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Skin Penetration Studies of *Arnica* Preparations and of their Sesquiterpene Lactones

Abstract

Alcoholic preparations of *Arnica montana* are widely used for the topical treatment of various inflammatory diseases. Sesquiterpene lactones (SLs) are mainly responsible for their anti-inflammatory activity. Here we have studied the penetration kinetics of *Arnica* tinctures prepared from dried *Arnica* flowers originating from different chemotypes as well as of their respective dominating SLs, helenalin isobutyrate and 11 α ,13-dihydrohelenalin acetate. Some alcoholic preparations of fresh *Arnica* flowers and an *Arnica* fresh plant gel were also included in the study. We used the stripping method with adhesive tape and pig skin as a model and determined the quantity of SLs in the stripped layers of the stratum corneum (SC). Thus, we observed the penetration into and permeation through this uppermost part of the skin. Whereas isolated SLs permeate through the SC only in a very small amount, permeation of SLs was much higher when they

were present in the tinctures. Furthermore, differences of permeation were observed between helenalin and dihydrohelenalin derivatives. Permeation through the SC could be determined for the tested *Arnica* preparations of fresh *Arnica* flowers with two preparations showing the best penetration behaviour of all the tested substances. Moreover, the effects of incubation time as well as of repeated applications were investigated with one preparation. Altogether, this study shows that a sufficient amount of SLs might permeate the skin barrier by using *Arnica* preparations to exert anti-inflammatory effects and that the topical use of plant preparations may be advantageous compared to the isolated compounds.

Key words

Arnica montana · Asteraceae · sesquiterpene lactones · skin penetration · herbal medicines

Introduction

Preparations of *Arnica* flowers have been extensively used in traditional medicine to treat a wide variety of different ailments. Alcoholic preparations have been approved by the German Commission E and the European Scientific Cooperative on Phytotherapy (ESCOP) for topical treatment of various inflammatory diseases [1]. The secondary metabolites that mediate these anti-inflamma-

tory effects are mainly sesquiterpene lactones (SLs) of the 10 α -methylpseudoguaianolide-type such as helenalin, 11 α ,13-dihydrohelenalin and their ester derivatives ([1] and references cited therein). The anti-inflammatory activity of *Arnica* preparations and their SLs has been proven in various *in vitro*, *ex vivo* and *in vivo* assays and most importantly also in initial clinical trials [2], [3]. Possible side effects such as contact allergy are also known [3]. However, pharmacokinetic studies have not been undertaken up to now.

Dedicated to Professor Adolf Nahrstedt on the occasion of his retirement as Editor-in-Chief of *Planta Medica*

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Here, we report on skin penetration studies of topically used *Arnica montana* preparations and their SLs. Using a stripping method with adhesive tape and pig ear skin as a model, we investigated the penetration into and the permeation through the stratum corneum (SC) [4], [5], [6]. This skin layer is regarded as the main barrier and the greatest obstacle to transdermal diffusion [7]. Pig ear skin is suitable for predicting penetration and permeation processes in human skin. The histological characteristics of pig and human skin have been reported to be comparable, with similarities in the epidermal composition, dermal structure, lipid content, histochemistry and general morphology [5], [8]. Permeability behaviours are almost the same in pig ear skin as in human skin [9].

We studied two *Arnica* tinctures from different chemotypes and their respective dominant SLs (for structures, see Fig. 1) for their penetration behaviour. To prove a possible enhancing effect of monoterpenes, which are also components in *Arnica* tinctures, solutions of both helenalin isobutyrate and 10-acetoxy-8,9-epoxythymol isobutyrate (Fig. 1) were investigated. Furthermore, four herbal medicines, three alcoholic preparations, and one gel, all prepared from fresh *Arnica* flowers, were included in the study. The effects of incubation time as well as of repeated applications were investigated with one preparation.

Materials and Methods

Materials

All organic solvents were of analytical grade and obtained from Merck (Darmstadt, Germany). Sephadex LH 20 was obtained from Pharmacia Biotech (Uppsala, Sweden). Santonin was purchased from Sigma (St. Louis, USA).

11 α ,13-Dihydrohelenalin acetate was isolated from flower heads of *Arnica montana* (Spanish chemotype) as previously described [10]. Helenalin isobutyrate was kindly provided by S. Hildebrandt (Hermal, Hamburg, Germany) and 10-acetoxy-8,9-epoxythymol isobutyrate was isolated from fresh plant extracts of *A. montana* flower heads. Identity was confirmed by NMR and MS analysis, and purity was evaluated by GC and TLC analyses.

Arnica tincture 1 (registration number 006511) was prepared from flowers of *A. montana*, Spanish chemotype, and contained mainly 11 α ,13-dihydrohelenalin esters [10]. *Arnica* tincture 2 (No. 020, deposited at the Institute of Pharmaceutical Biology, University of Freiburg) was prepared from *A. montana* flowers type "Arbo" by percolation according to the European Pharmacopoeia 1997. The tested *Arnica* preparations (preparations A, B, C (produced from *Arnica* tincture batch number 006169, no commercial products) and D (A. Vogel Rheuma Gel, batch number 009862)) were kindly provided by Bioforce (Roggwil, Switzerland) and were prepared from fresh *A. montana* flowers of type "Arbo" (information for formulation see Table 1).

Determination of the SL content in *Arnica* tinctures and in *Arnica* preparations

0.21 mg santonin (internal standard) in ethanolic solution was added to 10 mL *Arnica* tincture. After concentration under reduced pressure, further extraction and measurement was carried

Table 1 Composition (% m/m) of the *Arnica* preparations A, B, C and D

	Preparation A	Preparation B	Preparation C	Preparation D
<i>Arnica</i> tincture	50	50	50	50
Ethanol (94% m/m)	26.6			26.6
water, demineralised	23.4	30	37.5	15.9
methylhydroxypropyl-cellulose				2.5
glycerin (85% m/m)		5	5	5
medium chain triglycerides		10	2.5	
polyethylene glycol 40		5	5	

out as described [10]. 10.0 mL of the obtained solution were evaporated to dryness and the residue was dissolved in 0.5 mL methanol. This solution was measured by GC-MSD in order to identify the helenalin and dihydrohelenalin derivatives and by GC-FID for quantification. The total content of SLs was determined by addition of both the content of helenalin esters (calculated as helenalin methacrylate) and the content of 11 α ,13-dihydrohelenalin esters (calculated as 11 α ,13-dihydrohelenalin methacrylate) using the following equation [10]:

$$C_{SL} = C_{Hel} + C_{DHel} = \frac{(A_{Hel} * C_{San})}{A_{San} * 1.03} + \frac{A_{DHel} * C_{San}}{A_{San} * 0.96}$$

Equation (1)

Preparations A, B, C, and D were produced in such a way that in all cases the same *Arnica* tinctures were used at the same concentrations. The determination of SL content in preparation A was therefore sufficient.

Determination of the SL content: tincture 1 = 2.1 mM, tincture 2 = 2.5 mM, preparations A, B, C and D = 0.47 mM.

To be able to compare the penetration of the tinctures and the alcoholic solutions of SL 1 and 2 (ethanol/water, 7:3), a total content of 2.5 mM was necessary in each instance. Therefore, 10 mL of tincture 1 were evaporated and redissolved in 8.4 mL ethanol/water (7:3).

Penetration studies

Fresh ears were kindly provided by a slaughterhouse. These ears were not treated with boiling water and were used within 7 hours. The inner side of the ears was shaved and up to 3 application areas (20×50 mm) were marked with a line on each ear. A constant skin temperature of 32 °C was sustained using a thermostatic plate.

200 μ L of the sample were applied and gently distributed on the marked area. Except for the time course studies, the incubation time was always one hour. The horny layer of the epidermis was then removed by 23 strippings with adhesive tape. For each stripping, a tarred adhesive tape was applied to the marked area and pressed on with a roller (500 g) by rolling 10 times to and fro. For reasons of standardization and reproducibility, no additional

pressure was applied to the tape. After this procedure, the tape was stripped off quickly and firmly. The SC is normally removed after 16–20 strippings [6], [11]. This is indicated by the appearance of areas with a watery sheen, which comes from the stratum granulosum, the underlying layer [4].

The strips were weighed and the amount of the SC was determined by subtracting the tare. The depth of penetration was calculated by considering the area (10 cm²), assuming uniform coverage of the tapes and an approximate stratum corneum density of 1 g/cm³ [12].

Processing of strips

The SLs were redissolved by treating the strips with acetone. Santonin (2.62 µg) was added as an internal standard and the resulting samples were dried under reduced pressure. Each sample was redissolved in 200 µL methanol and centrifuged at 15 000 × g for 10 min. The supernatant was applied to a Sephadex column (2 cm³) and eluted with 2 mL methanol. The eluate was dried again under reduced pressure, redissolved in 80 µL methanol and analysed by GC. Studies with isolated SLs and *Arnica* tinctures were measured by GC-FID but studies of the preparations were measured by GC-MS because of the higher sensitivity and selectivity.

Analytical methods

The GC-MS method was validated according to [13]. A linear correlation was shown between 480 µM (160 ng on column) and 4.8 µM (1.6 ng on column; LOQ), the calibration function was $y = 0.049x + 0.0102$. A correlation coefficient of $r^2 = 0.999$ was calculated. Precision was below 3.7% and accuracy below 10.0%, even at the LOQ level. LOD ($S/N \geq 3$) was determined to be 0.48 µM (0.16 ng on column). Comparability was shown for the results of both the GC-FID and the GC-MS methods.

GC analyses were carried out with an HP6890 series GC system (Hewlett Packard, Wilmington, USA) equipped with a flame ionisation detector, using nitrogen as carrier gas. A fused silica capillary column (25 m × 0.25 mm I.D.) coated with 0.25 µm dimethylsiloxane was used (Optima 1, Machery-Nagel, Düren, Germany). The flow rate was set to 1.0 mL/min. The temperature profile started at 120 °C followed by a rate of 10 °C/min to 270 °C, which was held for 20 min. The injector and detector temperatures were 290 °C, the split ratio was set to 1 : 50, and the injection volume was 1.0 µL.

For GC-MS, an Agilent 5973 Network Mass Selective Detector (Agilent Technologies, Palo Alto, USA) at an ionisation energy of 70 eV was used. Identification of SLs in the quantitative analyses was achieved by holding the EI mass spectra between 40 and 400 amu. A SIM mode was used at 246 amu (santonin and dihydrohelenalin derivatives) and 244 amu (helenalin derivatives) when quantitative analyses were carried out in the penetration studies.

Data analysis

All values were obtained from three independent measurements. Data are presented as mean ± standard deviation and were analysed using the independent t test (2 groups). A p value < 0.05 is considered statistically significant.

Results and Discussion

SLs penetrate in and permeate through the SC

Penetration profiles of helenalin isobutyrate (SL 1) and dihydrohelenalin acetate (SL 2) (Fig. 1) being the dominating SLs in *A. montana* type “Arbo” and the Spanish chemotype, respectively, were studied (see Fig. 2). Each point in the figure represents the percentage amount of SL which has penetrated at least to the indicated depth of SC, being calculated by considering the weight of the tape and an approximate stratum corneum density of 1 g/cm³ [12]. With a semi-logarithmic scale, the penetration profiles could be mostly divided into two areas: A first area with a flat curve was observed to a depth of 12 µm, representing penetration into the SC. The next area was characterised by a more steeply falling curve indicating the subsequent stratum granulosum layer with a different penetrating behaviour. The first part of the penetration curve could be described by an exponential function:

$$P = a^{-b \cdot d} \quad \text{Equation (2)}$$

where P = penetration [%]; d = depth of horny layer [µm].

Therefore, a linear curve was found in the semi-logarithmic plot, implying that each stripped layer offers the same resistance to penetration [7]. Considering the exponential coefficient b [Equation (2)], we could demonstrate that this parameter can be used to characterise the extent of penetration: the smaller the coefficient (the flatter the curve), the better is the penetration into the horny layer.

Whereas SL 2 showed penetration into and permeation through the SC, SL 1 could only be detected up to a depth of 7 µm (Fig. 2). However, the standard deviation was high for the experiments with SL 1, thus the exponential coefficients of SL 1 and 2 did not differ significantly from one another (see Fig. 3). The reasons for the different behaviour can only be discussed. It is highly improbable that different reactivities of both SLs towards skin proteins with sulfhydryl groups could cause the differences in penetration, since we could show that there is no observable reaction between powdered SC and the highly reactive SL parthenolide (data not shown, method according to quantification of tannins, Deutscher Arzneimittelcodex, DAC). The different penetrations may be explained by the different octanol-water partition coefficients, which is discussed as an important descriptor for predicting skin permeability in addition to molecular weight [14]. Calculating log P values by CAChe® (Fujitsu Inc.) revealed better penetration for SL 2 (log P = 1.48) than for SL 1 (log P = 2.50) because the multilayer

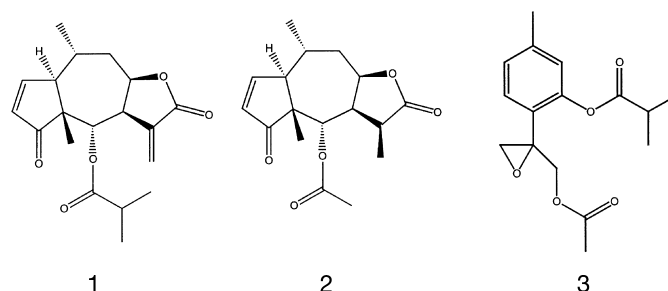


Fig. 1 Structures of the investigated sesquiterpene lactones helenalin isobutyrate (1), 11α,13-dihydrohelenalin acetate (2) and of the monoterpene 10-acetoxy-8,9-epoxythymol isobutyrate (3).

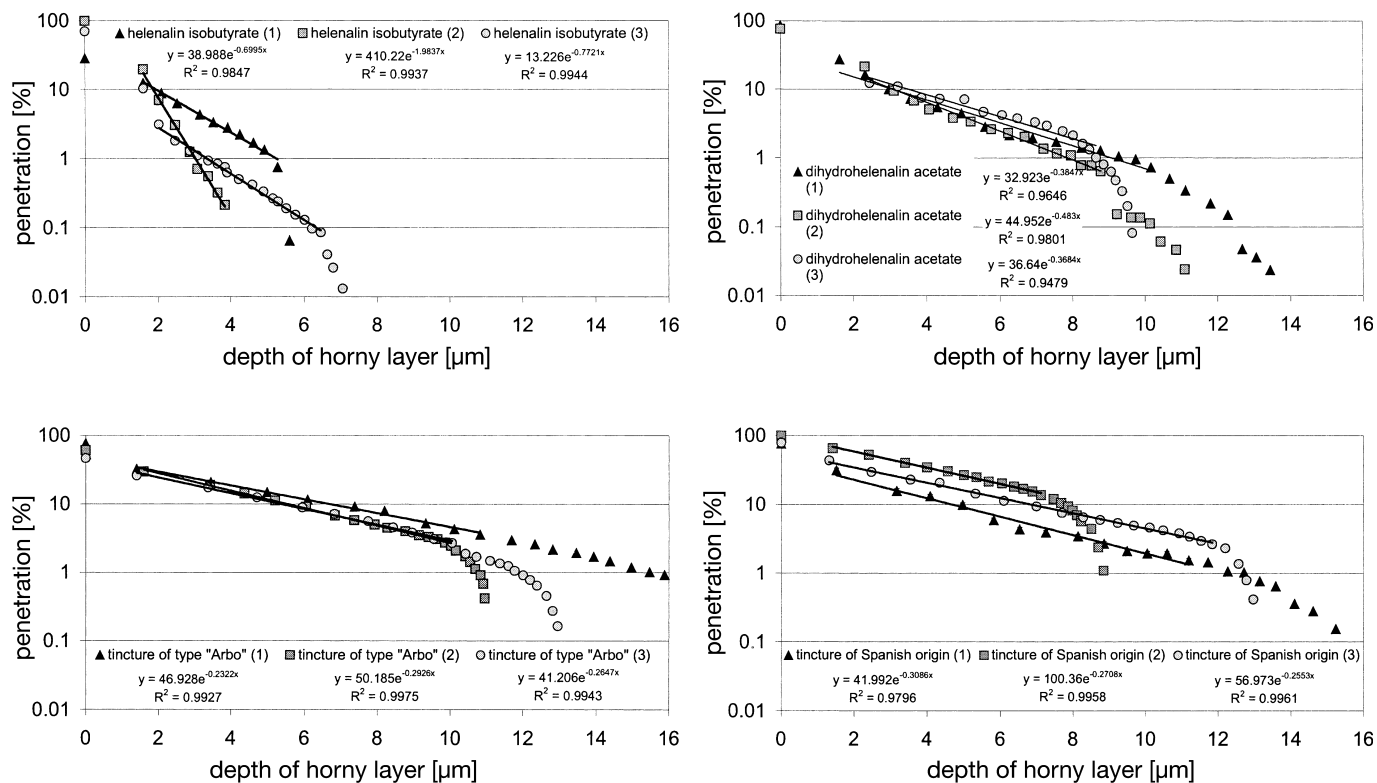


Fig. 2 Penetration profiles of SL 1 and 2 and of *Arnica* tinctures 1 and 2 (each contains a 2.5 mM concentration of SLs in ethanol-water, 7:3) in three independent experiments after one hour. Each point represents the percentage of SL which has penetrated at least to the indicated depth of SC.

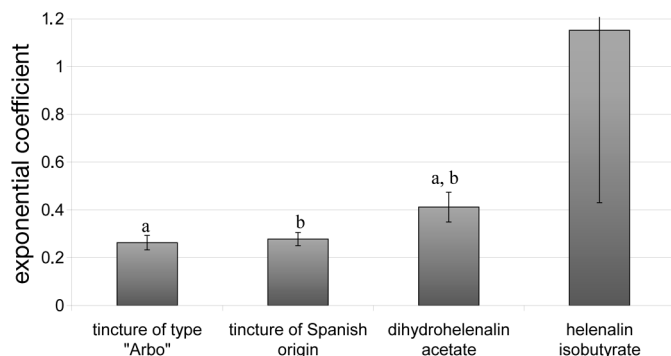


Fig. 3 Exponential coefficients of the penetration curves of SL 1 and 2 and of *Arnica* tinctures 1 and 2 (each with a 2.5 mM content of SL in ethanol-water, 7:3) of three independent experiments after one hour. The smaller the exponential coefficient the better is the penetration. An equal letter indicates statistical significance ($p > 0.05$), respectively.

system of alternating lipophilic and hydrophilic layers of the horny layer can be penetrated better by a substance with a log P value close to 0.0 [15].

SLs in tinctures penetrate better than isolated SLs

Investigation of *Arnica* tinctures from the Spanish chemotype and type "Arbo" showed that both tinctures possessed the same penetration behaviour (Fig. 2) and, consequently, nearly the same exponential coefficients (Fig. 3). Interestingly, if SLs were present in *Arnica* tinctures, a substantial increase of the penetration rate could be determined compared to SLs in ethanol/water (Figs. 2 and 3), although SLs and ethanol were present in the same

concentration. For example, only 1% of the applied SL 2 penetrated through a depth of 8 μm, whereas 8–10% of SLs present in *Arnica* tinctures penetrated through this depth (Fig. 2) indicating a 10-fold better penetration. However, there is no significant difference between the means of the exponential coefficients of SL 1 and of the tinctures due to the great difference of exponent b of SL 1.

The improved penetration of SLs in *Arnica* tinctures may be caused by enhancers, such as fatty acids, fatty alcohols, *n*-alkanes and monoterpenes, which are components in the essential oil of *Arnica* flowers [3] and thus also in *Arnica* tinctures. In particular, monoterpenes are of interest as useful vehicles for the delivery of drugs from external formulations [15], [16], [17]. Therefore, components of the essential oil in *Arnica* tinctures may also be responsible for the observed enhanced penetration.

A thymol derivative does not act as an enhancer at low concentrations

Thymol derivatives are important components of the essential oil [3]. In different tinctures, a maximum thymol derivatives content of 0.1% (1.13 mM calculated as 10-acetoxy-8,9-epoxythymol isobutyrate from the GC analysis carried out for determination of the SL content) could be detected (data not shown). To prove an enhanced activity of these secondary plant metabolites, we used an ethanol/water (7:3) solution containing 10-acetoxy-8,9-epoxythymol isobutyrate (Fig. 1, substance 3; 1.13 mM) and SL 1 (2.5 mM) and an ethanol/water (7:3) solution with solely SL 1 (2.5 mM) as a control for penetration studies on three different pig ears. No differences in the penetration profile could be detected between the two solutions (data not shown).

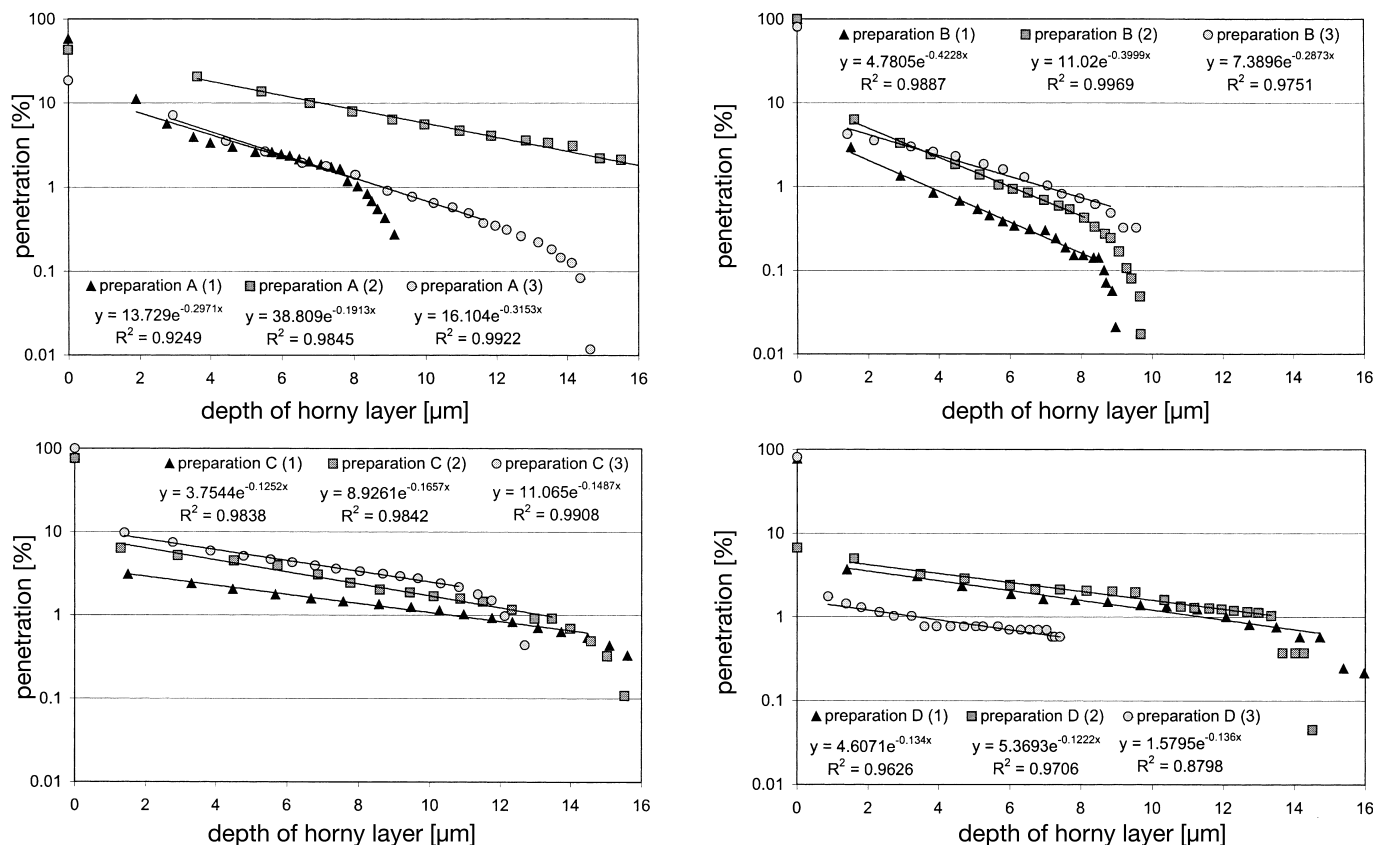


Fig. 4 Penetration curves of preparations A, B, C and D in three independent experiments after one hour. Each point represents the percentage of SL which has penetrated at least to the indicated depth of SC.

The missing enhancer activity may be explained by the low concentrations of thymol derivative **3** used in this investigation. Previously, thymol and other monoterpenes were used at concentrations of 2–5% (w/v) to cause an enhancement effect [18], [19]. Therefore, penetration studies have to be carried out with higher concentrations of substance **3**. However, it also has to be considered that the tinctures contain a variety of other substances which can contribute to the enhancement effect, such as the large amount of fatty acids in the essential oil (40–50%) [3].

SLs in *Arnica* preparations show sufficient penetration and permeation

Four different preparations (A, B, C and D) were used in our study. All preparations consisted of 50% of *Arnica* tincture prepared from fresh *A. montana* flowers. The SL content was determined to be 0.49 µM. Preparation A had no other additives, whereas preparation B contained 10% and preparation C 2.5% of a lipid. Preparation D was a gel formulation containing hydroxypropylmethylcellulose and glycerin.

All tested preparation showed penetration into and permeation through the SC (Fig. 4). Significant differences were observed between the means of the exponential coefficients of preparations A and B on the one hand and of C and D on the other hand (Fig. 5). Preparations C and D showed better penetration than A and B. The exponential coefficient of preparation A (Fig. 5) – a diluted *Arnica* tincture – is identical to those of the tested *Arnica* tinctures (Fig. 3).

The better penetration of SLs in preparation D could be explained by the slower drying of the gel compared to the pure *Arnica* tincture (preparation A). The poorer penetration of preparation B is associated with the large amount of lipids found therein. These lipids remain on the surface, thus forming a kind of depot for the lipophilic SLs. Interestingly, although preparation C also contains lipids, the penetration of SLs was substantially better. It has to be discussed whether the lower lipid concentration in this preparation might act as an enhancer, with this effect predomi-

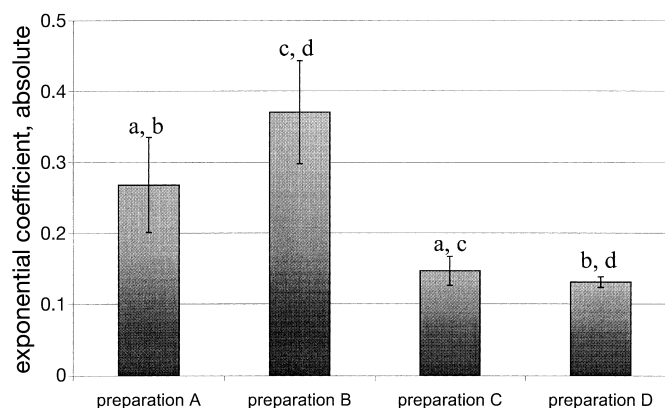


Fig. 5 Exponential coefficients of the penetration curves of preparations A, B, C and D in three independent experiments after one hour. The smaller the exponential coefficient the better is the penetration. An equal letter indicates statistical significance ($p > 0.05$), respectively.

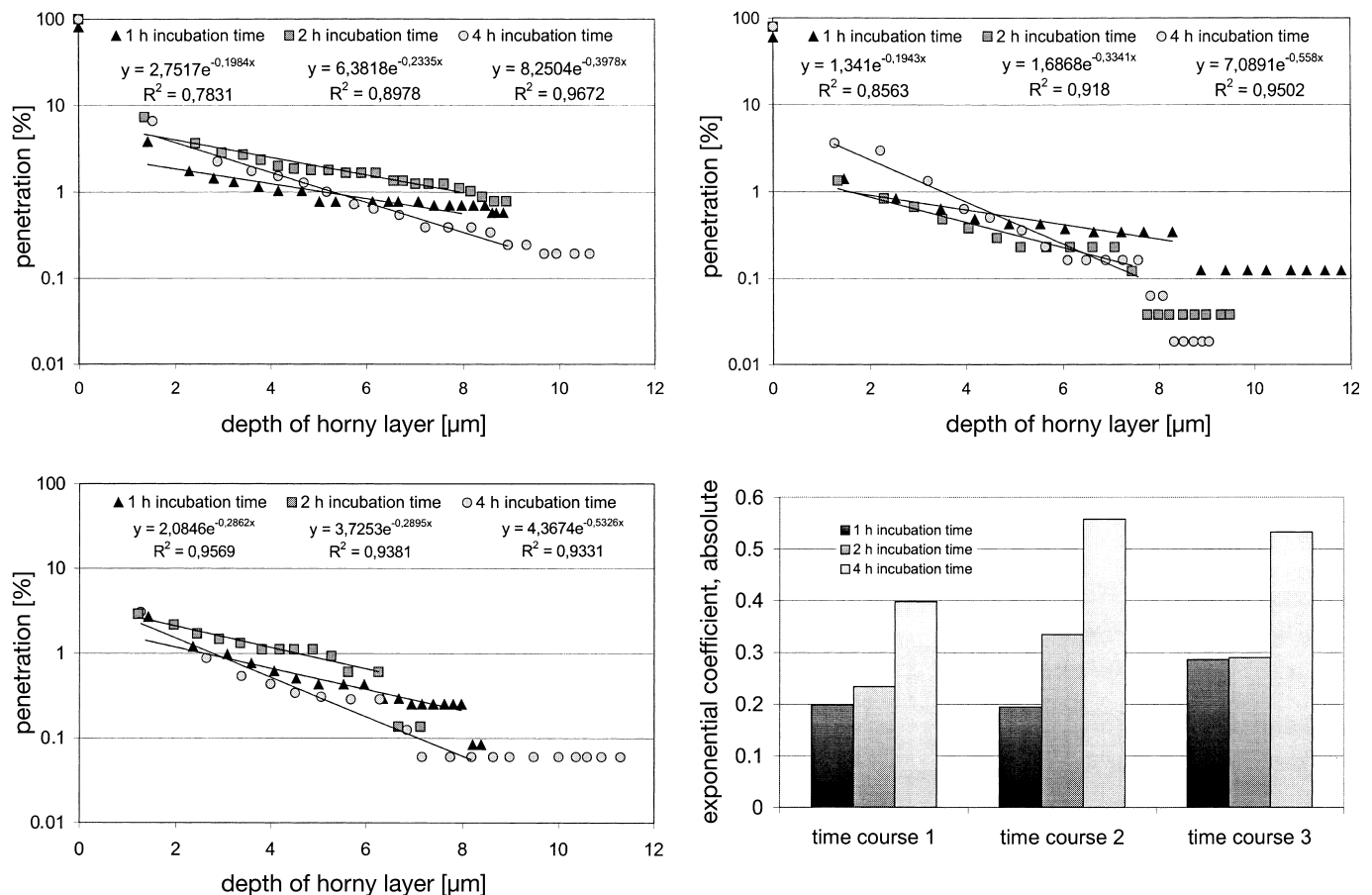


Fig. 6 a) Penetration curves of preparation D after an incubation time of one, two and four hours in three independent experiments. Each point represents the percentage of SLs which has penetrated at least to the indicated depth of SC. b) Exponential coefficients of the penetration curves of preparation D in three independent experiments after one, two and four hours. The smaller the exponential coefficient the better is the penetration.

nating over the depot effect. Moreover, this preparation did not dry up like preparation A.

Influence of incubation time on the penetration of SLs in the gel preparation

To study whether the penetration of SLs is time-dependent, preparation D was incubated for different periods of time, namely one, two and four hours (see Fig. 6). Again, penetration as well as permeation could be observed. The exponential coefficients using an incubation time of one hour were similar to those found in the previous investigations (Fig. 5).

Within the individual studies, an increase in exponential coefficient was produced by increasing the incubation time, indicating penetration of SLs into deeper layers of the skin, without a further penetration of SLs from the gel into the SC. This might be due to the drying of the gel over time, resulting in less movement of SLs from the gel into the SC.

Influence of repeated application on the penetration of SLs in the gel preparation

To simulate the repeated application of a gel, which often happens in practice, we applied 200 μL of the gel after 0, 3 and 6 hours to the same area without removing the former residues of the gel. After a further incubation period of 1 hour (a total of 7 hours) stripping was carried out. SLs were not detectable in the

whole horny layer after the expiration of this term. Their concentrations were below the limit of detection in a depth between 3 and 6 μm in the horny layer (Fig. 7). Accordingly, the exponential coefficient (0.568 ± 0.104) was much higher than when using a one hour incubation time (0.131 ± 0.007). Again, this effect could be due to the drying of the gel, which may lead to the formation of a membrane, thus preventing the movement of SLs out of the gel. Even repeated application did not redissolve this membrane.

However, before the results of the last two penetration studies can be converted into recommendations for application, some circumstances have to be considered. Due to the production of sweat, the surface of human horny layer is slightly moist and therefore the drying of tinctures and gels could be retarded. Nevertheless, the use of an *Arnica* gel for more than an incubation period of 2 hours and repeated application without removing the former residues of the gel does not seem to be efficient. But since the gel is rubbed into the skin in everyday practice, it is too early to draw final conclusions concerning clinical application.

In conclusion, it was shown that a sufficient amount of SLs might permeate the skin barrier and that plant preparations, such as those from *Arnica* flowers, may be advantageous compared to the isolated compounds. This fits well to our previous study which showed that SLs in ethanolic *Arnica* preparations exhibit

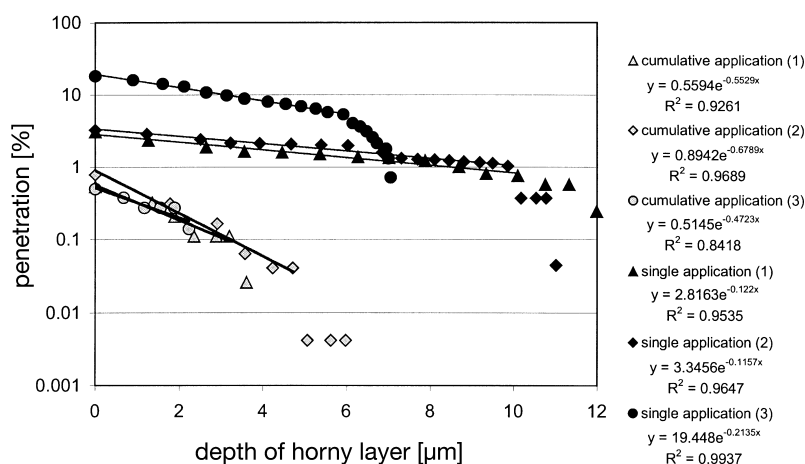


Fig. 7 Penetration curves of preparation D after cumulative application (0, 3 and 6 hours). Stripping was done one hour after the last application. As a control, penetration curves after a single application and one hour incubation time are shown, too. Each point represents the percentage of SLs which have penetrated at least to the indicated depth of SC. Three independent experiments were carried out.

a lower degree of protein binding [20]. Furthermore, our studies underline that knowledge on the penetration of drug preparations is important for the correct estimation of their biological activity.

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References

- Merfort I. *Arnica*: new insights on the molecular mode of action of a traditional medicinal plant. *Forsch Komplementarmed Klass Naturheilkd* 2003; 10 (Suppl 1): 45–8
- Knuesel O, Weber M, Suter A. *Arnica montana* gel in osteoarthritis of the knee: an open, multicenter clinical trial. *Adv Ther* 2002; 19: 209–18
- Merfort I. *Arnica*. In: Hänsel R, Keller K, Rimpler H, Schneider G (eds). *Handbuch der Pharmazeutischen Praxis* Springer-Verlag, Berlin, Heidelberg, New York, 1990: pp 342–57
- Peschka R. Herstellung und Entwicklung liposomenhaltiger Zubereitungen mit hydrophilen Arzneistoffen zur topischen Anwendung. Thesis, University Tübingen, 1994
- Lindemann U, Weigmann HJ, Schaefer H, Sterry W, Lademann J. Evaluation of the pseudo-absorption method to quantify human stratum corneum removed by tape stripping using protein absorption. *Skin Pharmacol Appl Skin Physiol* 2003; 16: 228–36
- Shah VP, Flynn GL, Yacobi A, Maibach HI, Bon C, Fleischer NM et al. Bioequivalence of topical dermatological dosage forms – methods of evaluation of bioequivalence. *AAPS/FDA Workshop on 'Bioequivalence*

of Topical Dermatological Dosage Forms - Methods of Evaluating Bioequivalence'. September 4–6, 1996, Bethesda, Md. *Skin Pharmacol Appl Skin Physiol* 1998; 11: 117–24

- Schaefer H, Stuetgen G, Zesch A, Schalla W, Gazith J. Quantitative determination of percutaneous absorption of radiolabelled drugs *in vitro* and *in vivo* by human skin. *Curr Problems Dermatol* 1978; 7: 80–94
- Nicolaides N, Fu HC, Rice GR. The skin surface lipids of man compared with those of eighteen species of animals. *J Invest Dermatol* 1968; 51: 83–9
- Schmook FP, Meingassner JG, Billich A. Comparison of human skin or epidermis models with human and animal skin in *in-vitro* percutaneous absorption. *Int J Pharm* 2001; 215: 51–6
- Willuhn G, Leven W. On the qualitative and quantitative analysis of the sesquiterpene lactones of *Arnicae flos* DAB 9. *Pharm Ztg Wiss* 1991; 136: 32–9
- Kalia YN, Alberti I, Sekkat N, Curdy C, Naik A, Guy RH. Normalization of stratum corneum barrier function and transepidermal water loss *in vivo*. *Pharm Res* 2000; 17: 1148–50
- Anderson RL, Cassidy JM. Variation in physical dimensions and chemical composition of human stratum corneum. *J Invest Dermatol* 1973; 61: 30–2
- U S. Department of Health and Human Services, Food and Drug Administration. Guidance for Industry. *Bioanalytical Method Validation*. <http://www.fda.gov/cder/guidance/index.htm>, 2001
- Kanikkannan N, Kandimalla K, Lamba SS, and Singh M. Structure-activity relationship of chemical penetration enhancers in transdermal drug delivery. *Curr Med Chem* 2000; 7: 593–608
- Williams AC, Barry BW. Skin absorption enhancers. *Crit Rev Ther Drug Carrier Syst* 1992; 9: 305–53
- Almirall M, Montana J, Escribano E, Obach R, Berrozpe JD. Effect of α -limonene, alpha-pinene and cineole on *in vitro* transdermal human skin penetration of chlorpromazine and haloperidol. *Arzneimittel-Forsch* 1996; 46: 676–80
- Moghimi HR, Williams AC, Barry BW. Enhancement by terpenes of 5-fluorouracil permeation through the stratum corneum: model solvent approach. *J Pharm Pharmacol* 1998; 50: 955–64
- El Kattan AF, Asbill CS, Kim N, Michniak BB. The effects of terpene enhancers on the percutaneous permeation of drugs with different lipophilicities. *Int J Pharm* 2001; 215: 229–40
- Gao S, Singh J. *In vitro* percutaneous absorption enhancement of a lipophilic drug tamoxifen by terpenes. *J Control Release* 1998; 51: 193–9
- Wagner S, Kratz F, Merfort I. *In vitro* behaviour of sesquiterpene lactones and sesquiterpene lactone containing plant preparations in human blood, plasma and human serum albumin solutions. *Planta Med* 2004; 70: 227–33