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Enhancement by Terpenes of 5-Fluorouracil Permeation through the Stratum Corneum: Model Solvent Approach

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Abstract

5-Fluorouracil permeates the stratum corneum through the intercellular pathway. 5-Fluorouracil is hydrophilic and, therefore, its partitioning from the aqueous region into the hydrocarbon interior of stratum corneum lipids is expected to be an important stage of its permeation and a target for some permeation enhancers. It has also been reported that complexation plays a role in the enhancement effect of some accelerants. These mechanisms have been investigated.

For partitioning-permeation studies, isooctane was chosen as a model of the hydrocarbon interior of stratum corneum lipid bilayers and the effects of 26 different terpene enhancers on the solubility of 5-fluorouracil in isooctane were measured. Results were then compared with the effects of the same enhancers on the permeation of 5-fluorouracil through the epidermis in man. The stoichiometry of interaction of cineole and limonene with 5-fluorouracil were also studied to reveal possible complex formation. Solubility studies revealed good correlation between solubility and enhancement ratios for the majority of terpenes, indicating that one mechanism by which terpenes increase permeation of the stratum corneum by 5-fluorouracil is by improvement of partitioning. Stoichiometry studies showed that cineole can form 1 : 1 or higher complexes with 5-fluorouracil. With limonene, only a weak 1 : 1 complex was indicated. Data obtained using epidermis from man show that the enhancement effect of cineole toward 5-fluorouracil is much higher than that of limonene.

These data reveal that terpenes might increase the permeation of 5-fluorouracil through the stratum corneum as a result of complex formation and a form of facilitated transport.

Before any topically applied drug can act either locally or systemically it must permeate the stratum corneum, the barrier of the skin which is usually its outermost layer (Berenson & Burch 1951). The stratum corneum is a multilayered wall-like structure in which keratin-rich corneocytes are embedded in an intercellular lipid-rich matrix (Figure 1). In this two-compartment system the only continuous phase is the intercellular domain in which the lipids are arranged in bilayers (Elias et al 1979). There are two possible pathways of drug permeation through the intact stratum corneum, intercellular and transcellular. The intercellular route is considered to be of major importance for permeation of most drugs through the stratum corneum (Moghimi et al 1996).

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The success of a transdermal formulation depends on the ability of the drug to penetrate the skin barrier at a sufficient rate. Unfortunately, many drugs do not permeate sufficiently well and for many years investigators have researched the usefulness of different methods of promoting skin permeation; one of these is addition of chemicals (enhancers or accelerants) to the skin. According to the lipid-protein-partitioning theory of skin penetration enhancement (Barry 1991), one way enhancers might act is to improve drug partitioning into the stratum corneum. This mechanism is the subject of this paper.

There is abundant evidence that at all levels of organization—membrane, cell and whole animal—the lipophilicity of compounds, which is normally

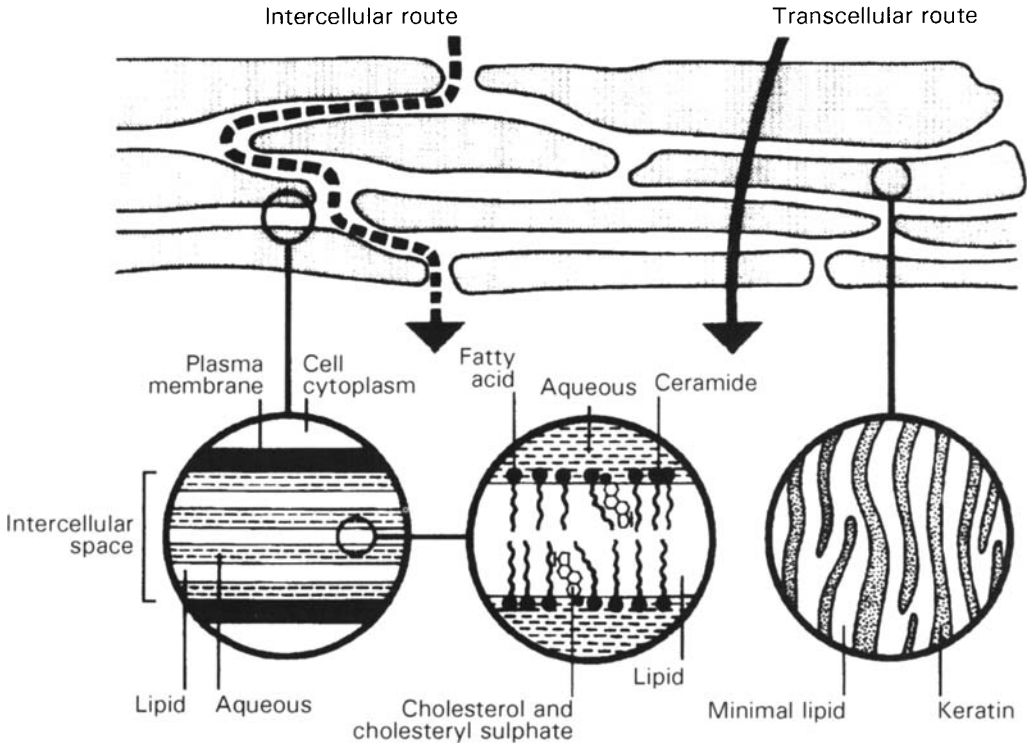

Figure 1. Schematic representation of the brick and mortar model of the stratum corneum and a very simplified lamellar organization of intercellular domains in which only major stratum corneum lipids are shown. Also illustrated are possible pathways of drug permeation through the intact stratum corneum.

defined by a partition coefficient, plays an important role in the biological activity of drugs (Hansch & Dunn 1972). As far as permeation through the lamellar structure of biological membranes is concerned the partition coefficient of drugs between the hydrocarbon interior of lipid bilayers and the aqueous domain plays an important role, and hence partitioning might be the rate-determining step in drug permeation (Stein 1981, 1986).

Studies using a lamellar mesomorphic matrix as a model for stratum corneum intercellular lipids provided evidence that 5-fluorouracil permeates the stratum corneum through the intercellular pathway (Moghimi et al 1996). Because 5-fluorouracil (Figure 2) is a hydrophilic drug (the octanol–water and isooctane–water partition coefficients of 5-fluorouracil are 0·150 and $1{\cdot}03 \times 10^{-4}$, respectively; Moghimi 1995), partitioning from the aqueous region and polar head-groups of the lamellar structure into the essentially hydrocarbon interior of lipid bilayers might possibly be an important step in the permeation of 5-fluorouracil through the intercellular lipids, and possibly a target for some penetration enhancers. This work investigates such a mechanism. In such an endeavour it is logical to study the effects of a wide range of chemical species. Because a variety of mono-, sesqui- and diterpenes and structurally similar chemicals of different chemical classes (hydrocarbons, ketones, ethers and alcohols) act as enhancers of the penetration of 5-fluorouracil through epidermal membranes in man (Williams 1990; Williams & Barry 1991; Cornwell 1993; Cornwell & Barry 1994), these compounds (26 terpenes and related substances) were used for studies of solubility-permeability relationships.

It is possible that some penetration enhancers act by increasing the partitioning of drug into the intercellular lipids via complexation and thereby increase the permeation of drugs by a form of facilitated transport. It was suggested that fatty acids and propranolol form complexes which readily partition into stratum corneum lipids and diffuse down to the viable epidermis where the complex dissociates in the interfacial region and the freed propranolol partitions in the water-rich

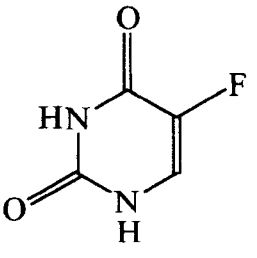

Figure 2. The molecular structure of 5-fluorouracil.

viable tissue (Ogiso & Shintani 1990). This mechanism was also investigated here for the action of two terpene penetration enhancers towards permeation of 5-fluorouracil through the stratum corneum.

Materials and Methods

Materials

Tritium-labelled 5-[6-^{3}H]fluorouracil in ethanol–water (7 : 3) with a radiochemical purity of 99% was supplied by NEN (DuPont) Research Products (Germany). Unlabelled 5-fluorouracil (99%), 2,2,4-trimethylpentane (isooctane, 99·7%+), 1*R*-(+)-α-pinene (98%), α-terpineol (97·8%), (+)-terpinen-4-ol (97%), (−)-carveol (mixture of isomers, 97%), geraniol (98%+), nerolidol (98%), farnesol (mixture of isomers, 95%), phytol (mixture of isomers, 98·9%), (−)-menthone (90%), *R*-(−)-carvone (98%), 1*R*-(−)-fenchone (98%+), (+)–limonene oxide (mixture of isomers, 97%), (−)-α-pinene oxide (98%), 7-oxabicyclo[2.2.1]heptane (98·5%), cyclopentene oxide (98%) and cyclohexene oxide (98%) were supplied by Aldrich (UK). 1,8-Cineole (99·5%), (+)–limonene (99·2%) and safrole (90%) were supplied by Sigma (UK). (+)-β-Cedrene (99·2%), (+)-longifolene (99·5%), (+)-aromadendrene (97·6%), (−)-*trans*-caryophyllene (99%),

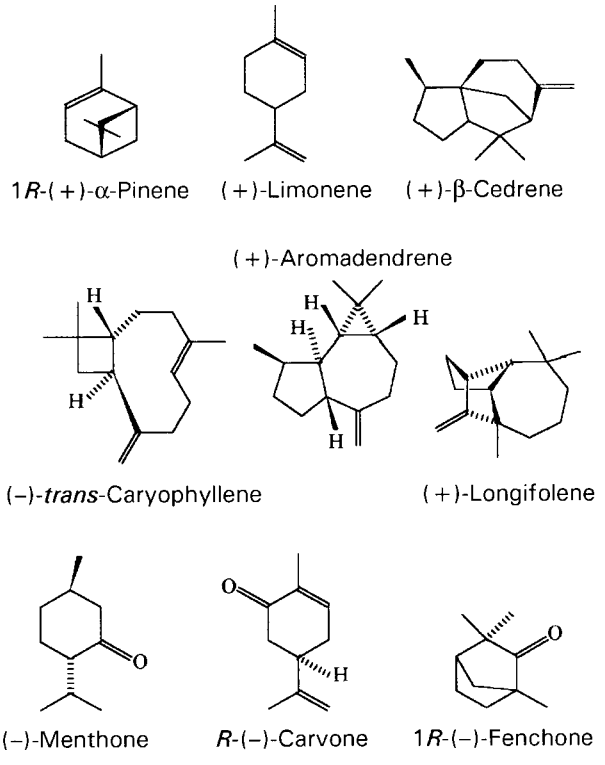

Figure 3. The chemical structures of hydrocarbon and ketone terpenes.

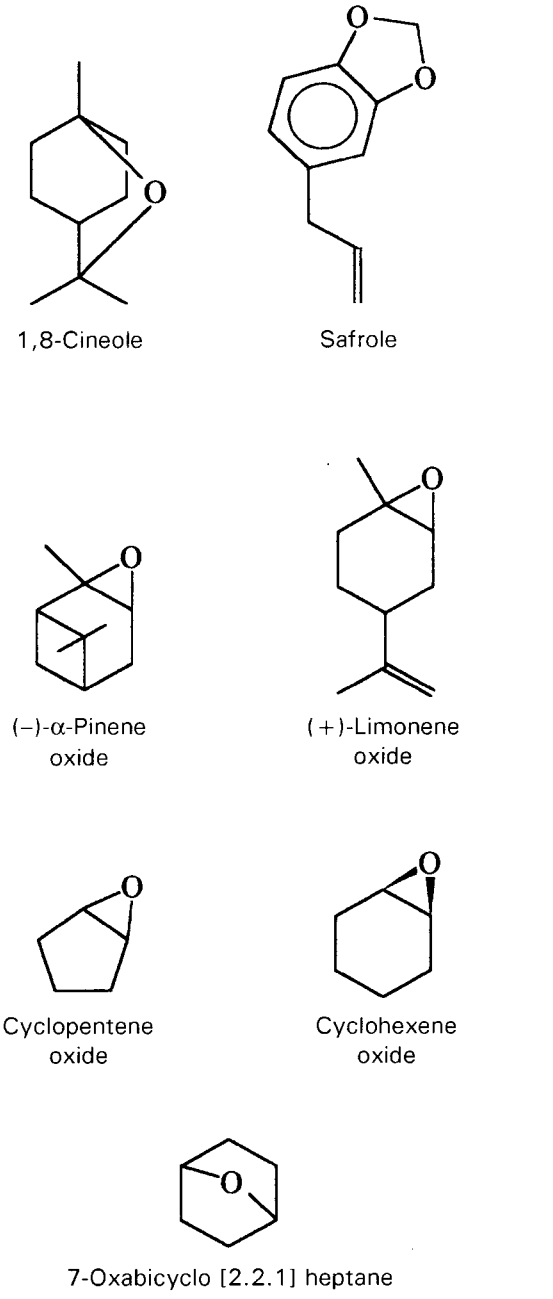

Figure 4. The chemical structures of ether terpenes and structurally related compounds.

(−)-guaiol (98·7%) and (+)-cedrol (99%+) were supplied by Fluka (Switzerland). (−)-α-Bisabolol (95%) was supplied by BASF Aktiengesellschaft (Germany). OptiPhase Hisafe 3 scintillation fluid was supplied by Fisons (UK) and 0·2 μm pore-size, 13-mm diameter PTFE membrane filters were purchased from Whatman (UK). The chemical structures of the terpenes and related substances are illustrated in Figure 3 (hydrocarbons and ketones), Figure 4 (ethers) and Figure 5 (alcohols).

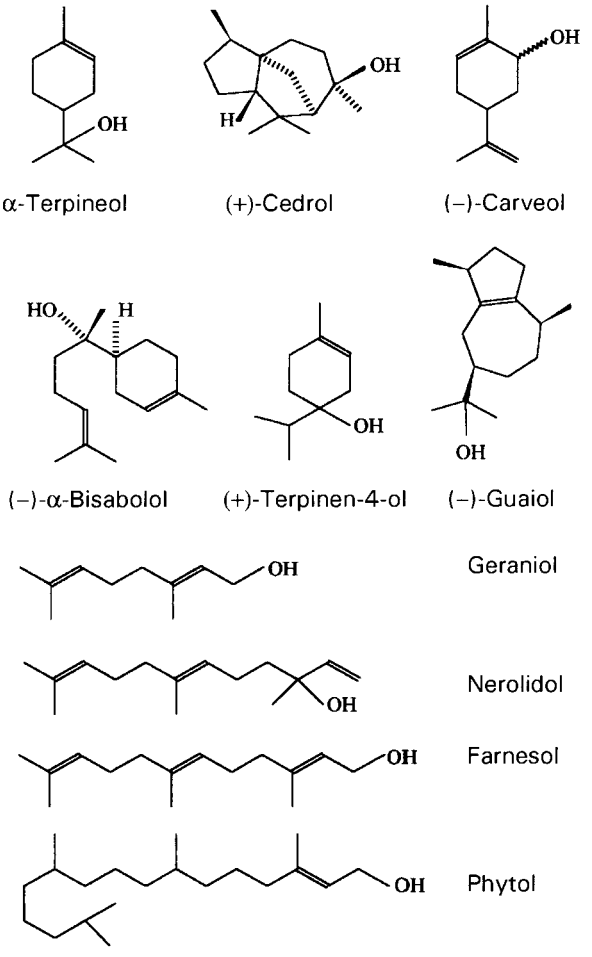

Figure 5. The chemical structures of alcohol terpenes.

Effects of terpenes on the solubility of 5-fluorouracil in isooctane

Isooctane, a non-polar, non-interactive hydrocarbon for which abundant solubility data are available in the literature (e.g. Grant & Higuchi 1990), was chosen as a simple model for the hydrocarbon interior of stratum corneum lipid bilayers. The solubilities of 5-fluorouracil in isooctane and isooctane–terpene mixtures were measured at 25°C as explained below. Solubility ratios (SR) were calculated as the solubilities of 5-fluorouracil in isooctane–terpene systems divided by the solubility of 5-fluorouracil in isooctane. Solubility ratios were compared with enhancement ratios (ER) measured at 32°C (the temperature of the stratum corneum). ER is defined as the permeability coefficient of 5-fluorouracil through enhancer-treated epidermis divided by that through untreated samples. Solubility studies were performed at a standard laboratory temperature of 25°C; we assumed that the difference between solubility ratios at 25 and 32°C is negligible, i.e. increasing the temperature increases the solubility in both solvent systems to approximately the same extent over this narrow temperature range.

For such an investigation a measure of the uptake of enhancers by intercellular lipids is needed. There have been no extensive studies of the exact amounts of terpenes taken up by stratum corneum intercellular lipids and these are not easily measured. Cornwell et al (1996) determined the uptake of limonene, cineole and nerolidol by the stratum corneum in man and from these results it was calculated that the concentration of these materials in stratum corneum intercellular lipids might reach 40–60% (w/w) of tissue treated with the neat terpenes. Accordingly, concentrations in isooctane of 3–4 M for monoterpenes and 1·5 M for sesqui- and diterpenes, which give a terpene concentration of approximately 40–60% (w/w), were used here. For solid terpenes, saturated solutions in isooctane were employed to correlate with permeation studies.

Stoichiometry

Solubility studies in mixed solvents can provide information about specific interactions (e.g. stoichiometry and stability constants). Limonene (a hydrocarbon monoterpene) and cineole (an ether monoterpene) were chosen for a preliminary study of the complexation behaviour of terpenes with 5-fluorouracil. Alcohols were not used because their self-association makes data interpretation difficult. Isooctane (a non-polar, non-interactive solvent) was maintained saturated with 5-fluorouracil (solute). Incremental amounts of cineole or limonene were added as interactive co-solvents or ligands to form a complex (or complexes) with the solute and the total amount of solute in solution was determined (see below). The concentrations used were 0·03–5·9 M for cineole and 0·03–6·1 M for limonene, in isooctane. Results were then interpreted to determine orders of complexes and stability constants from graphs based on models discussed by Fung & Higuchi (1971), Anderson et al (1980) and Grant & Higuchi (1990).

Solubility measurements

For each solubility determination, a methanolic solution of radiolabelled 5-fluorouracil (5 mg mL^{-1}, 0·04 mCi mL^{-1}; 1 mL) was evaporated to dryness under vacuum. Samples of isooctane, terpene, or isooctane–terpene mixture (6 mL) were then added and the systems were heated at 40°C for 30 min to dissolve as much drug as possible. Mixtures were then equilibrated with stirring for approximately 72 h in a water-bath at 25°C ± 0·1°C and the equilibrated suspension was filtered through a PTFE membrane. The first 1 mL of filtrate was discarded and then triplicate 1 mL

samples were used for drug determination by liquid scintillation counting by means of a Tri-Carb liquid scintillation analyser (model 1600 TR, Packard, USA). OptiPhase Hisafe 3 scintillation fluid (5–10 mL) was added to give a clear mixture. Radioactivity counts min^{-1} were then measured. Because water and other solvents quench sample activity, the volumes of quenching agents in all sample and standard vials were maintained constant.

The possibility of tritium exchange between the labelled drug and other components (e.g. water and alcohols) is a concern in such experiments. Here, tritium is covalently bound to a carbon atom and therefore its exchange with hydrogen atoms of other molecules is unlikely; Cornwell (1993) showed experimentally that there was no significant tritium label exchange between 5-[6-^{3}H]fluorouracil and water over prolonged periods.

Results and Discussion

Effect of terpenes on the solubility of 5-fluorouracil in isooctane—relation to permeation enhancement

Table 1 lists the solubility of 5-fluorouracil in isooctane and isooctane–terpene systems. All terpenes increased the solubility of 5-fluorouracil in isooctane; hydrocarbons had the least effect (solubility ratio (SR) = 3–21), alcohols were the strongest solubilizers (SR up to 2620) and the effects of ketones and ethers were between those of the hydrocarbons and alcohols (SR 63–215).

Relative solubilities of polar solutes in both polar and non-polar organic solvents are often unrelated to bulk properties of pure components but are highly sensitive to the functional groups of interacting molecules. When specific molecular interactions (e.g. hydrogen bonding) occur these are often the dominant factors in determining solubility. Abundant evidence supports the existence of molecular complexes between various hydrogen donors and hydrogen acceptors even for a weak hydrogen acceptor such as benzene (Fung & Higuchi 1971; Anderson et al 1980; Grant & Higuchi 1990).

All of the hydrocarbon terpenes used here have at least one carbon–carbon double bond in their structures (Figure 3). Because the π electrons, being exposed, are very polarizable (Grant & Higuchi 1990), terpene hydrocarbons can interact with 5-fluorouracil through dipole-induced-dipole or Debye forces. Despite some statements propounding their unimportance, Debye interactions might be crucial in explaining and promoting

Table 1. Effect of terpenes on the solubility of 5-fluorouracil in isooctane at 25°C and on the permeation of 5-fluorouracil through epidermal membranes of man at 32°C.

No.	Compound	Chemical type	Terpene molarity in isooctane	5-Fluorouracil solubility (μM)*	Solubility ratio†	Enhancement ratio‡
Isooctane		Hydrocarbon	0·0	3·5 ± 0·7	–	–
1	(+)-β-Cedrene	Hydrocarbon	1·5	11 ± 4	3·1	2·7§
2	(−)-*trans*-Caryophyllene	Hydrocarbon	1·5	20 ± 13	5·7	2·0§
3	1*R*-(+)-α-Pinene	Hydrocarbon	3·0	27 ± 11	7·7	1·2¶
4	(+)-Limonene	Hydrocarbon	3·0	33 ± 6	9·4	2·1¶
5	(+)-Longifolene	Hydrocarbon	1·5	34 ± 11	9·7	1·7§
6	(−)-Guaiol	Alcohol	Saturated	65 ± 23	19	3·8§
7	(+)-Aromadendrene	Hydrocarbon	1·5	72 ± 22	21	2·5§
8	Safrole	Ether	3·0	219 ± 79	63	5·0**
9	(+)-Cedrol	Alcohol	Saturated	293 ± 62	84	4·6§
10	*R*-(−)-Carvone	Ketone	3·0	341 ± 85	97	12¶
11	(+)-Limonene oxide	Ether	3·0	349 ± 52	100	11¶
12	Cyclopentene oxide	Ether	3·0	411 ± 125	117	31¶
13	(−)-Menthone	Ketone	3·0	429 ± 120	123	38¶
14	Cyclohexene oxide	Ether	3·0	473 ± 118	135	2·4¶
15	(−)-α-Pinene oxide	Ether	3·0	480 ± 113	137	14¶
16	1*R*-(−)-Fenchone	Ketone	3·0	502 ± 148	143	7·8**
17	1,8-Cineole	Ether	4·0	577 ± 14	165	94¶
18	7-Oxabicyclo[2.2.1]heptane	Ether	3·0	752 ± 161	215	92¶
19	Phytol	Alcohol	1·5	1325 ± 261	379	3·4††
20	Farnesol	Alcohol	1·5	1584 ± 271	453	14§
21	Nerolidol	Alcohol	1·5	1922 ± 480	549	23§
22	(−)-Carveol	Alcohol	3·0	2377 ± 683	679	20¶
23	(−)-α-Bisabolol	Alcohol	1·5	2418 ± 548	691	8·4§
24	Geraniol	Alcohol	3·0	3097 ± 748	885	18††
25	α-Terpineol	Alcohol	3·0	5920 ± 1184	1691	9·4¶
26	(+)-Terpinen-4-ol	Alcohol	3·0	9169 ± 2017	2620	10¶

* Mean ± s.d., n = 3–9. † Isooctane–terpene/isooctane solubilities. ‡ Terpene-treated/untreated permeability coefficients. § From Cornwell & Barry (1994). ¶ From Williams & Barry (1991). ** From Williams (1990). †† From Cornwell (1993).

selectivity in solubility phenomena (Grant & Higuchi 1990). Therefore, the increases in the solubility of 5-fluorouracil in isooctane in the presence of hydrocarbons might be attributable to Debye forces. Both 5-fluorouracil and alcohols can accept or donate and ketones and ethers can accept hydrogen bonds. Therefore the increase in the solubility of 5-fluorouracil in isooctane in the presence of these groups might be attributed to complexation of 5-fluorouracil with these terpenes as a result of hydrogen bonding. Solubility results indicate that the interaction of 5-fluorouracil with alcohols (Figure 5) is more than that with ketones (Figure 3) or ethers (Figure 4).

The permeability coefficient of 5-fluorouracil through the epidermis in man from its saturated aqueous solution at 32°C is $2{\cdot}46 \times 10^{-5}$ cm h^{-1} (Williams & Barry 1991). Effects of 12-h treatment of the epidermis with different terpenes on the permeation of 5-fluorouracil were also studied by Williams (1990), Williams & Barry (1991), Cornwell (1993) and Cornwell & Barry (1994). These experiments were performed in-vitro at 32°C by use of a saturated aqueous solution of 5-fluorouracil as the donor phase. Table 1 lists the effects of terpenes on the permeability coefficient of 5-fluorouracil through the epidermal membranes in terms of enhancement ratios. Among these terpenes hydrocarbons, which elicited the least change in the solubility of 5-fluorouracil in isooctane, had the least effect on 5-fluorouracil permeation (ER = 1–3). Alcohols, the strongest solubilizers of 5-fluorouracil in isooctane among the terpenes used, increased the permeability of 5-fluorouracil up to 23 times. Although some ketones and ethers led to enhancement ratios less than those elicited by alcohols or hydrocarbons, the highest enhancement ratios were afforded by ketones and ethers (ER of up to 94). The effects of ketones and ethers on the solubility of 5-fluorouracil were between those of hydrocarbons and alcohols. At first glance, these results might suggest a parabolic relationship between SR and ER for the systems studied here.

Considerable evidence suggests a parabolic relationship between lipophilicity and biological activity (Hansch & Dunn 1972), including membrane transport. The permeation of alcohols and steroids through the epidermis in man is parabolically dependent on the stratum corneum–water partition coefficient (Tai & Lien 1980). The percutaneous absorption of *para*-substituted phenols increased with increasing lipophilicity and then began to decrease with further increase in lipophilicity (Bucks et al 1991). The decrease in absorption was interpreted as a change in the rate-limiting step from diffusion through the stratum corneum to transfer of the penetrant across the stratum corneum-viable epidermis interface.

Figure 6A shows the double logarithmic plot (base 10) of solubility ratio against permeability ratio for all terpenes used with a parabolic curve fitted to the data using Grafit 3.01 (Erithacus Software):

$$\log ER = -0{\cdot}24(\log SR)^2 + 1{\cdot}34(\log SR) - 0{\cdot}65 \quad (1)$$

The plot illustrates scattering of data typical of skin permeation measurements. The curve shows that as the solubility ratio increases the ER increases and

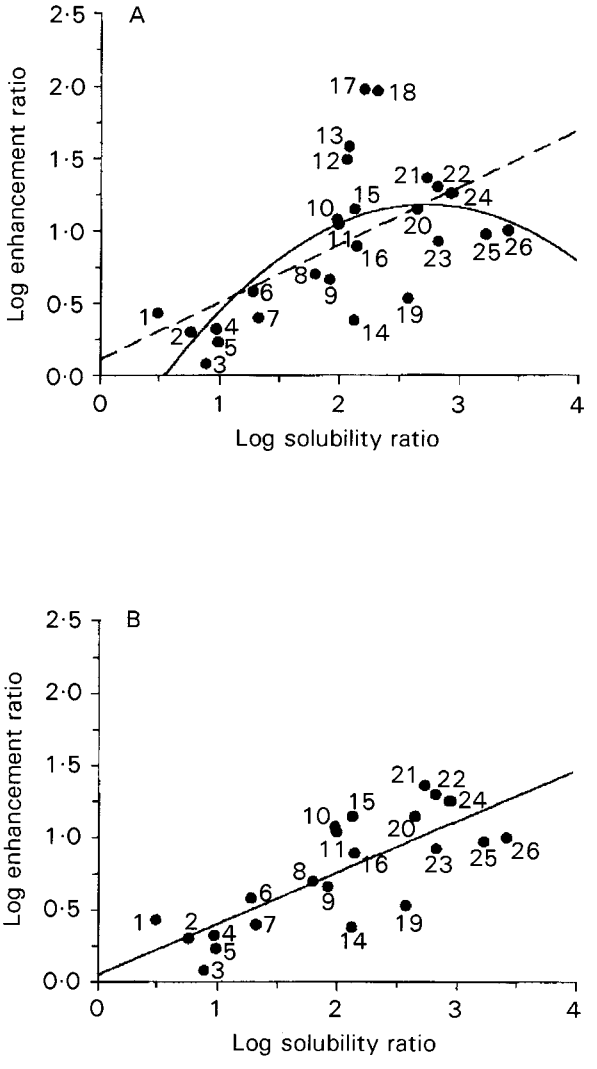

Figure 6. Relationship between enhancement ratio of 5-fluorouracil through terpene-treated epidermal membranes from man (terpene-treated/untreated permeability coefficients) and its solubility ratio in isooctane–terpene systems (isooctane–terpene–isooctane solubilities). A. Parabolic curve (—) and linear least-squares line (– – , r = 0·60) fitted to all data. B. Linear least-squares line (r = 0·78) fitted to data after exclusion of four terpenes (data points 12, 13, 17 and 18); see text for explanation. Numbering and source of permeability data as in Table 1.

reaches a maximum at an SR of approximately 500. With further SR increase the curve shows a slight decrease in the ER. If partitioning is the only mechanism involved, such behaviour suggests that partitioning from the aqueous domain into the hydrocarbon interior is the rate-limiting step in the permeation of 5-fluorouracil through intercellular lipids. Thus ER increases with SR until it reaches a maximum. A further alcohol-induced increase in the solubility of 5-fluorouracil in the hydrocarbon interior changes the rate-limiting step from partitioning into, to partitioning out of, the lipid bilayers, reducing ER. Such a phenomenon is not realistic in the systems used here if partitioning is the only mechanism acting. The parabolic fitted curve showed a maximum ER at an SR of approximately 500. Among the systems used here the highest solubility of 5-fluorouracil in isooctane–terpene systems was 1·2 mg mL^{-1} for isooctane–terpinen-4-ol (SR = 2600) which is still 10 times less than the solubility of 5-fluorouracil in water (12·2 mg mL^{-1}; Rudy & Senkowski 1973). Therefore, even in the presence of terpene alcohols, partitioning of 5-fluorouracil from hydrocarbon chains into the aqueous domain is not expected to be the rate-limiting step in the permeation of 5-fluorouracil through the intercellular lipids. Therefore, other mechanisms are probably responsible for the lower enhancement effects of alcohols relative to ethers and ketones.

Besides partitioning, diffusion through lipid bilayers also plays an important role in the permeation of drugs through the lamellar structure of biological membranes (Stein 1981). Diffusion is a size-dependent process and an increase in the diffusant volume as a result of molecular associations (e.g. hydrogen bonding) reduces the diffusion coefficient (Stein 1986; Erkey et al 1991). Although Stokesian diffusion in fluids is only slightly size-dependent (the diffusion coefficient is inversely proportional to the cube root of the molecular volume), non-Stokesian diffusion (e.g. diffusion through lipid bilayers) is substantially size-dependent (Stein 1986). For example, whereas resistance to diffusion of water, methanol, ethanol, urea, *n*-propanol and glycerol (maximum 5-fold difference in molecular weight) through the membranes of red cells from man (non-Stokesian diffusion) is steeply exponentially dependent on size, the resistance to diffusion of H_2, N_2, O_2, CO_2, urea, glycerol and glucose (up to almost 90-fold different in molecular weight) in water (Stokesian diffusion) is relatively insensitive to size (Stein 1986). Stein (1986) showed that the parabolic relationship between permeation of homologous series of *n*-alkanols through dog red-cell membranes and hexadecane–water partition coefficients on double logarithmic axes changed to a linear relationship after correction for size. 5-Fluorouracil can accept and donate hydrogen bonds at more than one site. Alcohols can also accept or donate hydrogen bonds. But ethers and ketones can only accept hydrogen bonds. Therefore, the lower enhancement effects of alcohols in comparison with those of ketones and ethers could be in part attributed to the larger sizes of 5-fluorouracil–alcohol complexes than those of 5-fluorouracil–ketone or 5-fluorouracil–ether complexes, possibly because of differences in the stoichiometry of the interactions. If this argument is correct, before any conclusion can be made about the importance of partitioning on the enhancement effects of these terpenes data should be corrected for size, a procedure which requires stoichiometry studies.

Other possible explanations for the higher ER of some ketones and ethers (especially cyclopentene oxide, menthone, cineole and 7-oxabicyclo[2.2.1]-heptane; data points 12, 13, 17 and 18, respectively) than of alcohols might involve their capacity to disrupt the bilayer and form pools. Thus, cineole creates liquid pools in the stratum corneum intercellular lipids and model lamellar systems, disrupts lipid bilayers and, as a result of these modifications, increases the permeation of 5-fluorouracil through such systems (Moghimi et al 1997). Preliminary studies using a synthetic membrane have also provided evidence that 7-oxabicyclo[2.2.1]heptane might act through the same mechanism (data not presented). We suppose that cyclopentene oxide and menthone for which the enhancement ratios were higher than for alcohols, act similarly. We might omit these four compounds from our solubility-permeability treatment. Stein (1986) reported a linear relationship between the logarithm of the permeability coefficients for red blood cells from man and the logarithm of drugs' hexadecane–water partition coefficients (r = 0·86). Linear correlation analysis of logER against logSR for all the compounds used in our study (Figure 6A) resulted in a correlation coefficient of 0·60 which is lower than that reported by Stein. After omission from our data of cineole, 7-oxabicyclo[2.2.1]heptane, cyclopentene oxide and menthone (for which ER values were very high) linear correlation analysis (Figure 6B, equation 2) resulted in a correlation coefficient of 0·78 which is in reasonable agreement with that reported by Stein (1986). These results indicate that the partitioning from the aqueous domain into the hydrocarbon interior of lipid bilayers might be an important step in the permeation of 5-fluorouracil through the epidermis in man and might be a target for some penetration enhancers; however, other

mechanisms are also involved. Note that omission of data points 12, 13, 17 and 18 is not simply to improve goodness-of-fit. These data have been omitted only because they are suspected to act through mechanisms other than increased partitioning.

$$\log ER = 0{\cdot}35(\log SR) + 0{\cdot}05 \quad (2)$$

For a full explanation of such data, more information is needed; enhancer concentrations in lipid bilayers are required for solubility studies. Some of the enhancers used, such as the alcohols, and in particular long straight-chain molecules, might be accommodated within lipid bilayers instead of dissolving in the hydrocarbon interior. The effects of enhancers on the corneocytes and the consistency of the lamellar intercellular structures are other parameters which should be taken into account. In such studies those enhancers which are proven to act through other mechanisms, such as pool formation or bilayer disruption, or both, should be excluded. All of these features are left for future investigations.

Stoichiometry of interaction of 5-fluorouracil and cineole in isooctane

As discussed elsewhere (Fung & Higuchi 1971; Anderson et al 1980; Grant & Higuchi 1990), when a non-interactive solvent contains solute (S) and ligand (L), and the ligand undergoes specific interactions with the solute, different solute–solute (S_n), ligand–ligand (L_m) and solute–ligand (S_nL_m) association species might exist in equilibrium (n and m refer to the stoichiometric aggregation numbers of S and L in associated species). The equilibrium concentration of each solute species follows the law of mass action. For solute–ligand complex species we can write, in general:

$$nS + mL \rightleftharpoons S_nL_m \quad (3)$$

A 1 : 1 or first-order complex between solute and ligand ($n = m = 1$) is expected at low concentrations of ligand. If such a complex is assumed, then:

$$[S]_t = [S]_0 + [(K_{1:1}[S]_0)/(1 + K_{1:1}[S]_0)][L]_t \quad (4)$$

where $[S]_t$ is the total solubility of the solute in the solution, $[S]_0$ is the intrinsic solubility in the non-interactive solvent in the absence of ligand, $K_{1:1}$ is the equilibrium constant and $[L]_t$ is the total concentration of ligand in the system. Thus, a plot of $[S]_t$ against $[L]_t$ gives a straight line with an intercept $[S]_0$ and a slope $K_{1:1}[S]/(1 + K_{1:1}[S]_0)$, from which $K_{1:1}$ can be calculated. Table 2 lists the molar solubilities of 5-fluorouracil, $[S]_t$, in isooctane–cineole mixtures containing 0·0 to 5·9 M cineole, $[L]_t$. Because cineole and isooctane are not completely miscible (there is a gap between cineole concentrations of almost 1·2 M to 3·5 M), to ensure that the mixtures used were in the miscibility range concentrations between 0·8 and 4·0 M were eliminated from the study and the data were analysed graphically as discussed above.

Table 2. Molar solubility of 5-fluorouracil in isooctane–cineole mixtures at 25°C.

Cineole concentration (M)	Solubility of 5-fluorouracil (μM)*	Solubility ratio†
0·00	3·5 ± 0·7	–
0·03	5·1 ± 2·4	1·5
0·06	9·9 ± 4·1	2·8
0·08	11 ± 4	3·1
0·30	29 ± 9	8·3
0·60	57 ± 15	16
0·80	73 ± 27	21
4·0	577 ± 14	165
4·5	839 ± 141	240
5·5	1420 ± 20	406
5·9 (pure cineole)	1723 ± 178	492

* Mean ± s.d., n = 3–9. †Isooctane–cineole/isooctane.

Figure 7A shows a graph of molar solubility of 5-fluorouracil, $[S]_t$, against cineole concentration, $[L]_t$, for the whole range of isooctane–cineole solvent systems. The graph is not linear for the whole range of cineole concentrations and the rising curve is indicative of formation of a complex (or of complexes) of higher order than S-L at high concentrations of cineole. Figure 7B is a graph of $[S]_t$ against $[L]_t$ for 5-fluorouracil solubility in isooctane–cineole for cineole concentrations of 0·0–0·8 M. In this low concentration range the relationship is linear with $r = 0{\cdot}999$, which indicates the presence of a first-order complex in the system. From the slope ($87{\cdot}9 \times 10^{-6}$) and intercept ($3{\cdot}53 \times 10^{-6}$) of this line the equilibrium constant ($K_{1:1}$) of the first-order complex between 5-fluorouracil and cineole in isooctane was calculated, by use of equation 4, to be 24·9 $dm^3\,mol^{-1}$. Owing to the lack of data on the concentration of the free ligand (cineole) in the system and the region of immiscibility, the solubility data were not analysed for determination of the stability constants of second- or higher-order complexes.

Ogiso & Shintani (1990) argued that fatty acids and propranolol form complexes which partition readily into stratum corneum lipids and diffuse down to the viable epidermis where the complex dissociates and propranolol partitions into the viable tissue. According to the current results, the same mechanism might be involved in the enhancement effects of cineole and possibly some other terpenes towards 5-fluorouracil.

Stoichiometry of interaction of 5-fluorouracil and limonene in isooctane

Table 3 lists the molar solubility, $[S]_t$, of 5-fluorouracil in isooctane–limonene mixtures containing 0·0 to 6·1 M limonene. Unlike cineole and isooctane, limonene and isooctane are completely miscible.

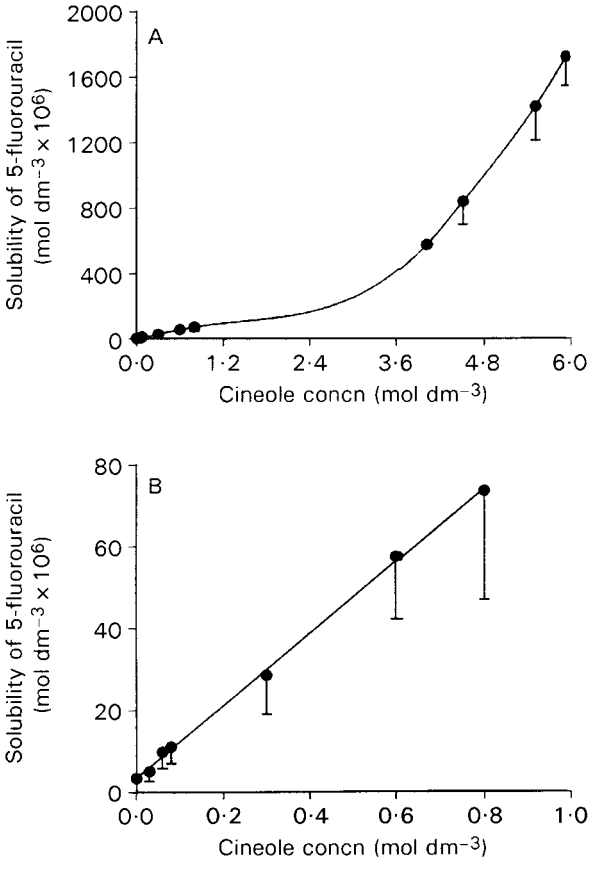

Figure 7. Complexing behaviour of cineole with 5-fluorouracil in isooctane at 25°C. A. Whole cineole concentration range in which the rising curve indicates formation of complex(es) of higher order than 1 : 1. B. Linear relationship at lower cineole concentrations (r = 0·999) shows the formation of a first-order complex. Error bar lines within the size of symbol are not shown.

Table 3. Molar solubility of 5-fluorouracil in isooctane–limonene mixtures at 25°C.

Limonene concentration (M)	Solubility of 5-fluorouracil (μM)*	Solubility ratio†
0·00	3·5 ± 0·7	–
0·03	5·9 ± 1·7	1·7
0·06	6·4 ± 2·1	1·8
0·08	7·4 ± 1·1	2·1
0·30	9·1 ± 1·0	2·6
0·60	16 ± 5	4·6
0·80	20 ± 8	5·7
3·0	33 ± 6	9·4
4·5	58 ± 4	17
6·1 (pure limonene)	80 ± 29	23

*Mean ± s.d., n = 3–9. †Isooctane–limonene/isooctane.

Figure 8 shows a graph of the molar solubility of 5-fluorouracil, $[S]_t$, against limonene concentration, $[L]_t$, for the whole range of isooctane–limonene solvent systems. The graph is linear for the whole limonene concentration range with r = 0·991 and shows that the 5-fluorouracil complex with limonene (if it exists) is first-order for the whole limonene range and that higher-order complexes either do not exist or are undetectable by this technique. From the slope ($11{\cdot}3 \times 10^{-6}$) and intercept ($6{\cdot}13 \times 10^{-6}$) of this line, $K_{1:1}$ was calculated (equation 4) to be 1·85 dm^3 mol^{-1}.

A complex might be said to be formed when, in the immediate vicinity of the solute, S, significantly more molecules of ligand, L, are found than would be predicted on a purely statistical basis. An apparent $K_{1:1}$ as much as 1 dm^3 mol^{-1} could be rationalized on a purely statistical basis and might not, therefore, reflect complex formation (Grant & Higuchi 1990). Also, when the $K_{1:1}$ values are small, non-specific contributions to the $K_{1:1}$ value also might be significant (Anderson et al 1980). The current results indicate that a 1 : 1 complex between 5-fluorouracil and limonene is a possibility.

Conclusions

Although partitioning of 5-fluorouracil between the hydrocarbon interior and the aqueous domain of stratum corneum intercellular lipids might be one factor in the permeation process and might be a target for penetration enhancers, more data are needed to understand fully the mechanisms of action of terpene penetration enhancers, such as

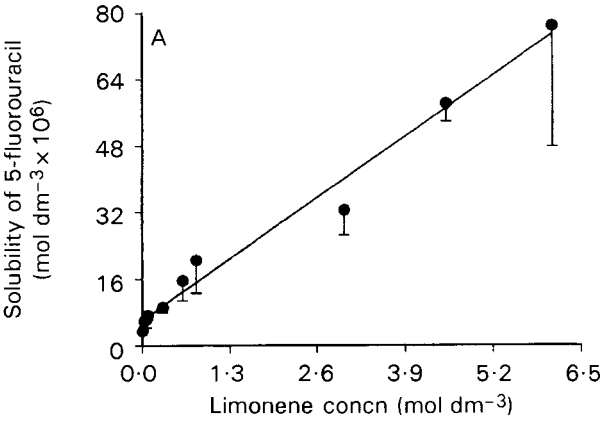

Figure 8. Molar solubility of 5-fluorouracil in isooctane in the presence of various molarities of limonene at 25°C. The straight line is the least-squares line fitted to data; r = 0·991. Error bar lines within the size of symbol are not shown.

their modification of the integrity and consistency of the intercellular lipid structure, their effects on the corneocytes, and the actual amounts of enhancers delivered to the intercellular lipid and protein domains.

Stoichiometry studies revealed that terpenes can form complexes with 5-fluorouracil. Ogiso & Shintani (1990) argued that complexation plays an important role in the enhancement effect of fatty acids towards propranolol. This mechanism might be involved to some extent in the enhancement effects of some terpenes toward a polar compound such as 5-fluorouracil.

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