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Research Article

l-Carvyl Esters as Penetration Enhancers for the Transdermal Delivery of 5-Fluorouracil

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Abstract. To develop effective and safe penetration enhancers, a series of *l*-carvyl esters, namely, 5-isopropenyl-2-methylcyclohex-2-en-1-yl heptanoate (C-HEP), 5-isopropenyl-2- methylcyclohex-2-en-1-yl octanoate (C-OCT), 5-isopropenyl-2-methylcyclohex-2-en-1-yl decanoate (C-DEC), 5-isopropenyl-2-methylcyclohex-2-en-1-yl dodecanoate (C-DOD), 5-isopropenyl-2-methylcyclohex-2-en-1-yl tetradecanoate (C-TET), and 5-isopropenyl-2-methylcyclohex-2-en-1-yl palmitate (C-PAL), was synthesized from *l*-carveol and saturated fatty acids (C7–C16). The volatility of *l*-carveol and *l*-carvyl esters was evaluated by a live weight loss experiment. The enhancing effects of *l*-carvyl esters on 5-fluorouracil (FU) were investigated in the *in vitro* permeation experiment on rat skin. The stratum corneum (SC) uptakes of the enhancers were tested *in vitro* by gas chromatography. Only the *l*-carvyl esters with a moderate SC uptake, namely, C-OCT (C8), C-DEC (C10), and C-DOD (C12), showed a potential to enhance FU skin permeation. An evident parabolic relationship was found between the permeation enhancement of FU and the SC uptake of the *l*-carvyl esters. The *l*-carvyl esters with a chain length of C8–C12 seemed to be favorable for FU.

KEY WORDS: *l*-carvyl ester; penetration enhancer; skin permeation; stratum corneum uptake; 5-fluorouracil.

INTRODUCTION

Penetration enhancers are often used as a key ingredient in transdermal products to promote drug permeation through the stratum corneum (SC), which is the main skin barrier. Although various advanced physical techniques, which include structure-based, electrically based, velocity-based, and miscellaneous delivery systems, have been developed, adding enhancers to transdermal products is one of the easiest methods in promoting the passing of drugs through the skin in practical applications (1,2).

Terpenes are considered as generally regarded-as-safe enhancers because of their natural origin (3). As one of the commonly studied terpenes, *l*-carveol is widely used in cosmetics (4). However, previous studies (5–7) showed that *l*-carveol exhibits a weak enhancing activity and high volatility, which limit its wide application in transdermal drug delivery.

In the design of penetration enhancers, an enhancer with a polar head and a hydrophobic chain is known to contribute a better enhancing potential by penetrating the SC and interacting with the polar groups of the SC lipid domain (8). Furthermore, Zhao *et al.* (9,10) successfully synthesized a series of *O*-acylmenthol derivatives and proved that they can efficiently enhance the skin permeation of various model drugs with different physicochemical properties.

In the current study, a series of *l*-carvyl esters were synthesized from *l*-carveol and saturated fatty acids with various carbon chains. The cyclic structure of *l*-carveol was defined as the polar head, and the fatty acids provided the hydrophobic chain. Moreover, the enhancing activities of these *l*-carvyl esters were evaluated using the model drug 5-fluorouracil (FU) in the *in vitro* skin permeation experiment. Several researchers agree that the enhancing activity of an enhancer may be determined by the amount of its uptake into the SC (11–13). However, the amount of an enhancer retained in the skin is rarely measured. In the present study, the skin retentions of *l*-carvyl esters were determined to investigate the underlying enhancing mechanism.

MATERIALS AND METHODS

Materials

l-Carveol was purchased from Alfa Aesar (Tianjin, China). FU was supplied by Beijing Fuankai technology Co., Ltd. (Beijing, China). Acetaniline was purchased from Beijing Xingjin Chemical Plant (Beijing, China). Isopropyl myristate (IPM), heptanoic acid, octanoic acid, decanoic acid, dodecanic acid, tetradecanoic acid, and palmitic acid were supplied by China National Medicines Co., Ltd. (Shanghai, China). Methanol and acetonitrile (HPLC grade) were obtained from the

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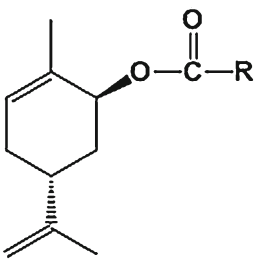
Yuwang Pharmaceutical Co., Ltd. (Shandong, China). All other chemicals were of the highest reagent grade available.

Synthesis of *l*-Carvyl Esters

General procedure for the synthesis of the *l*-carvyl esters: A solution with 5 mmol of *l*-carveol, 6 mmol of carboxylic acid, and 3% in total weight of *p*-toluene sulfonic acid in cyclohexane was heated to azeotropic reflux using water separator. The reaction was finished when no more water was separated. After being cooled, 50 mL of 5% Na_2CO_3 was added. The organic layer was separated and washed with 50 mL of saturated brine, then dried over anhydrous Na_2SO_4. The solvent was concentrated by evaporation under reduced pressure. The crude product was purified by a silica gel column chromatogram (eluting solvent: petroleum ether/ethyl acetate=19/1), whereby the esters were obtained as a colorless oil.

Chemical structures of the synthetic *l*-carvyl esters are shown in Fig. 1. The physicochemical parameters of the synthetic enhancers are shown in Table I. The purity of each compound was over 98% determined by gas chromatography (GC-14C, Shimadzu, Japan). The structures of the compounds were characterized by NMR (ARX-300, Bruker, Germany) and GC-MS (ZQ-2000, Waters, USA). The ^{1}H-NMR and MS data are as follows:

5-Isopropenyl-2-methylcyclohex-2-en-1-yl heptanoate (C-HEP) Yield, 58%. GC-MS (m/z), 264.3 (M^+); ^{1}H-NMR ($CDCl_3$, 600 MHz), 0.893 (t, 3H), 1.204~1.387 (br, 7H), 1.563~2.339 (m, 14H), 4.717~4.754 (m, 2H), 5.271~5.749 (m, 2H).
5-Isopropenyl-2-methylcyclohex-2-en-1-yl octanoate (C-OCT) Yield, 62%. GC-MS (m/z), 278.3 (M^+); ^{1}H-NMR ($CDCl_3$, 600 MHz), 0.861 (t, 3H), 1.212~1.365 (br, 9H), 1.621~2.357 (m, 14H), 4.716~4.751 (m, 2H), 5.272~5.753 (m, 2H).
5-Isopropenyl-2-methylcyclohex-2-en-1-yl decanoate (C-DEC) Yield, 55%. GC-MS (m/z), 306.3 (M^+); ^{1}H-NMR ($CDCl_3$, 300 MHz), 0.889 (t, 3H), 1.198~1.387 (br, 13H), 1.596~2.353 (m, 14H), 4.721~4.754 (m, 2H), 5.276~5.754 (m, 2H).
5-Isopropenyl-2-methylcyclohex-2-en-1-yl dodecanoate (C-DOD) Yield, 63%. GC-MS (m/z), 334.3 (M^+); ^{1}H-NMR ($CDCl_3$, 300 MHz), 0.902 (t, 3H), 1.219~1.1.417 (br, 18H), 1.633~2.369 (m, 13H), 4.712~4.744 (m, 2H), 5.268~5.744 (m, 2H).



l-carvyl ester

Abbr.	R	Abbr.	R
C-HEP	$(CH_2)_5CH_3$	C-DOD	$(CH_2)_{10}CH_3$
C-OCT	$(CH_2)_6CH_3$	C-TET	$(CH_2)_{12}CH_3$
C-DEC	$(CH_2)_8CH_3$	C-PAL	$(CH_2)_{14}CH_3$

Fig. 1. The chemical structure of *l*-carvyl esters

5-Isopropenyl-2-methylcyclohex-2-en-1-yl tetradecanoate (C-TET) Yield, 59%. GC-MS (m/z), 362.4 (M^+); ^{1}H-NMR ($CDCl_3$, 300 MHz), 0.902 (t, 3H), 1.213~1.415 (br, 22H), 1.628~2.370 (m, 13H), 4.721~4.751 (m, 2H), 5.272~5.755 (m, 2H).
5-Isopropenyl-2-methylcyclohex-2-en-1-yl palmitate (C-PAL) Yield, 60%. GC-MS (m/z), 390.4 (M^+); ^{1}H-NMR ($CDCl_3$, 300 MHz), 0.886 (t, 3H), 1.213~1.1.353 (br, 26H), 1.590~2.369 (m, 13H), 4.712~4.744 (m, 2H), 5.268~5.744 (m, 2H).

Volatilization of *l*-Carveol and *l*-Carvyl Esters

The live weight loss experiments were conducted under normal conditions to determine the volatilization rates of *l*-carveol and its esters. Briefly, an excessive amount of *l*-carveol or its esters was placed on a watch glass, and the total weight of enhancer and watch glass was determined at predetermined time intervals over a period of 320 h. The cumulative amount of enhancer losing at room temperature was plotted against time. The slope of the plot gave the volatilization rate of the enhancer.

Solubility Determination

The solubility of FU in IPM was determined by suspending an excessive amount of drug in IPM with or without the addition of enhancers at the selected concentration. The suspensions were agitated in a water bath at 32°C for 24 h. Subsequently, the amount of dissolved drug in IPM was determined by HPLC after centrifugation and appropriate dilution with methanol. All determinations were performed in triplicate.

Preparation of Excised Skin

Rat skin was prepared for the *in vitro* experiment. Male Wistar rats weighing 180–220 g (6–8 weeks old) used in all experiments were supplied by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). All experiments were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and also in accordance with the guidelines for animal use published by the Life Science Research Center of Shenyang Pharmaceutical University. All efforts were made to minimize animal suffering and to limit the number of animals used. The rats were anesthetized with urethane (20% (*w*/*v*), i.p.), and the hair from their abdomen was carefully shaved with a razor after removal of hair by electric clippers (model 900, TGC, Japan). Full-thickness skin (epidermis with SC and dermis) was excised immediately after sacrificing the animals. The integrity of the skin was carefully ascertained by microscope observation, and any skin with low uniformity was rejected. After the fat and subdermal tissue were removed, the skin was kept frozen at −70°C and used within 1 week.

In Vitro Permeation Experiments

The *in vitro* skin permeation experiments were conducted at 32°C using a two-chamber side-by-side diffusion cell with a volume of 2.5 mL and an effective diffusion area of 0.95 cm^2. A piece of full-thickness rat skin was mounted between the

Table I. Physicochemical Properties of IPM, *l*-Carveol, and *l*-Carvyl Esters

Enhancers	Parameters					
	Carbon chain	log*P*[a]	Molecular weight[a]	Molecular volume[a] (cm^3/mol)	δ[b] $(J/cm^3)^{1/2}$	Volatilization rate ($\mu g/cm^2/h$)
IPM	–	7.43	270.45	313.0	16.76	–
Carveol	–	2.55	152.23	160.2	21.03	35.24
C-HEP	C7	6.18	264.40	282.6	17.59	0.54
C-OCT	C8	6.71	278.43	298.9	17.54	0.07
C-DEC	C10	7.77	306.48	331.4	17.46	0
C-DOD	C12	8.83	334.53	383.8	17.39	0
C-TET	C14	9.90	362.59	396.1	17.34	0
C-PAL	C16	10.96	390.64	428.4	17.29	0

IPM isopropyl myristate, *C-HEP* 5-isopropenyl-2-methylcyclohex-2-en-1-yl heptanoate, *C-OCT* 5-isopropenyl-2- methylcyclohex-2-en-1-yl octanoate, *C-DEC* 5-isopropenyl-2-methylcyclohex-2-en-1-yl decanoate, *C-DOD* 5-isopropenyl-2-methylcyclohex-2-en-1-yl dodecanoate, *C-TET* 5-isopropenyl-2-methylcyclohex-2-en-1-yl tetradecanoate, *C-PAL* 5-isopropenyl-2-methylcyclohex-2-en-1-yl palmitate

[a] Calculated using ACD Labs software

[b] Solubility parameter, calculated by the approaches of Hoftyzer/Van Krevelen (21)

two half cells with the SC facing the donor cell. The drug suspensions with or without enhancers were used as donor solutions. The concentration of *l*-carveol was fixed at 1% (*w*/*w*), and the amount of *l*-carvyl esters was in the equal molar amount to *l*-carveol. In all the experiments, excess drug was maintained in the donor compartment. The receiver compartment was filled with 2.5 mL phosphate buffer solutions (pH 7.4). Both donor and receiver compartments were stirred with a star-head bar driven by a constant-speed synchronous motor at 600 rpm. Samples (each 2 mL) were withdrawn from the receiver solution at predetermined time intervals over a period of 8 h and replaced with an equal volume of fresh receiver solution to maintain sink conditions. All experiments were replicated at least four times. The drug concentration was determined by reversed phase HPLC with reference to a calibration curve. The HPLC condition was described in detail in our previous paper (9).

The amount of each drug permeating through the skin during a sampling interval was calculated based on the receptor-phase concentration and volume. The cumulative amount of drug permeating through the skin was plotted against time. The pseudo steady-state flux, J (in microgram per square centimeter per hour), was determined as the slope of the linear portion of the plot and expressed as

$$J = \frac{\mathrm{dQ}}{A\mathrm{dt}} = PC_s \tag{1}$$

$$P = \frac{KD}{L} \tag{2}$$

$$D' = \frac{D}{L^2} = \frac{1}{6T_{lag}} \tag{3}$$

$$K' = KL = \frac{P}{D'} \tag{4}$$

where Q (in microgram per square centimeter) is the amount of drug that permeated through the skin during the experimental time, C_s (in microgram per milliliter) is the saturated solubility of drugs in donor solutions, and P (in centimeter per hour) is the permeability coefficient of drug through the skin. The lag time (T_{lag}) was determined by extrapolating the linear portion of the curve to the abscissa. The diffusional path length (L), the diffusion coefficient (D), and the partition coefficient (K) could not be determined. However, the apparent diffusion parameter (D') and the apparent partition parameter (K') could be calculated using Eqs. 3 and 4, respectively. To evaluate the enhancing activity of each enhancer, the enhancing ratio (ER) was defined as follows:

$$\mathrm{ER} = \mathrm{Q}_{(\text{with enhancer})} / \mathrm{Q}_{(\text{without enhancer})} \tag{5}$$

Skin Retention Studies

The SC samples used in the present study originated from rat skin and were prepared by immersing the rat skin in a 5% trypsin (T-8918, Sigma) solution for 4 h at 37°C. The SC sheets were subsequently dried in a vacuum drying oven at room temperature and stored at −70°C.

To determine the skin uptake of enhancers from donor solutions, the SC was inserted into donor solutions for 8 h at 32°C. Then, the donor solution on the SC surface was gently wiped off with alcohol swabs. After that, the SC was cut and extracted with 0.2 mL of ethanol followed by sonication for 20 min. The sample was then centrifuged at 15,000 rpm for 5 min, and the supernatant was collected to determine the content of enhancers (*l*-carvyl esters) and IPM in skin using the GC method. The concentrations of IPM and enhancers in the SC were represented as the amount in the SC divided by the weight of skin, respectively.

GC analyses were performed on an Agilent 6890N gas chromatographer (Agilent, USA) equipped with a flame ionization detector. Analytical separation was achieved on a HP-5MS capillary column (30 m×0.25 mm i.d.) with 0.2 μm film thickness (Alltech, Illinois, USA). Nitrogen was used as carrier gas (with a constant flow rate of 2 mL/min). The air, hydrogen, and auxiliary gas (N_2) flow rates for the detector were kept at 300, 45, and 15 mL/min, respectively. Temperature setting was described as follows: injector, 270°C; detector, 280°C. In the case of *l*-carveol, the oven temperature was held

Table II. Skin Permeation Parameters of FU (mean ± SE, n=4)

Enhancers	J (μg/cm^2/h)	Q_8 (μg/cm^2)	T_{lag} (h)	C_s (μg/mL)	ER	P (cm/h)	$D' \times 10^3$ (h^{-1})	K' (cm)
Control	10.34±0.98	67.84±8.64	1.84±0.06	30.49	1	0.34	90.58	3.75
Carveol	6.33±0.49	37.30±3.56*	2.13±0.26	29.29	0.55	0.22	78.25	2.81
C-HEP	10.25±1.96	63.77±12.82	1.84±0.12	42.28	0.94	0.24	90.58	2.65
C-OCT	25.12±2.52$^{*, **}$	180.66±20.74$^{*, **}$	0.68±0.13$^{*, **}$	49.01	2.66	0.51	245.10	2.08
C-DEC	16.98±1.54$^{*, **}$	121.87±10.51$^{*, **}$	0.82±0.12$^{*, **}$	31.51	1.80	0.54	203.25	2.66
C-DOD	18.26±1.94$^{*, **}$	136.02±18.00$^{*, **}$	0.60±0.25$^{*, **}$	24.99	2.00	0.73	277.78	2.63
C-TET	3.19±0.42*	21.91±2.31*	1.09±0.21$^{*, **}$	23.85	0.32	0.13	152.91	0.85
C-PAL	7.21±0.92	54.72±8.11	0.42±0.22$^{*, **}$	28.18	0.81	0.26	396.83	0.66

J the steady-state flux, Q_8 the amount of drug that permeated through skin after a period of 8 h, C_s the saturated solubility of drugs in IPM, P the permeability coefficient of drug through skin, T_{lag} the lag time, D' the apparent diffusion parameter, K' the apparent partition parameter, ER= $Q_{(\text{with enhancer})}/Q_{(\text{without enhancer})}$, *C-HEP* 5-isopropenyl-2-methylcyclohex-2-en-1-yl heptanoate, *C-OCT* 5-isopropenyl-2- methylcyclohex-2-en-1-yl octanoate, *C-DEC* 5-isopropenyl-2-methylcyclohex-2-en-1-yl decanoate, *C-DOD* 5-isopropenyl-2-methylcyclohex-2-en-1-yl dodecanoate, *C-TET* 5-isopropenyl-2-methylcyclohex-2-en-1-yl tetradecanoate, *C-PAL* 5-isopropenyl-2-methylcyclohex-2-en-1-yl palmitate
$^{*}p<0.05$ *vs* control; $^{**}p<0.05$ *vs* carveol

at 120°C for 2 min, then programmed to 200°C at 10°C/min, and held for 8 min at 200°C. The retention times of *l*-carveol and IPM were 4.7 and 12 min, respectively. In the case of C-HEP, the initial temperature was 175°C for 2 min, programmed to 180°C at 1°C/min, and held for 5 min. The retention times of C-HEP and IPM were 8.5 and 9.1 min, respectively. For the formulation containing C-OCT, the oven temperature was held at 200°C for 10 min, and the retention time was 6.46 min for C-OCT and 4.98 min for IPM. The oven temperature for C-DEC testing was at 220°C for 10 min, and the retention times of C-DEC and IPM were 6.21 and 3.07 min, respectively. A temperature program with initial temperature of 220°C with equilibration time of 2 min and then a temperature gradient of 20°C/min with final temperature of 260°C and final time of 5 min was used for C-DOD with a retention time of 5.3 min. For C-TET, a temperature program with initial temperature of 220°C for 3 min and then a temperature gradient of 20°C/min with final temperature of 260°C and final time of 5 min was used with a retention time of 7.9 min. For C-PAL, the oven temperature was initially 220°C for 2 min and then programmed to increase to 280°C at 10°C/min and held for 5 min. The retention time of C-PAL was 9.4 min.

Data Analysis

Statistical analysis was carried out using Student's t test. Analysis of variance followed by Tukey–Kramer test was used in the case of multiple comparison. The level of significance was taken as $p<0.05$.

RESULTS

Volatilization of *l*-Carveol and *l*-Carvyl Esters

The volatilization rates of *l*-carveol and its esters are listed in Table I. *l*-Carveol exhibited the highest volatilization rate (35.24 μg/cm^2/h) among the tested enhancers. Among the *l*-carvyl esters, the most volatile ester was C-HEP with a volatilization rate of 0.54 μg/cm^2/h, which is approximately 1.5% of that of *l*-carveol, followed by C-OCT (0.07 μg/cm^2/h). No weight loss was observed for C-DEC, C-DOD, C-TET, and C-PAL. These results suggest that *l*-carveol is more volatile than *l*-carvyl esters.

In Vitro Permeation Study

In vitro permeation experiments were conducted to evaluate the enhancing activities of *l*-carvyl esters with different carbon chain lengths (C7–C16). The skin permeation of FU was based on the determined drug steady-state flux and Q_8. The permeation parameters of FU with or without enhancers across rat skin are listed in Table II. Compared with the control, the skin permeation of FU was greatly promoted by C-OCT, C-DEC, and C-DOD ($p<0.05$), whereas *l*-carveol and other *l*-carvyl esters exhibited neither no permeation enhancement for FU nor a negative effect. T_{lag} of FU was significantly reduced ($p<0.05$) by the synthesized enhancers except C-HEP. C_s of FU was improved by C-HEP, C-OCT, and C-DEC, and reduced by other enhancers tested here when comparing with that of the control. P of FU was promoted by C-DOD to an extent for approximately twofold comparing to

Table III. The SC Uptake of Donor Solvent and Penetration Enhancers (n=4)

	Skin retention (μmol/g)							
	Control	Carveol	C-HEP	C-OCT	C-DEC	C-DOD	C-TET	C-PAL
IPM	118.94±44.64	102.72±59.70	132.72±34.71	90.74±35.25	99.88±23.30	127.12±21.47	132.46±42.02	133.63±23.32
Enhancer	–	–	0.42±0.20	1.10±0.25	1.41±0.51	1.28±0.42	3.35±0.25	3.17±0.74

IPM isopropyl myristate, *C-HEP* 5-isopropenyl-2-methylcyclohex-2-en-1-yl heptanoate, *C-OCT* 5-isopropenyl-2- methylcyclohex-2-en-1-yl octanoate, *C-DEC* 5-isopropenyl-2-methylcyclohex-2-en-1-yl decanoate, *C-DOD* 5-isopropenyl-2-methylcyclohex-2-en-1-yl dodecanoate, *C-TET* 5-isopropenyl-2-methylcyclohex-2-en-1-yl tetradecanoate, *C-PAL* 5-isopropenyl-2-methylcyclohex-2-en-1-yl palmitate

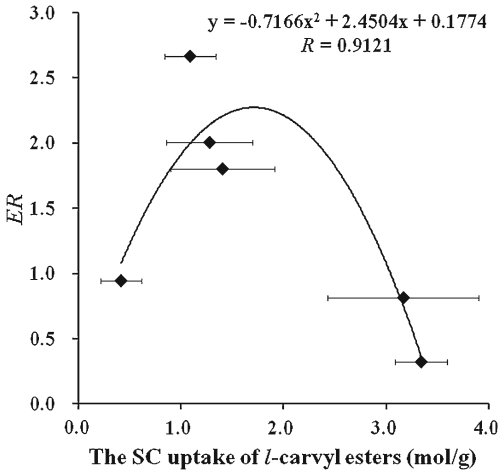


Fig. 2. The relationship between the permeation enhancement of FU and the SC uptake of *l*-carvyl esters

that of the control, followed by C-DEC and C-OCT. Other *l*-carvyl esters produced negative effects on *P*. These results suggested that C-OCT, C-DEC, and C-DOD were the most efficient enhancers for the skin permeation of FU. The efficient enhancers, namely, C-OCT, C-DEC, and C-DOD, contributed the moderate D' and K' values for FU across the skin. For the control, *l*-carveol, and C-HEP, a lower D' and a higher K' of FU were obtained; opposite results were found for C-TET and C-PAL.

SC Uptake of *l*-Carvyl Esters *In Vitro*

During the whole period of the permeation experiment, no *l*-carveol, *l*-carvyl esters, or IPM (donor solvent) were detected in the acceptor fluid by GC. The amounts of *l*-carveol, *l*-carvyl esters, and IPM in SC were listed in Table III. According to the data, no significant differences among these formulations ($p>0.05$) were observed in the SC uptakes of IPM, which were significantly higher than those of *l*-carveol and *l*-carvyl esters. *l*-Carveol was hardly determined in SC samples because of its high volatility (52 μg/cm^2/h). The order of the SC uptakes of *l*-carvyl esters from the IPM donor solutions was C-PAL, C-TET > C-DOD, C-DEC, C-OCT > C-HEP, in which no significant differences were observed between the SC uptakes of C-PAL and C-TET as well as among the SC uptakes of C-DOD, C-DEC, and C-OCT.

DISCUSSION

l-Carvyl esters exhibited lower volatility and higher permeation enhancement effect, thus making them better penetration enhancers than *l*-carveol. No enhancer was found in the acceptor fluid because highly lipophilic enhancers were hardly transported across the high lipophilic SC barrier, *i.e.*, *l*-carveol and *l*-carvyl esters were targeted to the SC. Table II reveals that only *l*-carvyl esters with a moderate hydrophobic carbon chain and moderate lipophilicity, such as C-OCT, C-DEC, and C-DOD, exhibited better enhancing potential. The permeation enhancement of FU mainly depended on the SC uptake of enhancers rather than on the SC uptake of IPM (Table III). Figure 2 shows an evident parabolic relationship between the permeation enhancement of FU and the SC uptake of *l*-carvyl esters, suggesting that only *l*-carvyl esters with a moderate SC uptake (~1.7 μmol/g) can provide a better enhancement. Therefore, the SC uptake of *l*-carvyl esters is a key factor for the skin permeation of FU.

A rare study has reported the possible relationship between SC uptake and permeation enhancement. However, several studies have revealed that the parabolic relationship also occurred between the concentration of enhancers in the formulations and skin permeation enhancement (9,14–16). It was hypothesized that the enhancer uptake by the skin would increase with enhancer concentration. Thus, it seemed acceptable for the similar parabolic relationship between the SC uptake of enhancers and skin permeation in this study.

The SC layer, as the active enhancer site, is a semipolar microenvironment that is closely mimicked by liquid *n*-octanol (17,18). The potency of enhancers is attributed to their partition between the aqueous phase and the intercellular lipid domain. Enhancers with a large polar head group and a lipid carbon chain are known to cause a reduction of the cholesterol–cholesterol interference and cholesterol–ceramide interactions in SC. These enhancers are also known to increase the fluidity of the SC lipid bilayers, thereby facilitating the skin

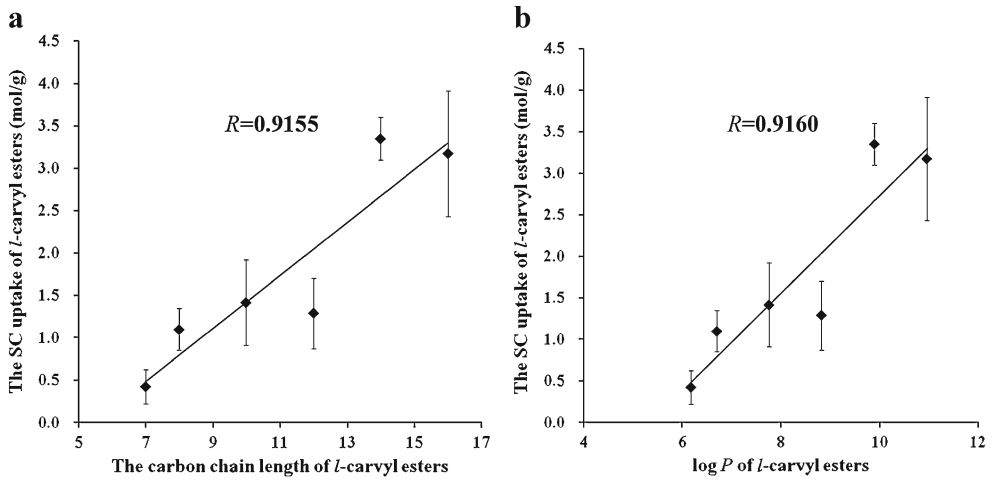


Fig. 3. The effect of carbon chain length **a** and lipophilicity **b** of *l*-carvyl esters on the SC uptake of *l*-carvyl esters

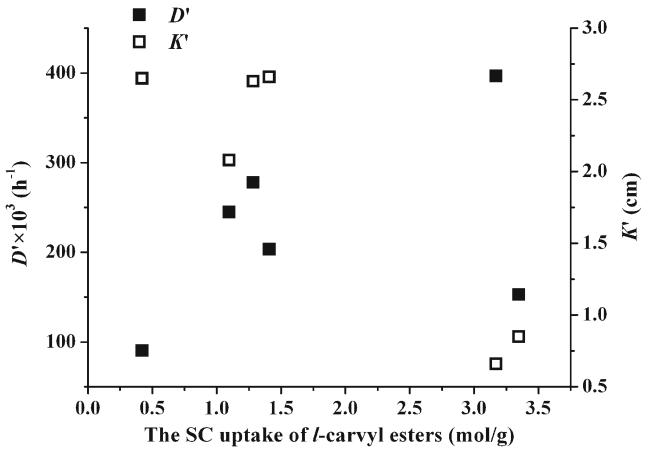


Fig. 4. The effect of the SC uptake of *l*-carvyl esters on the apparent diffusion parameter (D') and the apparent partition parameter (K')

permeation of drugs (19,20). Thus, the lipophilicities and carbon chain length of *l*-carvyl esters likely exhibited important effects on the SC uptakes of *l*-carvyl esters. A linear relationship was observed between the SC uptakes of *l*-carvyl esters and the carbon chain lengths as well as the lipophilicities of *l*-carvyl esters (Fig. 3). Therefore, *l*-carvyl esters with a high log *P* and a long carbon chain can be distributed uniformly across the modified SC intercellular lipid domain and penetrate the ceramide matrix, thereby disrupting ceramide packing and increasing drug skin diffusivity. On the one hand, shorter *l*-carvyl ester chains have insufficient disturbance for the SC intercellular lipid domain or insufficient lipophilicity for skin uptake. On the other hand, longer *l*-carvyl ester chains exhibit a much higher affinity to the lipid domain in the SC, thereby retarding their own permeation and that of FU. Furthermore, the skin permeation of a molecule across the skin is a complex process that involves continuous diffusion and partition. In Fig. 4, the apparent diffusion parameter generally increases with the SC uptake of *l*-carvyl esters as the apparent partition parameter decreases. Therefore, maximum permeation enhancement was observed in the *l*-carvyl esters containing C8–C12 carbon chains, which resulted in the moderate D' and K' values of FU.

CONCLUSIONS

l-Carvyl esters that were synthesized from *l*-carveol and unbranched fatty acids (C7–C16) exhibited lower volatility and higher permeation enhancement effect compared with *l*-carveol. The FU skin permeation was enhanced respectively to an extent of 2.66-, 1.80-, and 2.00-fold by C-OCT, C-DEC, and C-DOD compared to the control. The skin permeation enhancement was found to be related to enhancer uptake by SC. A parabolic relationship between SC uptakes of enhancers and skin permeation enhancement was observed. Only the *l*-carvyl esters with a moderate SC uptake, including C-OCT, C-DEC, and C-DOD (C8–C12), have the potential to enhance FU skin permeation.

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