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Effect of enhancers on percutaneous absorption of osthol across the excised full thickness rat skin

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Fructus cnidii, which has been mainly used for treatment of dermatosis with external use [1–6], is a Chinese traditional medicine of *Cnidium monnieri* (L.) *Cuss* described in the Pharmacopoeia of the People's Republic of China 2000 edition [7]. In recent years, the development of a topical dosage form of Fructus cnidii has been attracted increasing attention. A number of preparations containing osthol, 7-methoxy-8-(3-methyl-2-butenyl)-2*H*-1-benzopyran-2-one, which is the active principle from Fructus cnidii, are being introduced for the topical treatment of psoriasis in China. We have not found *in vitro* data concerning the percutaneous absorption fluxes of osthol through excised rat or human skin. Therefore, we studied the *in vitro* percutaneous absorption of osthol across excised full-thickness rat skin. Furthermore, we assessed the ability of three penetration enhancers, such as chenopodium, menthol and Azone®, on the *in vitro* percutaneous absorption of osthol.

The osthol steady-state fluxes (Jss) through the skin were calculated by plotting the cumulative amount of drug penetrated the skin against time and determining the slope of the linear portion of the curve and the X-intercept values (lag time, T_{lag}) by linear regression analysis ($R > 0.98$). The effectiveness of penetration enhancers (enhancement factor, EF) was determined by comparing the osthol steady state flux in the presence of enhancers with control. The permeability coefficient (P_m) can be evaluated by dividing the steady-state flux by the donor phase concentration (C_{SS}). These parameters, as calculated for the permeation of osthol are listed in Table 1. As shown, chenopodium, menthol and Azone could actually enhance the delivery of osthol across excised rat skin, and the EF is 3.09, 3.41 and 4.38, respectively.

Percutaneous penetration enhancers may act by altering the diffusion characteristics of the skin or by modifying the SC/vehicle partitioning behavior of the drug. To better understand the enhancement mechanism of the enhancers used, we examined the values of two permeation parameters of the drug, i.e. apparent diffusion coefficient

Table 1: Permeation parameters for osthol across the rat skin (n = 6)

Enhancer	J_{ss} (μ g/cm²/h)	EF	t_{lag} (h)	P_m (10^{-4} cm/h)
Azone	3.23 ± 0.41	4.38	0.16 ± 0.03	4.82
Menthol	2.52 ± 0.35	3.41	0.45 ± 0.07	3.76
Chenopodium	2.28 ± 0.34	3.09	0.89 ± 0.13	3.40
Control	0.74 ± 0.11	–	0.98 ± 0.12	1.10

Table 2: Apparent diffusion coefficients and SC/medium partition coefficient calculated from the permeation data for osthol across the rat skin

Enhancer	D_{app} (10^{-2} cm²/h)	$K_{app} \times 10^{-5}$
Azone	3.61	2.62
Menthol	1.25	2.04
Chenopodium	0.63	1.85
Control	0.57	5.99

(D_{app}) and apparent SC/vehicle partition coefficient (K_{app}), which were calculated from obtained percutaneous absorption data.

The diffusion coefficient was calculated based on eq. (1) [8]

$$D_{app} = h^2/6t_{lag} \quad (1)$$

where h is the barrier thickness. Assuming that the SC is the main rate-limiting barrier, h is 18.4 μm for rat skin [9].

The apparent SC/vehicle partition coefficient can be calculated indirectly from the equation:

$$K_{app} = P_m h/D_{app} \quad (2)$$

As shown in Table 2, compared to the control, chenopodium, menthol and Azone could increase the diffusion coefficients of osthol to 1.10, 2.18, 6.30 times, while decreasing the SC/medium partition coefficients to 0.31, 0.34, 0.44 times, respectively. It is indicated that the three enhancers modify the barrier function of stratum corneum, reduce the resistance of drug transport through the skin and increase the diffusion coefficients of osthol. Our findings are in accordance with the suggestion of several authors [10–13] that Azone exerts its effect on the drug apparent diffusion coefficient by altering the packing of the bilayer tails within the intercellular space. Menthol and chenopodium are considered to destroy the lipid barrier between the keratinized cells and to increase the drug partition into skin from the aqueous base [14], like the widely used terpine enhancer. However, the last enhancement mechanism is not consistent with the findings obtained in our experiments.

Experimental

1. Materials

Osthol was prepared in our laboratory by a super fluid extraction (CO_2) technique and recrystallization in ethanol. Azone was purchased from Guangzhou Chemical agents Inc. (Guangzhou City, China). Menthol and chenopodium were supplied by Hua Bao Natural Pharmaceutical Oil Factory (Yongji county, Jiangxi province, China). All other materials were of analytical grade.

2. In vitro skin permeation experiments

Samples of rat skin were mounted in modified Valia-Chien diffusion cells with Stratum corneum towards the donor compartment. The cells were stirred and thermostated at 32 ± 0.5 °C during all the experiments. The exposed skin surface area was 0.75 cm² and the receptor and donor compartment volume was 4.5 ml.

The donor cell was filled with 5% Azone, menthol, chenopodium propylene glycol solution, and propylene glycol (control) respectively, while the receptor compartment contained normal saline (NS). One hour later, the cells were emptied and rinsed with NS. Then the donor cell was filled up with saturated osthol NS solution, while the receptor compartment was filled with NS again. Each experiment was run for 12 h using six different diffusion cells (n = 6). At intervals, samples of receiving solution were withdrawn and replaced with fresh NS. The samples were analyzed for osthol content by HPLC.

3. High-performance liquid chromatography

The HPLC apparatus consisted of a Waters 510 system (Waters-Millipore Corporation, Milford, MA) equipped with a 20 μl loop and a Waters 486 detector. Chromatography was performed on a Lichrosphere 100 C_{18} R. P.

Column Diamonsil C_{18} (200 mm × 4.6 mm, 5 μm). The mobile phase was methanol-water (80 : 20). Detection wavelength was 323 ± 1 nm. The flow rate was set at 1.0 ml/min. The retention time was of 8.1 min for osthol.

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Chemcial constituents from the marine sponge *Iotrochoto birotulata*

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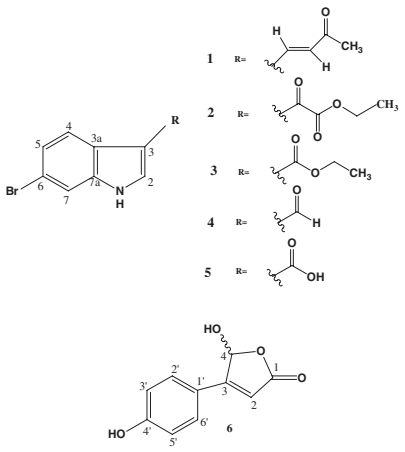
In continuation to investigate bioactive secondary metabolites from Chinese marine organisms, the sponge *Iotrochoto birotulata* [1–5] was collected from Southern China sea, a tropical area in southern Hainan island. The MeOH extract of *I. birotulata* was partitioned between H_2O and EtOAc, and the EtOAc extract was concentrated and subjected to column chromatography repeatedly to afford six compounds (**1**–**6**). The basic structural pattern of **1** to **5** was that of 6-bromoindole analogues, and **2** to **5** were identified as (6-bromo-indol-3-yl)-oxo-acetic acid ethyl ester (**2**) [6], bromoester (**3**) [7–8], 6-bromoindole-3-carboxylic acid (**4**) [9] and 6-bromoindole-3-carbaldehyde (**5**) [9], by comparison of the physical and chemical properties as well as the spectral data with those reported in the literature. Compound **2** was obtained previously as synthesized product [6], but the ^1H and ^{13}C NMR data have not been concluded before we elucidated them by extensive 2D NMR spectroscopy. Compound **6** was identical to hydroxybutenolide [10] due to an extensive 2D NMR spectral analysis. This is the first report to reveal the species *I. birotulata* containing brominated indole derivatives.



Compound **1** was isolated as a white powder. Its positive ESI-MS spectrum showed the molecular ion peak at *m/z* 264 and 266 with integration 1 : 1, suggesting the presence of a bromine element. The molecular formula $C_{12}H_{10}NOBr$ was established by the negative HRESIMS spectrometry data (*m/z* 261.9850, calcd. for 261.9872). The IR spectrum exhibited absorptions at 3160, 1707,