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[1]Department of Pharmacognosy, Medical University of Lublin, Poland
[2]Chair of Cosmetology, University of Information, Technology and Management in Rzeszow, Rzeszow, Poland
[3]Main Pharmaceutical Inspectorate, Warsaw, Poland

ELWIRA SIENIAWSKA[1], JOANNA FLISZKIEWICZ [1],
KATARZYNA BOROWSKA [2],
TOMASZ BAJ[1], ZOFIA ULZ[3], KAZIMIERZ GŁOWNIAK[1]

The 8-MOP identification and isolation from the plant material and percutaneous permeation in the modified Flynn's cell

Izolacja i identyfikacja 8-MOP z materiału roślinnego i badania przenikalności w modyfikowanej komorze Flynna

INTRODUCTION

Xanthotoxin (8-methoxypsoralen, methoxsalen) is a natural furanocoumarin consisting of coumarin annulated with furan. It's biosynthetic route in plants continues with the activation of dimethylallylpyrophosphate (DMAPP), produced via the mevalonate pathway. Then the carbocation is formed via the cleavage of the diphosphates. Umbelliferone 6-prenyltransferase catalyzes a C-alkylation between DMAPP and umbelliferone at the activated position ortho to the phenol, yielding demethylsuberosin. Hydroxylation catalyzed by the marmesin synthase yields marmesin, which hydroxylated by psorales synthase gives psoralen. Psoralen 8-monooxygenase hydroxylation gives xanthotoxol which is followed by a methylation via the xanthotoxol O-methylotransferase and S-adenosyl methionine to yield xanthotoxin [8]. Xanthotoxin can be found particularly in Apiaceae and Rutaceae species. It was isolated from *Esenbeckia* species [25,34], *Peucedanum luxurians* [6], *Tetradium daniellii* [32], *Murraya koenigii* [2], *Sideritis taurica* [36] and *Cnidium monnieri* [19]. *Ammi majus* L. and *Ruta graveolens* L. plants were found to be one of the richest natural sources of xanthotoxin and other linear furanocoumarins [9,22]. The most studied family concerning furanocoumarins is undoubtedly Apiaceae, with more than 19 genus investigated for their production [5,21,27]. Psoralen, bergapten, xanthotoxin and isopimpinellin are present in almost all species of this family. The highest concentrations of xanthotoxin were detected in *Heracleum mantegazzianum*, with about 4 mg g^{-1} DW [28]. These concentration is comparable or slightly higher than this measured in *Ruta* species [21]. Tables 1-3 below presents typical 8-MOP identification and isolation techniques.

Table 1. Classic TLC xanthotoxin identification methods

Eluting mixture	**Species**	**Ref.**
Silica gel		
• benzen – ethyl acetate (9:1) • dichloromethan – ethyl acetate (99:1) • cyclohexan – dichloromethan – diizopropyl ether (6:3:1).	*Myrrhis odorata* L.	[38]
• benzen – ethyl acetate (95:5)	*Heracleum spp.*	[11]
• methanol – water (6:4)	*Heracleum sosnowsky*	[37]
• cyclohexan – ethyl acetate (95:5)	*Angelica archangelica* L.	[15]
Multiple development • ethyl acetate – n-heptan (35:65)	*Heracleum spp.*	[13]
1. ethyl acetate – n-heptan (35:65) 2. methanol – water (55:45)	*Heracleum spp.*	[13]
Florisil		
• 10% 2-propanol in dichloromethan – n-heptan (7:3) • 15% ethyl acetate in benzen	*Archangelica officinalis*	[37]
• 15% ethyl acetate in benzen	*Archangelica officinalis*	[33]
1. 20% diizopropyl ether in dichloromethan – n-heptan (7/3) 2. ethyl acetate – benzen (1:4)	*Archangelica officinalis*	[33]
Aluminium oxide		
• hexan – benzen – methanol (5:4:1)	*Peucedanum morrissonii*	[35]

Table 2. Typical LC systems for xanthotoxin extraction

Eluting mixture	**Species**	**Ref.**
Silica gel – gradient mode		
1. n-hexan, 2. n- hexan – ethyl acetate, 3. ethyl acetate, 4. ethyl acetate – methanol, 5. methanol	*Angelica sylvestris*	[17]
1. n-heptan – dichloromethan –diizopropyl ether (50:47:3), 2. gradient of dichloromethan (47%, 57%, 77%), 3. dichloromethan – diizopropyl ether (97:3)	*Archangelica officinalis*	[29]
Florisil		
5% acetonitryl in dichloromethan – n-heptan (7:3)	Heracleum sosnowsky	[37]
3% acetonitryl in 70% dichloromethan in heptan	*Pastinaca sativa,* *Angelica officianalis*	[33]

Table 3. Typical HPLC systems for xanthotoxin analysis

Eluting mixture	**Species**	**Ref.**
methanol – water (65:35)	*Archangelica officinalis*	[29]
methanol – water gradient (8:2, 7:3, 6:4)	*Archangelica officinalis*	[3]
acetonitryl – water (60:40)	*Heracleum spp.*	[11]
methanol – water (7:3)	*Archangelica officinalis*	[24]
A: water – phosphoric acid (99,7:0,3) B: acetonitryl – water – phosphoric acid (79,7:20:0,3) gradient of A in B: 0-5 min 30-20%, 5-6 min 20:19%, 6-7 min 19-17%, 7-10 min 17-15%, 10-15 min 15-12%, 15-20 min 12-0 %, 20-25 min 0-70%	*Heracleum candicans*	[4]
methanol – water gradient of methanol in water: 30-45% (0-10 min) 45-60% (10-25 min) 60-65% (25-35 min) 65-75% (35-45 min) 75-85% (45-60 min) 85-90% (60-65 min)	*Angelica dauhurica*	[14]
acetonitryl – water (55:45)	Brazilian furanocoumarins products	[12], [16]

Xanthotoxin and other furanocoumarins play an important defensive role in the plants. They are phytoalexins which possess antifungal and antibacterial properties. Xanthotoxin is found constitutively in *Heraculeum mantegazzianum* [10] but is induced in celery (*Apium graveolens)*, parsnip (*Pastinaca sativa*) parsley (*Petroselinum hortense*), *Glennia littoralis* [20] and *Conium maculatum* [3].

Xanthotoxin possess antileukodermal activity and antitumor properties. It is also active, inhibiting the growth of HeLa cells [1]. Regarding the clinical use of furocoumarins 8-Metoxypsoralen (8-MOP) is used as an oral and topical photoactive chemical for the treatment of three dermatological disorders: vitiligo, psoriasis, and mycosis fungoides. Psoriaris is a genetic, dermal disease connected with keratinocytes proliferation and cytokines activation [26]. 8-Metoxypsoralen provided during PUVA therapy produces monoadducts with skin cells DNA, reduces cell proliferation by 73% and suppresses dermal T lymphocytes and Interleukin-2 receptors. Thaus PUVA (8-methoxypsoralen administration followed by ultraviolet A radiation) therapy) decreases immunological and epidermal activation in psoriaris [18]. The efficacy of PUVA has been repeatedly demonstrated and in some cases PUVA has become the choice of treatment [20].

The skin is the largest organ of the body (15% of body weight and its main functions are to help maintain water homeostasis and to protect against biological, chemical and mechanical hazards. Skin penetration studies play an essential role in the optimization of drug and formulation design in dermal

and transdermal delivery. Thus, quantitative dermal penetration data is highly needed. The permeation of chemicals through the skin can be measured by *in vivo* and *in vitro* techniques. Frequently this has been done by *in vitro* techniques because of the simplicity of the experimental conditions. In *in vitro* permeation techniques Fllyn type diffusion cells can be used [30]. The diffusion cell is composed of donor and acceptor compartment divided by the skin membrane. Investigated compound is added to the donor solution and effect of permeation is assessed by sampling acceptor solution and assaying the study compound concentration in the acceptor solution.

The aim of this work was to assign the 8-MOP transport profile through the skin using Flynn type diffusion cell.

MATERIAL AND METHODS

8-metoksypsoralen was purchased from Sigma-Aldrich Chemie GmbH (Munich, Germany), Na_2HPO_4, NaH_2PO_4 and solvents of analytical grade from POCh S.A (Gliwice, Poland), Oxoralen from Gerot Pharmazeutica, Wien, Austria.

Skin sample preparation. The studies were carried out using the pigs ears [31]. The ears of pigs were freshly slaughtered. The hair of the ears was carefully removed with scissors. The ears were washed with water and dried using soft tissue, after which they were stored at 4–8 °C. Five samples were removed from the same pig ear. The subcutaneous tissue was removed 1h before the experiments and the skin was cut into pieces using a scalpel. Approval for these experiments has been obtained from the First Local Independent Ethics Committee in Lublin.

Flynn type cell experiment. Skin pieces were placed in all glass Flynn type diffusion cell between the donor and acceptor compartment. The absorption surface area was 5 cm^2. Twenty six ml of 1% 8-MOP solution was deposited in the donor chamber. The donor and acceptor chamber were occluded to prevent evaporation. The 26 ml volume of the acceptor chamber was filled with a acceptor solution (phosphate buffer pH 7). The donor and acceptor solutions in were continuously agitated with a magnetic stirrer. The fluid in the acceptor chamber was removed every 30 min, measured spectrophotometrically and poured back into the acceptor solution.

Determination of 8 – MOP concentration in acceptor solutions. The absorbance of the receiver solution was monitored at analytical wavelength (8-MOP λ_{max} in the donor solution) every 30.0 min over a 3.5 h period. The experiment was repeated five times. At the appropriate time, aliquots were removed from the receiver cells. The determination of 8-MOP concentration was carried out by a spectrophotometer (Helios β, Unicam, Warsaw, Poland) and concentration on the basis of Beer's law was calculated. Beer's Law states that molar absorptivity is constant and the absorbance is proportional to concentration for a given substance dissolved in a given solute and measured at a given wavelength [7].

$$A_\lambda = \varepsilon \, c \, L$$

A_λ- maximal absorbance wavelength
ε - molar extinction coefficient for the dissolved substance
c - sample concentration
L - light path length in centimeters [7].

Determination of the thickness of the skin. The thickness of the different skin pieces was estimated dividing the skin surface by its mass.

RESULTS AND DISCUSSION

The mean values and standard deviations of the drug concentrations determined in the acceptor compartment at different times after application are given in Table 4. The percutaneous permeation profiles obtained using different donor solutions are presented on the Fig. 1. Flynn cell is the side by side diffusion cell developed by Flynn and Arbor to evaluate topical products intended for human use (Fig. 2).

Table 4. 8-MOP concentration in the acceptor compartment after appropriate time of experiment

	Concentration in the acceptor solution (mg%)	
Time (h)	Donor: 1% 8-MOP in the phosphate buffer pH 7.4 + 20 % EtOH acceptor: phosphate buffer pH 7.4 **A experiment**	Donor: Oxoralen (liquid) acceptor: phosphate buffer pH 7.4 **B experiment**
0.5	0.107+/-0.13	0.004+/-0.11
1.0	0.126+/-0.,12	0.010+/-0.13
1.5	0.135+/-0.17	0.013+/-0.18
2.0	0.142+/-0.14	0.014+/-0.11
2.5	0.161+/-0.13	0.018+/-0.20
3.0	0.171+/-0.15	0.019+/-0.14
3.5	-	0.021+/-0.11

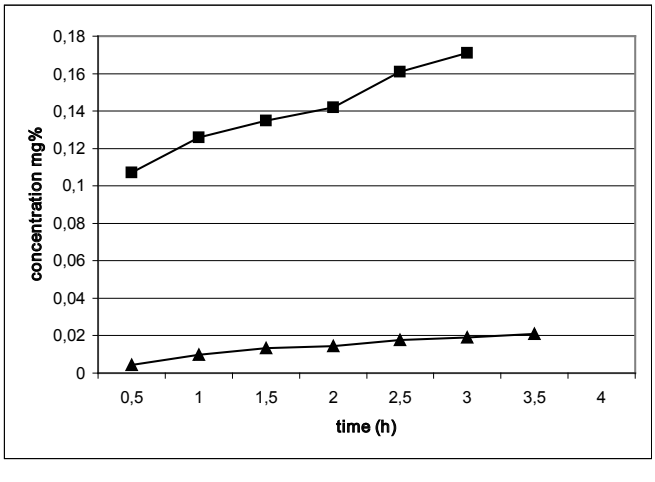

Fig. 1. The 8-MOP permeation profiles determined for two different donor solutions: ■- phosphate buffer pH 7.4; ▲- Oxoralen solution

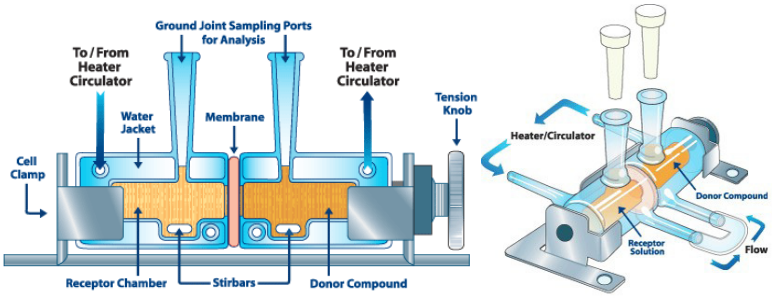

Fig. 2. Fluid/fluid Flynn cell. (www.permegear.com)

They are standard infinite dose cells in a side by side configuration, offering controlled stirring rate and system temperature control. These cells have been employed in the development of delivery systems [30]. The two *in vitro* experiments yielded the different parameters to describe the drug transport through the skin barrier. 8-metoxypsoralen suspended in the phosphate buffer with additional 20% volume of ethanol (A experiment**)** penetrates the membrane quite fast. Changes in the concentration measured every 30 min are significant. Solutio Oxoralen (B experiment) has less affinity to skin, however the permeation profile is quite stable. The first measurement in the A experiment gave the 0.107 mg% of the 8-MOP in the acceptor compartment, whereas in the B experiment this value was near zero. In B experiment the 8-MOP concentration in the donor solution increases very slowl and after 3.5 hours it reaches only 0.021 mg%. The differences in the permeation can be described by the different solubility of 8-MOP in the donor solutions. The 8-MOP molecule is practically insoluble in water, therefore it makes a suspension in phosphate buffer and has strong affinity to lipophilic structures like skin membrane. The donor solution is supersaturated with this compound thus it penetrates easily through the skin. The Oxoralen donor solution (experiment B) enables very good solubility of 8-MOP and prompts equilibrium between the donor solution and skin concentration of 8-MOP. Thus the quantity of 8-MOP in the acceptor solution is quite inconsiderable, however very stable. In summary, the better permeation profile was obtained with phosphate buffer donor solution.

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