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Comparative study on the *in vitro* human skin permeation of monoterpenes and phenylpropanoids applied in rose oil and in form of neat single compounds

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Essential oils are ingredients of cosmetic and health care products as well as massage oil used in aromatherapy. There is no doubt that essential oils and their components are able to permeate human skin. But information is rare dealing with percutanous absorption of essential oils in more detail. In this paper we investigated the *in vitro* skin permeation of monoterpenes and phenylpropanoids applied in pure rose oil and in form of neat single substances. We found that the application form had an exceeding influence on the skin permeation behaviour of the compounds. For substances applied in rose oil a clear relationship between their lipophilic character, chemical structure, and skin permeation could be confirmed. Regarding the P_{app}-values the substances are ranked in the order: monoterpene hydrocarbons < monoterpene alcohols < monoterpene ketons < phenylpropanoids. In contrast, for neat single substances there were no relationships between their lipophilic characters, structures and skin permeation. Furthermore, except for α-pinene and isomenthone, the P_{app}-values of all other substances were several times higher when applied in pure native rose oil than in their neat form. This suggests that co-operative interactions between essential oil components may promote skin permeation behaviour of essential oil and its components.

1. Introduction

Rose oil (rose absolute) is the essential oil extracted from the petals of damask rose, *Rosa damascena* (Rosaceae), which is widely grown in Bulgaria, Turkey, Russia, India, Iran, and China. Rose absolute is obtained through solvent extraction or supercritical carbon dioxide extraction. The oil is a yellow or colorless liquid with a deep-sweet, rich and tenacious floral rose-like odour (Arctander 1960). The complex mixture of more than 300 compounds contains mainly monoterpenes and phenylpropanoids. Besides phenylethanol, citronellol and geraniol are the major components of the blossom (Arctander 1960; Guenther 1952; Lawrence 1991).

Rose absolute is known as the queen of oils and its feminine properties make it emotionally smoothing. It is used in high-price perfumes, especially in floral and oriental bases and in small amounts to round off synthetic compositions. Rose oil is also an excellent skincare oil; it is perfect for dry and mature, but also for aged skin. It is used for palpitations, poor circulation, relieving cardiac congestion, digestive problems due to emotional upset, inflamed gallbladder and liver, jaundice, but also asthma, cough, hay fever, and sore throats (Lawless 1992; Price 1993; Rose 1992a, b; Ryman 1991; Sheppard-Hanger 1995). External application of rose oil is useful in smoothing irritated skin. Rose oil is utilized to counter depression, anxiety, grief, and negative feelings (Grieve 1971). It offers a pleasant, usually relaxing ambiance due to its very pleasant nature.

The ingredients of essential oils have lipophilic properties, and therefore essential oils are thought to be absorbed through the skin. In the middle of the last century, Strähli et al. (1940) demonstrated that after dermal application of essential oils, the essential oil compounds appeared in respired air after a certain time. Despite the frequent dermal use of rose oil the knowledge of skin permeation of the oil and its different compounds is rare. The percutaneous permeation of some terpenes has been proven in some studies (Cal 2006; Jaeger et al. 1992; Fuchs et al. 1997; Lademann et al. 2006; Schuster et al. 1986). Considering the phenylpropanoids, there is hardly any study available analyzing skin permeation of these compounds. Furthermore, question poses whether the application form has an influence on skin permeation behaviour of monoterpenes and phenylpropanoids, the bioactive compounds of rose oil. The knowledge on skin permeation of rose oil components is an important prerequisite to assess their skin and systemic bioavailability, respectively. This information is of common interest because some rose oil ingredients are suspected of genotoxic side effect.

In this study, we comparatively investigated the permeation of the major rose oil components applied in pure rose oil and in form of isolated single substances through excised abdominal human skin.

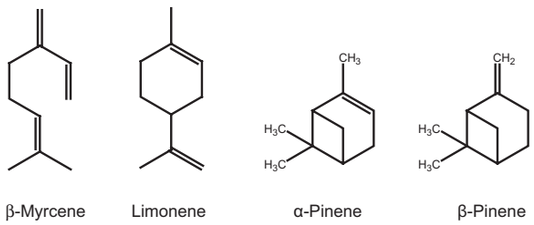
2. Investigations and results

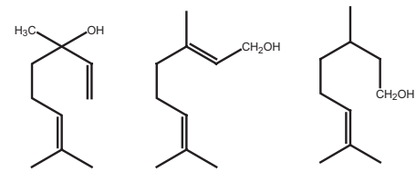
Monoterpenes such as β-myrcene, limonene, α-pinene, β-pinene, linalool, geraniol, citronellol, isomenthone are typical components of rose oil. Furthermore, rose oil comprises also the phenylpropanoids eugenol, methyleugenol, and phenylethanol.

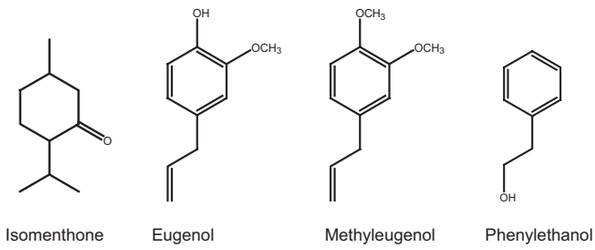
Table 1: Concentration of the main constituents of the investigated rose oil (means ± SD; n = 3)

Substances	Concentration (mg/ml)
β-Myrcene	4.584 ± 0.173
Limonene	11.724 ± 0.486
α-Pinene	7.188 ± 0.363
β-Pinene	1.067 ± 0.046
Linalool	15.742 ± 1.178
Geraniol	106.847 ± 0.696
β-Citronellol	458.838 ± 4.495
Isomenthon	1.132 ± 0.033
Eugenol	3.350 ± 0.154
Methyleugenol	2.054 ± 0.064
Phenylethanol	15.607 ± 1.993

The quantitative composition of rose oil with respect to the investigated substances is shown in Table 1.







We investigated the permeation behaviour of these compounds through heat separated human epidermis (HSHE) applied in form of pure native rose oil and neat single substances. The P_{app}-values of the investigated substances are shown in Table 2. The steady-state flux and the P_{app}-value were assessed from *in vitro* experiments in which the donor concentration of the penetrants was maintained more or less constant (infinite dose conditions) while the receptor phase provided sink-conditions. Over the time the flux increased to a steady-state value. The P_{app}-value was simply calculated from the slope of the linear portion of the graph of the cumulative amount penetrated as a function of time (Geinoz et al. 2004). The apparent permeability coefficient is described by the quotient of the flux (J) and the donor concentration (C_{Donor}). Concerning rose oil, the concentration of the main constituents in mg/ml were used as C_{Donor} values (see Table 1):

$$P_{app} = \frac{J}{C_{Donor}} \quad (1)$$

2.1. Skin permeation from native pure rose oil

Considering the ranking of the P_{app}-values of the ingredients of rose oil, the monoterpene hydrocarbons α-pinene, limonene, and β-myrcene showed only small apparent permeability coefficients ($1.43 \times 10^{-5} - 1.57 \times 10^{-5}$ cm/s). The skin permeation of the monoterpene alcohols β-citronellol, geraniol, and linalool was about twice as high ($2.74 \times 10^{-5} - 3.87 \times 10^{-5}$ cm/s) compared to the skin permeation behaviour of the hydrocarbons. It was remarkable that the monoterpene hydrocarbon β-pinene behaved different from the other hydrocarbons tested. The P_{app}-value of β-pinene was about four times higher (5.76×10^{-5} cm/s) compared to that of β-myrcene, limonene, and α-pinene. The skin permeation behaviour of the monoterpene keton isomenthone (P_{app}-value: 5.26×10^{-5} cm/s) was comparable to that of β-pinene. Considering the skin permeation behaviour of the phenylpropanoids, methyleugenol showed the smallest P_{app}-value (5.23×10^{-5} cm/s), comparable to the P_{app}-values of β-pinene and isomenthone. The P_{app}-value of phenylethanol was about twice as high (1.63×10^{-4} cm/s) as the apparent permeability coefficient of methyleugenol. The P_{app}-value of eugenol (9.14×10^{-5} cm/s) lied in between.

2.2. Skin permeation of neat single substances

Considering the ranking of the neat single substances according to their P_{app}-values it becomes evident that the skin permeation behaviour of the neat single substances was different from their behaviour as ingredients of rose oil (Table 2).
Of all substances tested, the monoterpene hydrocarbon limonene and the monoterpene alcohol geraniol showed by far the lowest permeation behaviour with P_{app}-values of 2.00×10^{-7} cm/s and 6.78×10^{-6} cm/s, respectively. In contrast, the monoterpene hydrocarbon β-myrcene (1.26×10^{-5} cm/s), the monoterpene alcohols β-citronellol (1.61×10^{-5} cm/s), linalool (2.03×10^{-5} cm/s), and the phenylpropanoids methyleugenol (1.70×10^{-5} cm/s) and eugenol (2.61×10^{-5} cm/s) clearly revealed a better skin permeation. The P_{app}-values of β-pinene (4.84×10^{-5} cm/s), phenylethanol (4.71×10^{-5} cm/s), and α-pinene (6.49×10^{-5} cm/s) were about twice and three times as high, respectively. The P_{app}-value of isomenthone was by far the highest (2.88×10^{-4} cm/s).

3. Discussion

The purpose of this study was to get more information about the *in vitro* percutaneous permeation of rose oil and its major components. Therefore, skin permeation behaviour of eight monoterpenes, namely α-pinene, limonene, β-myrcene, β-citronellol, geraniol, linalool, isomenthone and three phenylpropanoids, namely methyleugenol, eugenol, phenylethanol, applied in pure native rose oil or in form of neat single substances, were explored using heat separated human epidermis in static Franz diffusion cells. The P_{app}-values of all investigated substances are summarized in Table 2 and ranked according ascending values. Regarding the P_{app}-value ranking of the investigated substances it becomes obvious that there are some important differences in skin permeation behaviour of monoterpenes and phenylpropanoids when applied in form of a complex mixture like rose oil and neat single components, respectively.

Table 2: P_{app}-values (apparent permeability coefficients) [cm/s] of monoterpenes as ingredients of pure rose oil and as neat single substances (means ± SD; n = 12 and 3, respectively), ranked according ascending values; octanol-water-partition coefficient (log P)

Substances	P_{app} (cm/s) Pure native rose oil	Log P (25 °C)	Substances	P_{app} (cm/s) Neat single substances	Log P (25 °C)
α-Pinene	$1.43 \times 10^{-5} \pm 8.10 \times 10^{-6}$	4.37	**Limonene***	$\mathbf{2.00 \times 10^{-7} \pm 3.93 \times 10^{-8}}$	4.45
Limonene*	$\mathbf{1.54 \times 10^{-5} \pm 7.16 \times 10^{-6}}$	4.45	**Geraniol***	$\mathbf{6.78 \times 10^{-6} \pm 1.27 \times 10^{-7}}$	3.28
β-Myrcene	$1.57 \times 10^{-5} \pm 7.37 \times 10^{-6}$	4.58	β-Myrcene	$1.26 \times 10^{-5} \pm 5.57 \times 10^{-6}$	4.58
β-Citronellol	$2.74 \times 10^{-5} \pm 1.40 \times 10^{-5}$	3.38	β-Citronellol	$1.61 \times 10^{-5} \pm 1.10 \times 10^{-5}$	3.38
Geraniol*	$\mathbf{3.22 \times 10^{-5} \pm 1.53 \times 10^{-5}}$	3.28	**Methyleugenol***	$\mathbf{1.70 \times 10^{-5} \pm 5.43 \times 10^{-6}}$	2.97
Linalool*	$\mathbf{3.87 \times 10^{-5} \pm 1.48 \times 10^{-5}}$	3.28	**Linalool***	$\mathbf{2.03 \times 10^{-5} \pm 3.95 \times 10^{-6}}$	3.28
Isomenthone	$5.26 \times 10^{-5} \pm 1{,}93 \times 10^{-5}$	2.63	**Eugenol***	$2.61 \times 10^{-5} \pm 2.23 \times 10^{-6}$	2.20
Methyleugenol*	$\mathbf{5.23 \times 10^{-5} \pm 2.11 \times 10^{-5}}$	2.97	β-Pinene	$4.48 \times 10^{-5} \pm 7.26 \times 10^{-6}$	4.37
β-Pinene	$5.76 \times 10^{-5} \pm 5.17 \times 10^{-5}$	4.37	**Phenylethanol***	$\mathbf{4.71 \times 10^{-5} \pm 4.44 \times 10^{-6}}$	2.63
Eugenol*	$\mathbf{9.14 \times 10^{-5} \pm 4.67 \times 10^{-5}}$	2.20	α-Pinene	$6.49 \times 10^{-5} \pm 9.39 \times 10^{-6}$	4.37
Phenylethanol*	$\mathbf{1.63 \times 10^{-4} \pm 5.80 \times 10^{-5}}$	1.36	Isomenthone	$2.88 \times 10^{-4} \pm 1.71 \times 10^{-5}$	2.63

*$p < 0.01$, high significant differences of P_{app}-values regarding substances as ingredient of pure rose oil and as pure single substances

In case of rose oil, one can consider a clear relationship between the skin permeation behaviour, the chemical structures and the lipophilic properties of the compounds in question. Except for β-pinene, all investigated monoterpene hydrocarbons displayed the smallest permeation coefficients (1.43×10^{-5} to 1.57×10^{-5} cm/s), followed by the alcohols (2.74×10^{-5} to 3.87×10^{-5} cm/s), the keton isomenthone (5.26×10^{-5} cm/s), and the phenylpropanoids methyleugenol (5.23×10^{-5} cm/s), eugenol (9.14×10^{-5} cm/s), and phenylethanol (1.63×10^{-4} cm/s). In addition the skin permeation behaviour of the investigated substances correlate not only with molecular features but also with the octanol-water-partition coefficient (log P) (Table 2). Higher log P-values resulted in lower P_{app}-values. This means that moderately polar components, such as isomenthone, methyleugenol, eugenol and phenylethanol, had a higher permeation rate than lipophilic compounds (Roberts and Walter 1998).

Furthermore, focusing phenylpropanoids differences between chemical structures and skin permeation become more evident. Phenylpropanoids are aromatic compounds with C6-C3 skeleton. Regarding the different chemical structures of the investigated phenylpropanoids, it is likely that due to the different substituents at the aromatic ring system a steric hindrance appeared. Phenylethanol with no additional substituent penetrated skin the best. Methyleugenol with two methoxy groups at the aromatic ring system revealed the smallest P_{app}-value. This effect could also be observed in connection with the diffusion rates of various β-lactam antibiotics through the porin channels of *Escherichia coli* (Yoshimura and Nikaido 1985). The introduction of different side chains amongst methoxy groups led to a retardation of penetration through the porin channels.

Unexpectedly, the skin permeation behaviour of β-pinene was much higher than of the monoterpene hydrocarbons and alcohols. Its P_{app}-value (5.76×10^{-5} cm/s) was in the range of isomenthone and methyleugenol, both showing small log P-values. So, due to the log P-value, one could expect that the P_{app}-value of β-pinene would be similar to that of α-pinene (1.43×10^{-5} cm/s) or the other monoterpene hydrocarbons. Although differences in the skin permeation behaviour of α- and β-pinene have already been reported previously (Cal 2007; Mackay et al. 2001) the obvious discrepancy can not only be addressed to different melting points, solubility and structural differences of the compounds under consideration.

Focusing the skin permeation behaviour of the investigated neat single substances, we found evident differences in comparison to the same substances applied in rose oil. In contrast to rose oil, the ranking of the substances in question according to their P_{app}-values did not display a clear correlation between chemical structures, log P-values and skin permeation behaviour. For instance, the monoterpene hydrocarbon limonene and the monoterpene alcohol geraniol permeated the heat separated human epidermis the worst, followed by β-myrcene, β-citronellol, methyleugenol, linalool, and eugenol with P_{app}-values of 1.26×10^{-5} to 2.61×10^{-5} cm/s. In contrast to rose oil, the monoterpene hydrocarbons β-pinene and α-pinene permeated heat separated human epidermis as well as phenylethanol, the best penetrant when applied in rose oil.

Conclusion: In our study, apart from α-pinene and isomenthone, it is evident that the skin permeation of all monoterpenes and phenylpropanoids tested were several times higher when applied in pure native rose oil than in form of neat single substances; the differences were highly significant for geraniol, limonene, linalool, methyleugenol, eugenol, and phenylethanol. These findings correspond very well with data of a former investigation done by Cal and co-workers (Cal 2003; Cal and Sznitowska 2006). They found that selected monoterpenes permeated human skin barrier more effectively when applied in pure lavender oil. In a further investigation, Schmitt et al. (2009) explored the skin permeation of an artificial mixture of selected monoterpenes and phenylpropanoids. It was found that limonene revealed an enhancing effect on the skin permeation of citronellol and eugenol while β-pinene increased the P_{app}-value of methyleugenol but not of geraniol (Schmitt et al. 2009). Based on these findings, there is every reason to believe that co-operative interactions between rose oil components may enhance their skin permeation behaviour. Those interactions may also explain the differences in skin permeation behaviour of α-pinene and β-pinene when applied in rose oil. In addition, skin permeation of eugenol and methyleugenol were promoted when applied in rose oil. This result may be of common interest for further studies on the systemic bioavailability of both substances.

4. Experimental

4.1. Materials

Rose oil (Oleum Rosea verum, Rosa × damascena) was obtained from Caelo, Hilden, Germany. The ingredients of the essential oil were identified via GC/GC-MS.

The standard substances tridecane, myrcene, limonene, linalool, geraniol, β-citronellol, isomenthone, eugenol, methyleugenol, and phenylethanol were purchased from Fluka, München, Germany; α-pinene and β-pinene were obtained from Sigma-Aldrich Laborchemikalien GmbH, Steinheim, Germany and ethanol was purchased from Mallinckrodt Baker B.V.,

Deventer, Netherlands; n-hexane was obtained from Merck KG, Darmstadt, Germany, tert-butyl methyl ether from Carl Roth GmbH, Karlsruhe, Germany.

4.2. Skin samples

Abdominal skin from Caucasian female patients who had undergone plastic surgery was used. Approval from the Ethical Committee of the Ruprecht-Karls-University, Heidelberg, Germany was available. Immediately after excision the subcutaneous fatty tissue was removed using a scalpel, the skin was wrapped in aluminum foil and stored in polyethylene bags at –26 °C until use. Previous experiments had shown that neither the penetration characteristics nor the thickness of the stratum corneum (SC) were diminished after a freezing period of 3 and 6 months, respectively (Bronaugh et al. 1985; Harrison et al. 1984; Schaefer and Loth 1996). Heat-separated human epidermis (HSHE) was prepared according to Kligmann et al. (1963) for permeation experiments. Skin discs with a diameter of 30 mm were punched out of the frozen skin. After thawing, the skin pieces were cleaned with Ringer solution and put in a water bath of 60 °C for 90 s. Then the stratum corneum (SC) and the viable epidermis were carefully peeled off using forceps.

4.3. Permeation experiments

In the Franz-Diffusion Cell (FD-C) the HSHE and a cellulose membrane (MC 10000; Medicell, London, UK) were positioned between the donor compartment and the acceptor compartment containing 50% (V/V) ethanol as receptor medium. 50% ethanol was used according to the OECD Guidelines TG 428 (2004a) and No. 28 (2004b) to provide sink conditions over the whole experimental time. After 30 min of hydration, 1000 µl of native rose oil or 1000 µl of the single substances were added to the donor compartment. During the whole experimental time, the acceptor fluid was kept at 32 ± 1 °C using an incubator. The acceptor medium was permanently mixed with a magnetic stirrer (400 rpm, 7 mm). After 0, 3, 6, 9, 12, 24, and 27 h samples were withdrawn and immediately replaced by preheated acceptor fluid. The samples were analyzed via gas chromatography.

4.4. GC-Method

The samples were extracted with n-hexane/tert-butyl methyl ether (1:1) containing tridecane as internal standard. A gas chromatograph (Varian 3400) equipped with a flame ionization detector (FID) and a capillary column was used. The supplier of the column (OV-1 bonded 0.25 µm, 30m × 0.25 mm ID) was Ohio Valley Specialty Company, Marietta, Ohio, USA. The temperature of the injector was 250 °C, the split 1:30. Helium (15 psi column head pressure) was the carrier gas and the detector was heated to 300 °C. Temperature program: the initial column temperature was 40 °C (holding time 2 min), up to 105 °C the temperature was rising at 3 °C/min, up to 140 °C it was rising at 6 °C/min and up to 300 °C the temperature was rising at 10 °C/min (holding time 10 min). The data were analyzed with the software Peak Simple, Version 2.91 (SRI Instruments, Torrance, CA, USA).

4.5. GC/MS-Method

A gas chromatograph (1090 Series II, Hewlett-Packard, Bad Homburg) was directly coupled to a quadrupole mass spectrometer (SSQ 7000, Thermo-Finnigan, Bremen).
The temperature of the injector was 250 °C, the split 1:10. Helium was used as the carrier gas (15 psi column head pressure). Dimensions and phase of the used capillary column were the same like in GC (see 4.4). Mass spectra (70 eV) were recorded in MID Mode (m/z: 40–400) and analyzed with the software Xcalibur, Version 1.2, Thermo-Finnigan, Bremen). Characterization of the individual constituents of the samples was achieved by comparison of their mass spectra and GC retention indices (RIs) with that of authentic compounds.

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