donor compartment, and the donor drug solution was replenished every 8 h. Under these conditions, ES is at maximal

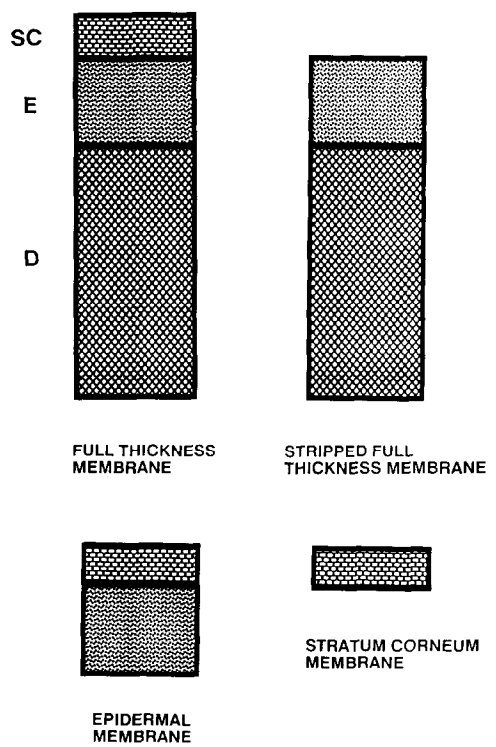

Fig. 2. A diagrammatical representation of skin membranes employed in this study (not to scale). SC, stratum corneum; E, nucleate epidermis; D, dermis.

thermodynamic activity throughout the diffusion experiment with negligible donor depletion. Samples of the receptor solution (2 ml) were collected every hour for 24 h, to which 5 ml OptiPhase HiSafe II scintillation fluid (Pharmacia) was added, and the radiolabelled drug determined by liquid scintillation counting (Packard Tri-Carb 460). The permeant solution was washed from the membrane with 0.002% aqueous sodium azide and replaced with 150 μl of a terpene. After 12 h treatment, the terpene was washed from the membrane and ES permeation was redetermined as above. Linear regression analysis of the pseudo steady-state diffusion results after the lag time allows evaluation of the permeability coefficient (Kp) of the drug in the membrane before and after terpene treatment. As a measure of the penetration enhancing activity of the terpenes, the enhancement ratio (ER) was calculated as (Goodman and Barry, 1988):

$$ER = \frac{\text{Kp after terpene treatment}}{\text{Kp before terpene treatment}} \quad (1)$$

Values reported are mean ratios from 4–13 replicates.

Permeation across the epidermis and dermis and clearance into the aqueous receptor fluid may provide the rate determining step in the permeation of very highly lipophilic drugs. The barrier to ES diffusion was therefore investigated using skin membranes composed of a variety of layers: stratum corneum alone, epidermis (including stratum corneum), full thickness skin (stratum corneum, nucleate epidermis and some dermal tissue) and tape stripped full thickness skin (stratum corneum removed). Oestradiol is not a very highly lipophilic drug (log P octanol/water = 2.29) and has a small but significant aqueous solubility (0.003 mg/ml at 30°C). Thus, removal of the drug into the flow through receptor fluid is unlikely to provide a significant resistance to drug permeation, but passage across epidermal/dermal tissue may.

Partitioning experiments

The method used to assess the effects of terpenes on ES partitioning into stratum corneum membranes was as described previously (Williams and Barry, 1991). Fully hydrated stratum corneum samples were equilibrated in a terpene for 12 h. The tissues were blotted dry and placed in a saturated radiolabelled aqueous solution of ES for 4 h. The samples were blotted dry, solubilised and the drug determined by liquid scintillation counting. Triplicate partition coefficients (stratum corneum/water) were determined using tissue samples from three different human sources. Controls were stratum corneum samples untreated with terpene. The results were expressed as a partition ratio P_R where:

$$P_R = \frac{\text{partition coefficient after terpene treatment}}{\text{partition coefficient with untreated membrane}} \quad (2)$$

Solubility studies

The solubility of ES in the terpenes was determined by the method of Williams and Barry (1991). Terpenes were saturated with radiolabelled crystals of ES and the saturated drug concentration determined in triplicate by liquid scintillation counting.

Results and Discussion

The experimental design for permeation studies of determining Kp, treating the membranes with a terpene and then redetermining Kp, allows each piece of tissue to act as its own control, thereby reducing errors due to the biological variability of human skin. The conditions for drug delivery were optimised by the use of saturated drug solutions, replenished every 8 h, which maintains the permeant at near maximal thermodynamic activity. Typical permeation profiles under these conditions are in Fig. 3. From the diffusion experiments, the mean permeability coefficient of aqueous ES through normal (untreated) human epidermal membranes at 32°C is $3.68 \pm 0.36 \times 10^{-3}$ cm/h ($n = 144$). This result shows good agreement with literature values of 3.2×10^{-3} cm/h (Goodman and Barry, 1988) and 5.2×10^{-3} cm/h (Michaels et al., 1975; Flynn and Stewart, 1988).

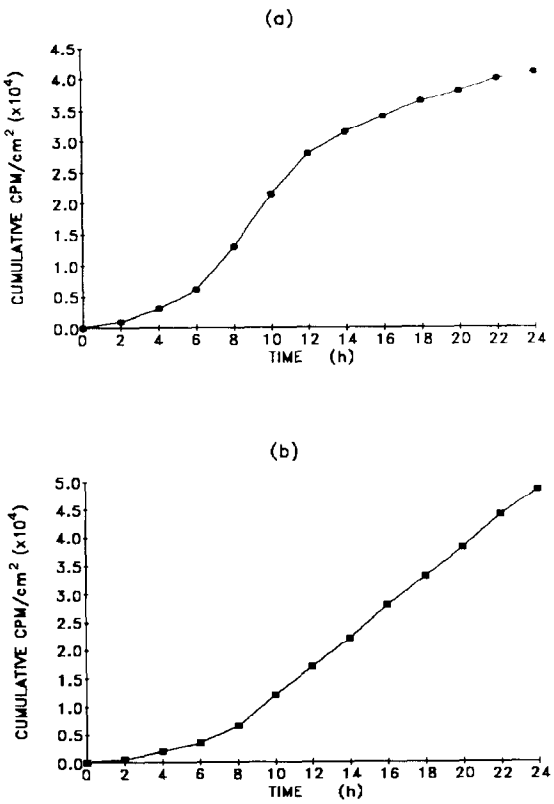

Fig. 3. Typical permeation profiles of aqueous oestradiol through human epidermal membranes showing the effects of donor replenishment. a. Donor not replenished. b. Donor replenished. Every second experimental value plotted.

For highly lipophilic drugs, the rate determining step in transdermal permeation may reside in the partitioning process into the essentially aqueous nucleate epidermis and dermis, and/or in clearance from the skin into the systemic circulation. To investigate the rate limiting step for ES permeation, a variety of skin membranes were employed in diffusion experiments. Relevant data are summarised in Table 1.

These results illustrate no significant difference in ES permeation through stratum corneum, epidermal or full thickness membrane with an aqueous receptor fluid ($P = 0.05$). This clearly illustrates that intact stratum corneum is the main barrier to oestradiol permeation. The resistance to drug permeation provided by various skin layers may be calculated as the reciprocal of the drug permeability coefficient. It should be noted that the resistance of each membrane includes those contributions arising from donor and receptor stationary layers. Due to the natural variability of human skin, no significant difference exists between the resistances of stratum corneum, epidermal and full thickness membranes ($P = 0.05$). However, the stratum corneum provides significantly greater resistance to drug permeation than stripped full thickness skin (E/D in Table 1; $P = 0.05$). After removal of the stratum corneum the permeability coefficient of ES increases by a factor of 5 compared with full thickness skin with the stratum corneum intact. This result implies that the *maximum* enhancement effect that an accelerant such as a terpene may induce is a 5-fold increase in permeability corresponding to full removal of the barrier resistance of the stratum corneum. Clearly, if the barrier nature of the stratum corneum is diminished by the use of penetration enhancers, then the resistance provided by epidermal/dermal tissue will exert a proportionally greater influence on oestradiol permeation.

Table 2 shows data on penetration enhancing activities of the terpenes. These results demonstrate that the hydrocarbon terpenes are acceler-

TABLE 1

The permeability coefficient (Kp) of aqueous oestradiol permeating through various layers of human skin, with SE

Membrane	Description	Kp (cm/h, $\times 10^3$)	Tissue resistance (h/cm)	*n*
SC	Stratum corneum	4.47 ± 0.77	224 ± 39	3
SC/E	Epidermal	3.68 ± 0.36	272 ± 27	144
SC/E/D	Full thickness	2.45 ± 0.42	408 ± 89	6
E/D	Stripped full thickness	13.0 ± 1.23	76.9 ± 7.8	8

SC, stratum corneum; E, epidermis; D, dermis; *n*, number of replicates.

TABLE 2

The mean permeability coefficients (Kp), with standard error of the mean, of aqueous oestradiol through epidermal membranes before and after treatment with a terpene, with mean (and SE) enhancement ratios (ER) and the number of replicates (n)

Terpene	Kp (cm/h, $\times 10^3$)		ER	*n*
	Initial (control)	Treated		
Hydrocarbons				
α-Pinene	3.72 ± 0.61	10.1 ± 0.93	3.09 ± 0.89	12
d-Limonene	2.89 ± 0.47	8.40 ± 0.41	3.75 ± 1.32	12
3-Carene	1.57 ± 0.32	6.57 ± 0.69	4.36 ± 1.02	4
Alcohols				
α-Terpineol	5.98 ± 0.73	2.24 ± 0.38	0.33 ± 0.10	6
Terpinen-4-ol	2.52 ± 0.49	1.63 ± 0.68	0.45 ± 0.10	5
Carveol	2.36 ± 0.49	1.06 ± 0.31	0.42 ± 0.10	9
Ketones				
Carvone	4.09 ± 1.06	0.40 ± 0.17	0.10 ± 0.04	9
Pulegone	3.01 ± 1.11	0.98 ± 0.26	0.34 ± 0.08	13
Piperitone	3.54 ± 0.89	0.63 ± 0.18	0.17 ± 0.02	5
Menthone	3.22 ± 0.71	1.01 ± 0.35	0.36 ± 0.17	7
Oxides				
Cyclohexene oxide	3.33 ± 0.59	4.68 ± 0.84	1.42 ± 0.75	6
α-Pinene oxide	2.68 ± 0.31	5.14 ± 1.29	1.90 ± 0.87	5
Limonene oxide	2.29 ± 0.64	3.46 ± 1.00	1.61 ± 0.76	5
Ascaridole	3.29 ± 0.87	15.8 ± 4.12	4.75 ± 1.34	8
7-Oxabicyclo-[2.2.1]heptane	2.94 ± 0.99	12.7 ± 3.43	4.93 ± 1.50	4
1,8-Cineole	3.26 ± 1.24	15.1 ± 3.78	4.40 ± 0.57	7

ants for the lipophilic drug, providing enhancement ratios of between 3 and 4. The alcohol and ketone terpenes did not enhance oestradiol permeation and may in fact hinder passage of the lipophilic drug. The oxide terpenes show varied accelerant activities; cyclohexene oxide, limonene oxide and α-pinene oxide provide no significant increase in oestradiol permeation ($P = 0.05$) whereas ascaridole, 7-oxabicyclo[2.2.1]heptane and 1,8-cineole all induce a 4–5-fold increase in diffusion of the lipophilic drug. It is interesting to note that the ineffective oxide terpenes all contain a 1,2-oxygen linkage and may broadly be classified as epoxides. The effective oxide terpenes contain 1,4-(ascaridole, 7-oxabicyclo[2.2.1]-heptane) or 1,8-(1,8-cineole) oxygen linkages and may be classed as cyclic ethers. It has been suggested that structural conformations may be a factor in determining penetration enhancer activities of terpenes (Williams and Barry, 1991).

The mechanisms underlying terpene penetration enhancement were investigated by partitioning and solubility studies. Terpene effects on oestradiol partitioning into isolated fully hydrated stratum corneum membranes were assessed (Table 3). The control (non-terpene treated) partition coefficient (P) was 14.5, providing a log P (stratum corneum/water) of 1.16. Literature P values include 46 for human stratum corneum (Scheuplein et al., 1969), 58.9 for male abdominal hairless mouse skin (Valia and Chien, 1984) and 80 for rat stratum corneum (Wepierre et al., 1990). The partition coefficient (octanol/water), which gives a rank order approximation to partitioning into the stratum corneum (Barry, 1983), for oestradiol is 195. Thus, the results from this

TABLE 3

The effects of terpenes on the partitioning of oestradiol from aqueous solution into fully hydrated stratum corneum membranes, and the solubility of oestradiol in the terpenes

Terpene	P ± SE	P_R ± SE	Solubility (mg/ml)
Control (water)	14.5 ± 1.79	1.00	0.003
α-Pinene	13.6 ± 0.19	0.99 ± 0.15	0.011
d-Limonene	13.5 ± 0.77	0.96 ± 0.08	0.025
3-Carene	32.1 ± 3.02	1.93 ± 0.17	0.046
α-Terpineol	54.6 ± 19.9	3.29 ± 1.21	5.00
Terpinen-4-ol	29.5 ± 3.56	2.04 ± 0.06	6.96
Carveol	28.3 ± 3.95	2.10 ± 0.46	5.27
Carvone	47.4 ± 5.94	3.31 ± 0.25	12.80
Pulegone	61.7 ± 2.06	4.56 ± 0.81	12.33
Piperitone	56.0 ± 5.51	3.94 ± 0.27	13.06
Menthone	100 ± 14.1	7.26 ± 1.15	5.60
Cyclohexene oxide	23.5 ± 1.76	1.68 ± 0.18	7.55
α-Pinene oxide	30.6 ± 3.25	2.34 ± 0.60	6.83
Limonene oxide	37.7 ± 5.71	2.65 ± 0.30	7.36
Ascaridole	71.8 ± 14.9	4.30 ± 0.86	0.291
7-Oxabicyclo[2.2.1]heptane	21.3 ± 0.66	1.57 ± 0.27	64.57
1,8-Cineole	30.0 ± 2.37	2.11 ± 0.12	8.02

P, partition coefficient, stratum corneum/water, mean of 3 replicates, with SE. P_R = partition ratio = P/control = P/14.5, mean of 3 individual values, with SE.

study and the literature confirm that the stratum corneum provides a more polar environment than octanol.

Terpene treatment generally increases partitioning of the lipophilic drug into the stratum corneum, as illustrated by the partition ratio, P_R, where:

$$P_R = \frac{\text{partition coefficient after terpene treatment}}{\text{partition coefficient with untreated membrane}} \quad (3)$$

However, it is evident from Table 3 that treatment with *d*-limonene and α-pinene does not improve such partitioning whereas the other hydrocarbon tested, 3-carene, provides a near doubling in partitioning. Improved partitioning would be expected considering the solubility of ES in the terpenes; the drug is more soluble in all the terpenes than in water, and is more soluble in oxygen-containing terpenes than in the hydrocarbons (Table 3). No mathematical relationship exists between the partition ratios and drug solubilities, although a trend is apparent with the hydrocarbons having little or no effect on partitioning and providing a drug solubility of 0.011–0.046 mg/ml compared with the ketones which improve partitioning approximately 4-fold and provide a greater drug solubility of 5.6–13 mg/ml.

Terpene effects on the apparent lag time to pseudo steady-state diffusion have been used to gain an insight into the accelerant effects towards 5-fluorouracil permeation (Williams and Barry, 1991). However, oestradiol permeation through untreated epidermal membranes provides a relatively short lag time of 4.42 ± 0.36 h (SE; $n = 93$). Consequently, variations in the apparent lag times are not sufficient for a critical analysis of the modes of action of terpene penetration enhancers. The inconsistent nature of the apparent lag time, due to the biological variability of human skin, is well illustrated by the above value; the 93 replicate values provide a standard deviation of 79%. Additionally, the lag time above is considerably lower than literature values of 19 h (Mollgaard and Hoelgaard, 1983) and 103 h (Scheuplein et al., 1969).

The oestradiol work has shown that hydrocarbon and cyclic ether terpenes are effective accelerants whereas alcohols, ketones and epoxides

are not. Hydrocarbon terpenes, including limonene, increased percutaneous absorption of indomethacin in rats (Nagai et al., 1989; Okabe et al., 1989), by direct effects on the barrier nature of skin. This implies that the hydrocarbons act by increasing diffusivity of the drug in the stratum corneum. Indomethacin (log P octanol/water 4.27), like oestradiol (log P 2.29), is a lipophilic drug and the authors reported that oxygen-containing terpenes, such as carvone, α-terpineol and 1,8-cineole were ineffective. Our results partially support this view; hydrocarbon terpenes promoted ES permeation whereas alcohol and ketone terpenes did not. However, this study also demonstrated that the cyclic ether terpenes (ascaridole, 7-oxabicyclo[2.2.1]heptane and 1,8-cineole) promoted ES permeation across human epidermal membranes, to a similar extent as the hydrocarbons. Another study employing lipophilic drugs and terpene penetration enhancers found that terpenes containing polar groups did not act as enhancers (Hori et al., 1989).

A comparison of the terpene activities towards the polar drug 5-fluorouracil (Williams and Barry, 1991) and oestradiol is given in Fig. 4a. The terpenes apparently are more active towards 5-fluorouracil (5-FU) than towards ES. The enhancement ratios of 1,8-cineole, an effective accelerant for both drugs, are 94.5 and 4.40 for 5-FU and ES respectively. However, a comparison between these results may be misleading, particularly in respect of the apparent low value for ES, as the scope for enhancement of these drugs varies considerably. The enhancement ratio for 5-FU provided by tape stripping the stratum corneum from full thickness human skin is 1045; the corresponding value for ES is only 5.3. We can deal with this consideration by treating the skin as a laminate with barriers in series.

The resistance (R) of intact, full thickness human skin to drug diffusion may be described mathematically by (Barry, 1983):

$$R = \frac{h_{SC}}{D_{SC}K_{SC}} + \frac{h_E}{D_E K_E} + \frac{h_D}{D_D K_D} \qquad (4)$$

where the subscripts SC, E and D refer to the stratum corneum, nucleate epidermis and dermis

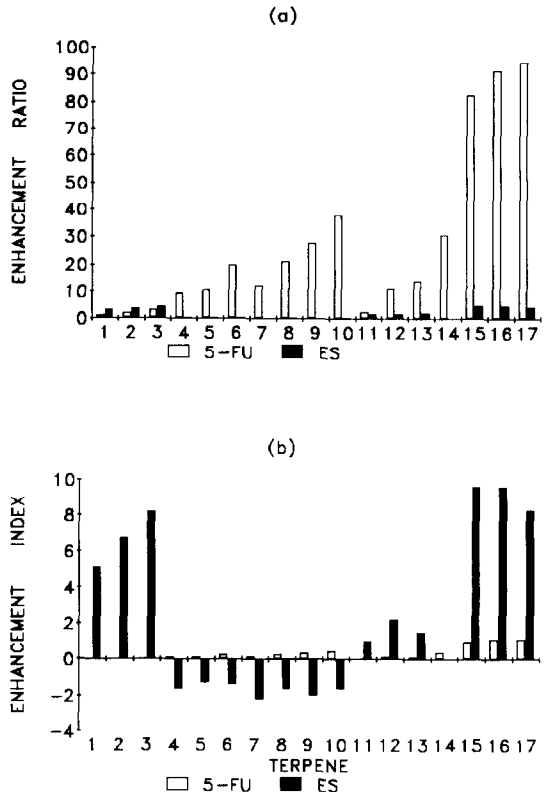

Fig. 4. a. The mean enhancement ratios of terpenes towards 5-fluorouracil and oestradiol. b. The enhancement indices calculated from Eqn 7. 1, α-pinene; 2, d-limonene; 3, 3-carene; 4, α-terpineol; 5, terpinen-4-ol; 6, carveol; 7, carvone; 8, pulegone; 9, piperitone; 10, menthone; 11, cyclohexene oxide; 12, limonene oxide; 13, α-pinene oxide; 14, cyclopentene oxide; 15, ascaridole; 16, 7-oxabicyclo[2.2.1]heptane; 17, 1,8-cineole.

respectively, h, D and K refer to the thickness, diffusion coefficient and partition coefficient of the various layers, and stationary layers and clearance are neglected.

Assuming that the nucleate epidermis and dermis provide similar barrier properties per unit thickness, then:

$$R = \frac{h_{SC}}{D_{SC}K_{SC}} + \frac{h}{DK} \qquad (5)$$

where $h = h_E + h_D$, $D = D_E = D_D$ and $K = K_E = K_D$. A problem with tape stripping studies arises from the mechanical weakness of epidermal

membranes; it is not practical to tape-strip the stratum corneum from epidermal membranes. Thus, the maximum enhancement ratios for the drugs obtained with the barrier layer removed were determined using stripped full thickness skin samples, approximately 400 μm thick. These ER values are then compared with data obtained using epidermal membranes, approximately 80 μm thick of which approximately 30 μm is fully hydrated stratum corneum and 50 μm nucleate epidermis (Marks, 1981; Sato et al., 1991). Since the nucleate epidermal tissue represents approximately one eighth of the stripped full thickness skin, then for epidermal membranes used in diffusion studies Eqn 5 may be rewritten as:

$$R = \frac{h_{SC}}{D_{SC}K_{SC}} + \frac{h}{8DK} \qquad (6)$$

With the stratum corneum intact, drug permeation across the bulk of the skin (nucleate epidermis and dermis) is rapid for hydrophilic and moderately lipophilic molecules compared with passage across the stratum corneum. However, when the barrier layer is removed, permeation across the nucleate epidermal and dermal tissue becomes rate limiting. Assuming that the epidermis and dermis provide similar resistances to drug permeation per unit thickness, a correction may be made for the differences in skin sample thickness between the two experimental protocols; the permeability coefficients of the drugs will be approximately 8 times greater through 50 μm of nucleate epidermis than through 400 μm epidermal/dermal tissue. Thus, the maximum achievable enhancement ratios through nucleate epidermal membranes can be corrected to approximately 8400 for 5-FU and 42 for ES. Clearly these two values are approximations, but they are more appropriate than the maximum enhancement ratios taken simply from the stripped full thickness tissue studies. Literature reports of penetration enhancers seldom provide data for drug permeation through epidermal tissue with the stratum corneum removed. In such cases an estimate of drug permeability coefficients may be calculated from the drug diffusivity in aqueous systems, assuming the epidermis to be a porous hydrogel.

To describe the activities of penetration enhancers in a more informative way, we propose that an enhancement index (EI) may be useful. The maximum achievable enhancement is dependent, in part, on the partition coefficient of the model permeant. The log P (octanol/water) of the drug is thus provided as a superscript to the enhancement index, and the maximum enhancement ratio provided by stripping the stratum corneum from nucleate epidermal membranes, corrected as discussed above, is given in a subscript. These two values provide information which places into context the enhancement effect. The enhancement index is then calculated as the *fraction* of the maximum achievable enhancement ratio induced by accelerant treatment, expressed as a percentage. However, our definition of the enhancement ratio, which is the ratio of drug Kp after to that before accelerant treatment, dictates that an enhancement ratio of 1.0 indicates that an accelerant has no penetration enhancing activity. To correct for this, the enhancement index may be defined as:

$$EI^{\text{permeant log P}}_{\text{maximum ER}} = \frac{\text{(Enhancement ratio after accelerant treatment)} - 1}{\text{(Maximum enhancement ratio, stratum corneum removed)} - 1} \times 100 \qquad (7)$$

The enhancement indices of the terpenes as calculated towards the model drugs are listed in Table 4. These values show that, for example, while the enhancement ratio for 1,8-cineole towards 5-FU is large, in terms of the maximum achievable enhancement the terpene activity is low, $EI^{P\ 0.89}_{M8400} = 1.1\%$. Contrariwise, for oestradiol, although the ER is *apparently* low, $EI^{P2.29}_{M42} = 8.3\%$, i.e., the terpene shows 8 times more activity towards the lipophilic drug as compared with the polar drug based on assessments of the maximum achievable effect. Table 4 illustrates that the hydrocarbon and oxide terpenes are more active, in terms of the maximum possible enhancement, towards ES than towards 5-FU, whereas the alcohol and ketone terpenes are

TABLE 4

The enhancement ratios (ER) and indices (EI) of some monoterpenes, towards a model polar penetrant, 5-fluorouracil (log P = − 0.89) and a model lipophilic penetrant, oestradiol (log P = 2.3). EI values quoted to 2 significant figures

Terpene	5-Fluorouracil		Oestradiol	
	ER	$EI^{P=0.89}_{M8400}$ (%)	ER	$EI^{P2.3}_{M42}$ (%)
α-Pinene	1.2	0.0024	3.09	5.1
Limonene	2.1	0.013	3.75	6.7
3-Carene	3.2	0.026	4.36	8.2
α-Terpineol	9.4	0.10	0.33 [a]	− 1.6
Terpinen-4-ol	10.4	0.11	0.45 [a]	− 1.3
Carveol	20.0	0.23	0.42 [a]	− 1.4
Carvone	12.2	0.13	0.10 [a]	− 2.2
Pulegone	21.2	0.24	0.34 [a]	− 1.6
Piperitone	27.7	0.32	0.17 [a]	− 2.0
Menthone	37.9	0.44	0.36 [a]	− 1.6
Cyclohexene oxide	2.4	0.017	1.42	1.0
α-Pinene oxide	13.7	0.15	1.90	2.2
Limonene oxide	11.0	0.12	1.61	1.5
Cyclopentene oxide	30.9	0.35	–	–
Ascaridole	82.5	0.97	4.75	9.1
7-Oxabicyclo-[2.2.1]heptane	91.7	1.1	4.93	9.6
1,8-Cineole	94.5	1.1	4.40	8.3

[a] Enhancement ratio less than 1.00.

P, log partition coefficient of the permeant; M, maximum enhancement with stratum corneum removed.

more active towards the polar drug. For ES, the hydrocarbon and cyclic ether terpenes produce between 5 and 10% of the maximum enhancement, i.e., up to 10% of the stratum corneum resistance is removed, a value at which the resistance of other skin layers or drug clearance into the receptor fluid may become significant. For 5-FU, the hydrocarbons remove less than 0.1% of the barrier resistance and even the relatively effective cyclic ether terpenes remove only 1% of the stratum corneum resistance. 'Accelerants' providing enhancement ratios of less than 1.0 inhibit drug permeation. Such agents provide a negative enhancement index.

The use of enhancement indices demonstrates that comparisons between values obtained from permeation studies may at first sight be misleading and could be viewed more profitably with respect to the maximum permeation rate a molecule may achieve through skin after all the stratum corneum resistance is removed. This is illustrated in Fig. 4 which compares the enhancement ratios and enhancement indices of the terpenes towards the two drugs. An apparently effective penetration enhancer, increasing the drug permeability coefficient markedly, may actually be poor in relation to the maximum values achievable, although the marked increase in drug flux may still prove to be clinically acceptable. Thus, the ER provides a practical measure of enhancement while the EI informs the investigator how close he has approached the limit of complete chemical removal of the stratum corneum barrier.

Another way of looking at the phenomenon is simply that hydrophilic drugs have great potential for enhancement because their permeability coefficients are low; lipophilic drugs have much less potential because their unmodified coefficients already approach a maximum value (Flynn and Stewart, 1988).

The expression of penetration enhancement by the enhancement index implies the assumptions: (1) that the accelerant acts only to alter the barrier nature of the stratum corneum; (2) that the stratum corneum is the rate limiting barrier to drug diffusion; and (3) that the vehicle does not alter the solvent nature of nucleate epidermal/dermal tissue in tape stripped skin.

This third assumption merits further consideration. When aqueous vehicles are employed in permeation studies, the maximum drug flux may be obtained through stripped full thickness skin (stratum corneum removed) as described in this report; assuming that the nucleate epidermis and dermis are porous hydrogels, then no appreciable changes in the solvent nature of the skin samples should occur on removal of the stratum corneum and on direct contact with aqueous donor. Similarly, for non-aqueous vehicles which are essentially immiscible with dermal tissue (and water), for example liquid paraffin, permeant diffusion through skin with the stratum corneum removed should also be capable of experimental determination. However, for non-aqueous miscible vehicles such as ethanol, DMSO, propylene glycol, etc., the solvent may partition into the epidermis

or dermis in sufficient quantities to alter the solvent nature of the tissue. For example, an ethanolic vehicle for drug delivery may not drastically alter the solvent properties of epidermal/dermal tissue when applied to skin with an intact stratum corneum, but with the horny layer removed the vehicle may radically alter the solvent nature of the remaining skin thus modifying partitioning and solubilisation phenomena. Hence, with a non-aqueous miscible system the partition coefficients for epidermal and dermal tissues will be altered (K_E and K_D in Eqn 4, respectively). In vivo, to attempt to increase drug permeation by altering the solvent nature of the epidermis and dermis would presumably cause irritation of the viable tissue and hence be impracticable for clinical use.

When using non-aqueous miscible vehicles such as ethanol, the term $h/8DK$ (Eqn 6) may be calculated theoretically: D can be approximated to the diffusion coefficient through thickened water or a protein gel with allowance for the volume fraction of free water in the gel; diffusion coefficients through nucleate epidermis or dermis are of the order of one-tenth those in bulk water (Flynn, 1989). The thickness, h, can be measured directly. For K, the partition coefficient may be calculated as:

$$K = \frac{\text{solubility of drug in water (or protein gel)}}{\text{solubility in applied vehicle}} \qquad (8)$$

Thus, for Eqn 6 we can calculate the resistance and its reciprocal, the permeability coefficient, of nucleate epidermal membranes (stratum corneum removed) to a drug applied from a vehicle such as ethanol or propylene glycol.

In conclusion, this study has shown that some cyclic monoterpenes are effective penetration enhancers for oestradiol. Hydrocarbon and cyclic ether terpenes induced a near 4-fold increase in drug permeability coefficient in human epidermal membranes, but other terpenes (alcohols, ketones and epoxides) provided no enhancement for this lipophilic drug. The terpenes may act by increasing diffusivity of the drug in the stratum corneum, and cyclic ether terpenes increased drug partitioning.

A novel concept for interpretation of permeation enhancement data has been introduced. The penetration enhancing abilities of chemicals may be more usefully described if viewed with respect to the maximum achievable drug permeation, i.e., that with the barrier layer of the skin removed. This concept has been formalised as an enhancement index which provides information as to the log partition coefficient (octanol/water) and the maximum achievable permeation enhancement of a drug, together with a measure of a penetration enhancer's activity towards the permeant expressed as a percentage of the maximum achievable effect.

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