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The effect of terpenoid esters on membrane structure investigated by fluorescence and Fourier-transform infrared spectroscopy

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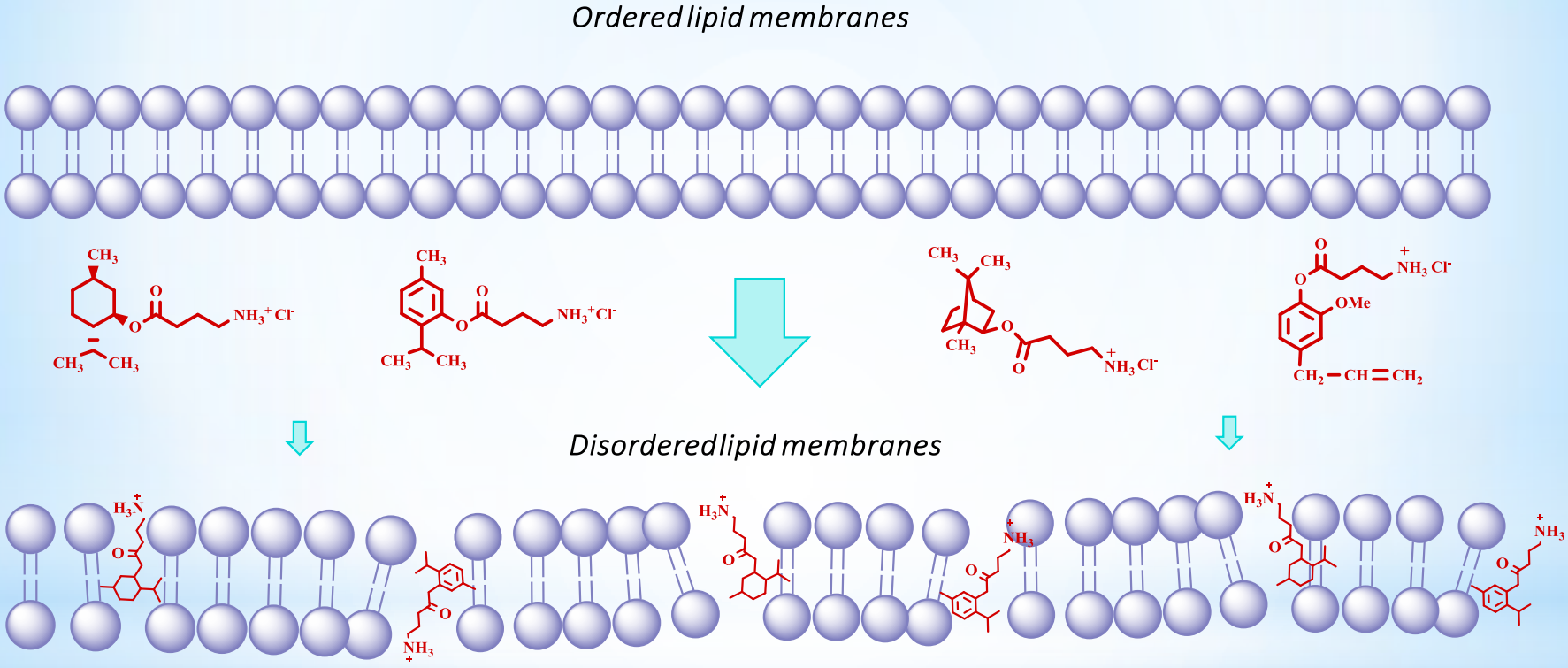


Abstract:

The influence of esters based on gamma-aminobutyric acid (GABA) and mono-/bicyclic terpenoids on membrane structure was investigated. The mechanism of action for terpenoid esters on phospholipids of artificial membranes and lipids isolated from the rat stratum corneum was studied by fluorescence and FT-IR spectroscopy.

We report here, that inclusion of monocyclic terpenoid esters in phospholipid liposomes leads to growth of excimer to monomer ratio (I_E/I_M) indicating a decrease of membrane microviscosity. Another mechanism of influence on biomembranes was proposed for ester of bicyclic borneol – in this case a high ratio of vibronic peak intensities (I_1/I_3) was revealed.

The addition of terpenoid esters appears in the FT-IR spectra as intensity reduction of absorption bands associated with C=O, P=O and P–O–C groups of lecithin phospholipids. Similar results were obtained after esters addition to lipids isolated from stratum corneum indicating a decrease of hydrogen bonds number between polar groups of lipids.

Keywords: terpenoids; fluorescence probe; FT-IR spectroscopy; liposomes; stratum corneum.

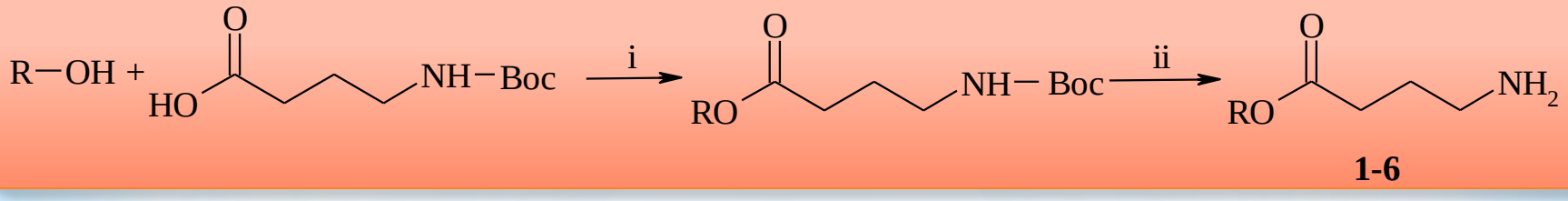
Introduction

Since the discovery and detailed structure determination of transient receptor potential (TRP) channels, a significant amount of naturally occurring substances were identified as modulators of these molecular targets. Among them, terpenes and their derivatives attract great attention when applied topically due to binding to TRP channels in nerve endings or non-neuron skin cells. Despite the presence of own pharmacological activity, terpenes are widely used as penetration enhancers in transdermal delivery.

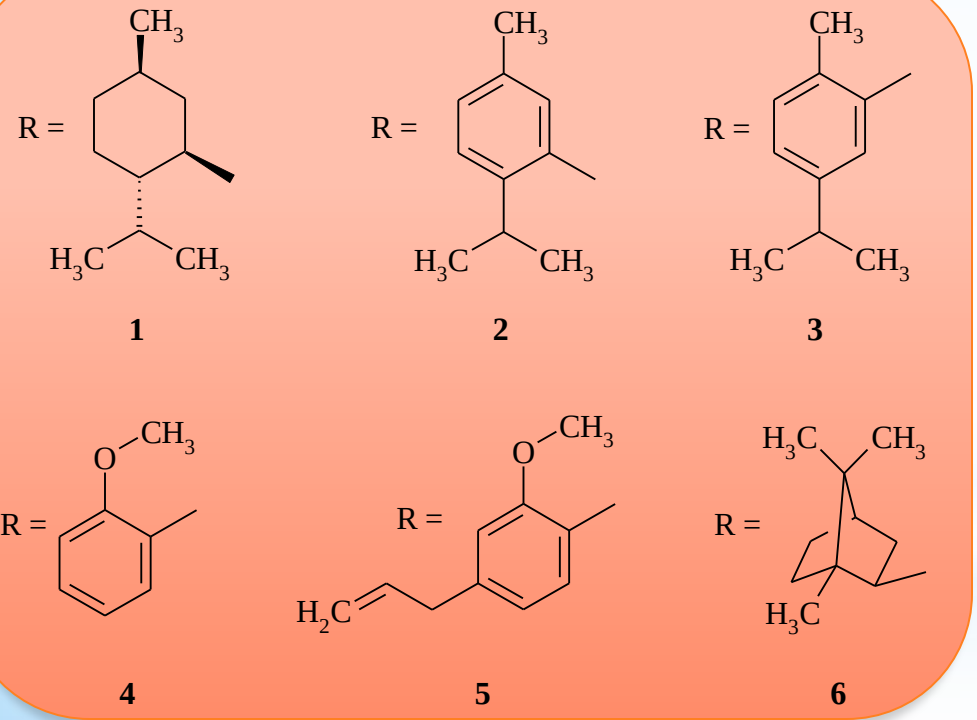
Additionally to TRP channels $GABA_B$ receptors were also found to localize in the periphery; intriguing in this case is GABA presence at the terminal endings of corneal nociceptors. Given the above, combination of terpenoid and GABA residues in one molecule is expedient for development of novel transdermal therapeutic system. Recently, the esters based on mono-/bicyclic terpenoids and GABA were synthesized and found to possess analgesic and anti-inflammatory effect after their transdermal delivery.

Despite the high efficiency of the aforementioned esters via topical application, their mechanism of interaction with membrane lipids has not been studied and described. Thus, the present paper is devoted to understanding the influence of terpenoid esters on phospholipids of artificial membranes and lipids isolated from the stratum corneum (SC). For this purpose instrumental methods such as fluorescence and Fourier transform infrared spectroscopy (FT-IR) have been used.

Results and discussion



Synthetic pathway of compounds **1**–**6**. *Reagents and conditions*: (i) DMAP, CH_2Cl_2, rt, 10 min; DCC, 0 °C, 30 min; rt, 10 h; (ii) HCl, CH_3COOH. All esters were prepared as hydrochlorides.



Esters based on the corresponding terpenoids (**1**−**6**) were synthesized using DCC/DMAP coupling method followed by deprotection of the amino groups in the HCl/CH_3COOH medium.

Investigation of esters' influence on membrane permeability using method of fluorescence probe

Chloroform solution of pyrene + methanol solution of terpenoid esters + chloroform solution of lecithin in a molar ratio 1:10:100

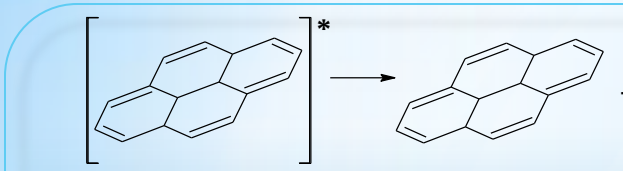
The solvents were removed by slow evaporation under vacuum at 40 °C

The dried mixture was resuspended in 25 ml deionized water and vigorously stirred for 10 min

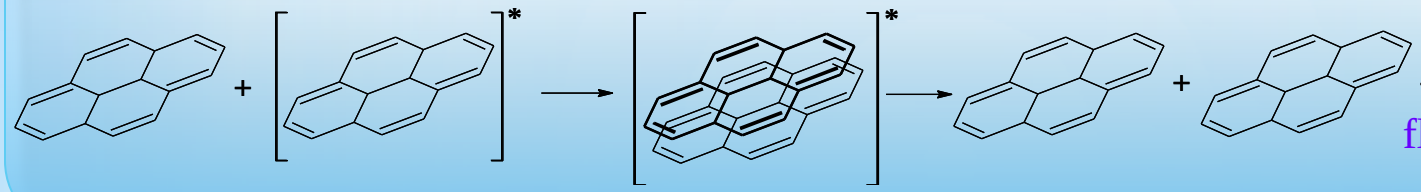
The resulting emulsion was then sonicated for 10 min at 22 kHz frequency

Steady-state fluorescence spectra of samples containing pyrene were recorded on a Horiba Jobin-Yvon Fluorog-FL 3-22 spectrophotometer equipped with a 450W Xe lamp

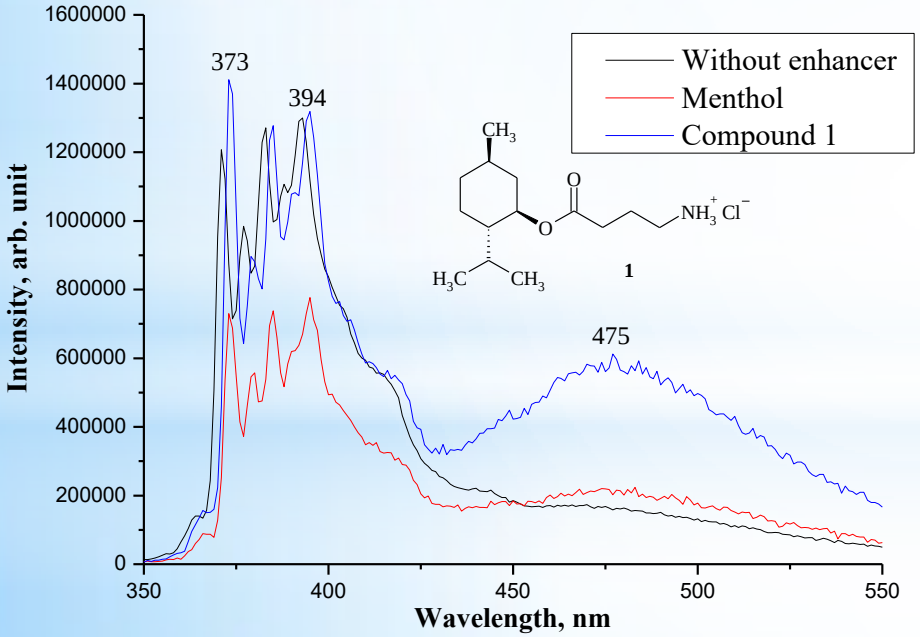
The influence of terpenoids and their esters on membrane microviscosity and polarity

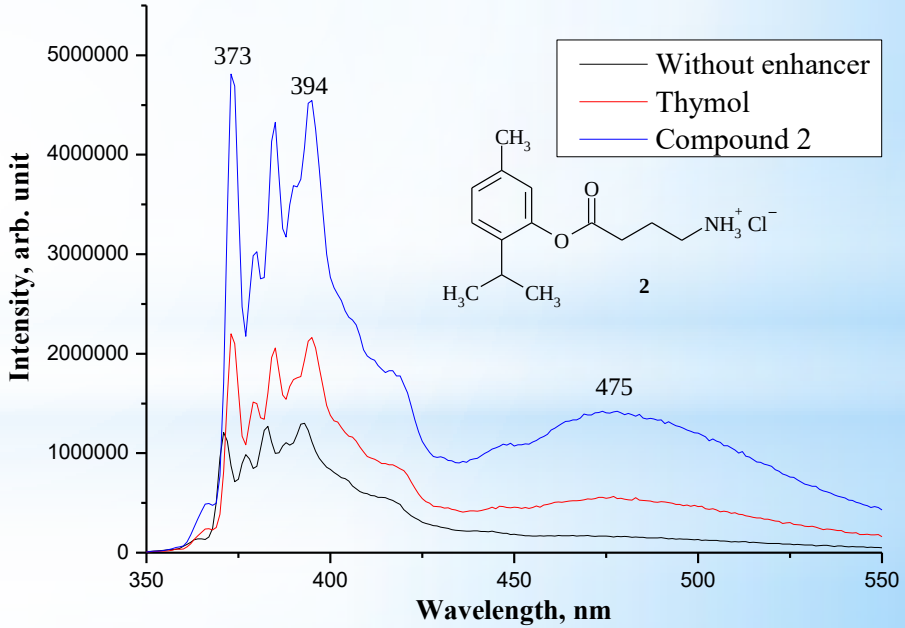


+ $h\nu_M$ - monomer fluorescence (370-395 mn)



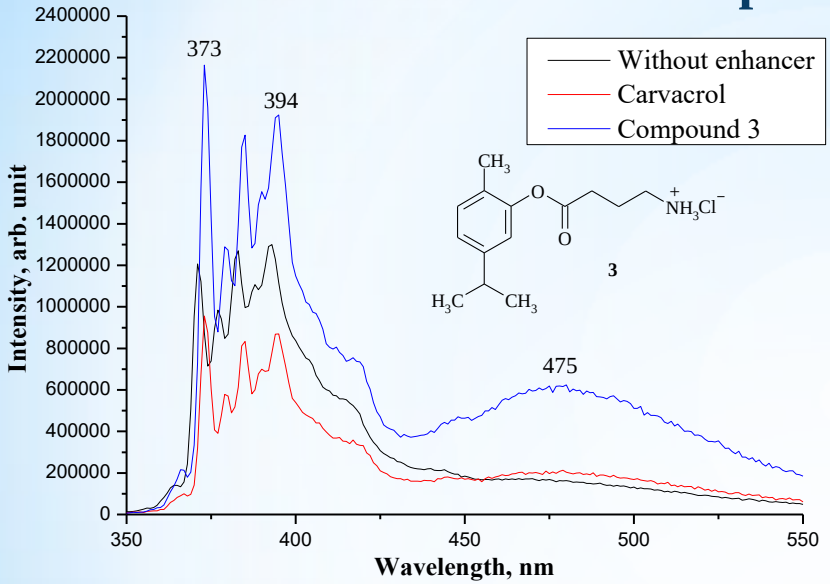
+ + + $h\nu_E$ - excimer fluorescence (470-480 nm)

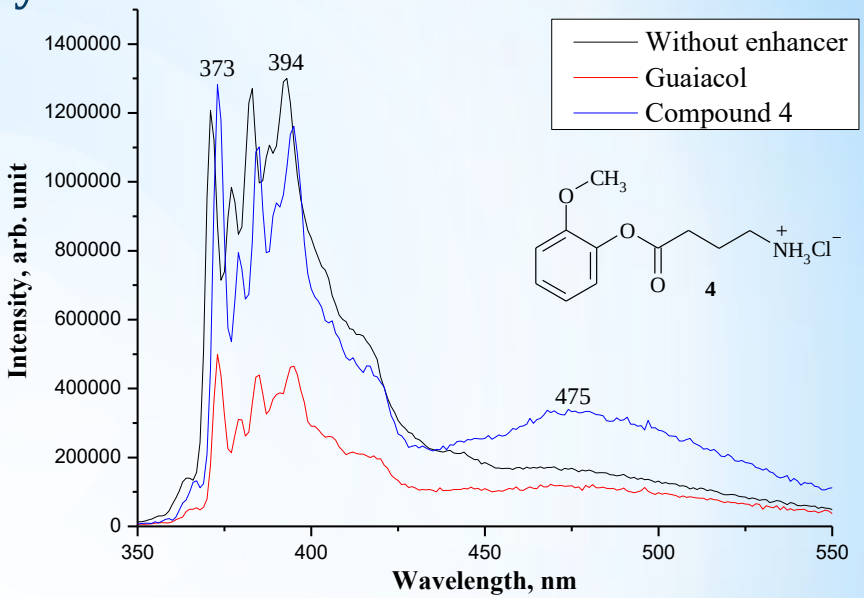




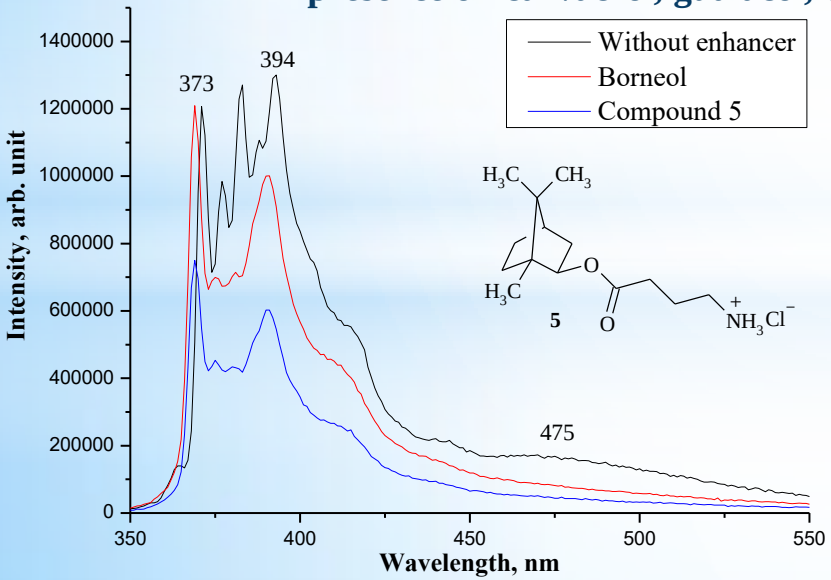
Fluorescence emission spectra of pyrene incorporated into liposome membranes (control, black line) and in the presence of menthol, thymol and their GABA esters

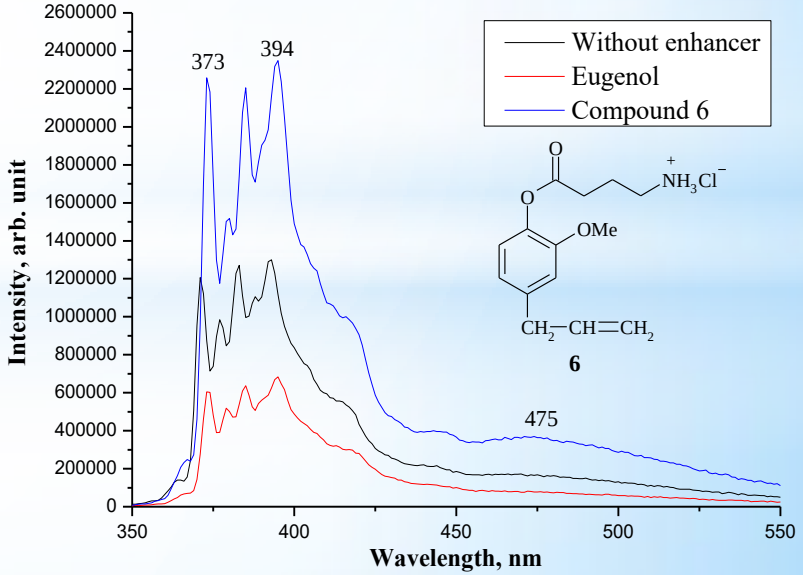
The influence of terpenoids and their esters on membrane microviscosity and polarity





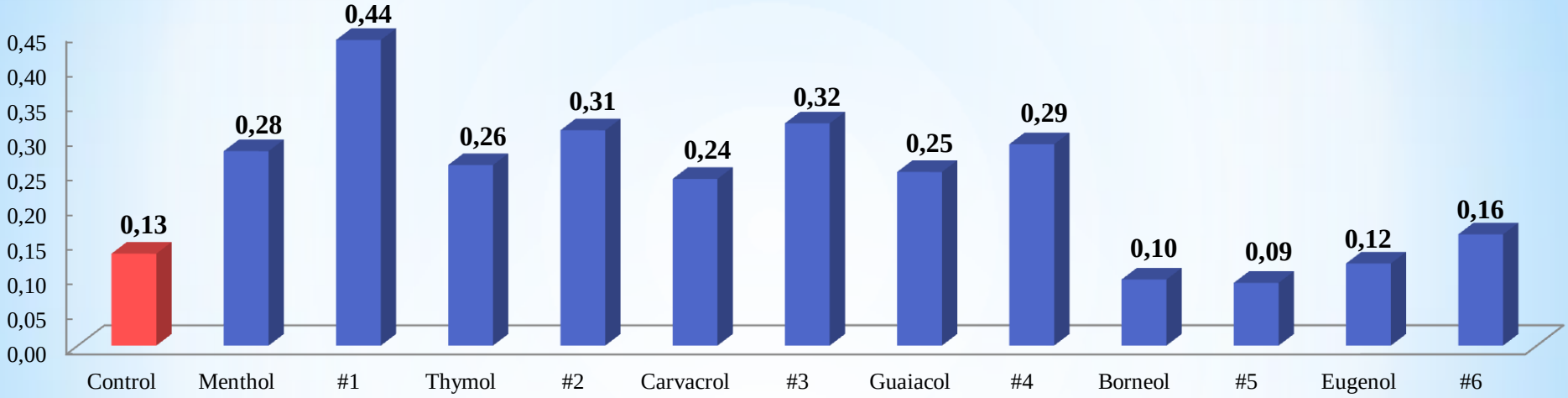
Fluorescence emission spectra of pyrene incorporated into liposome membranes (control, black line) and in the presence of carvacrol, guaiacol, borneol, eugenol and their GABA esters



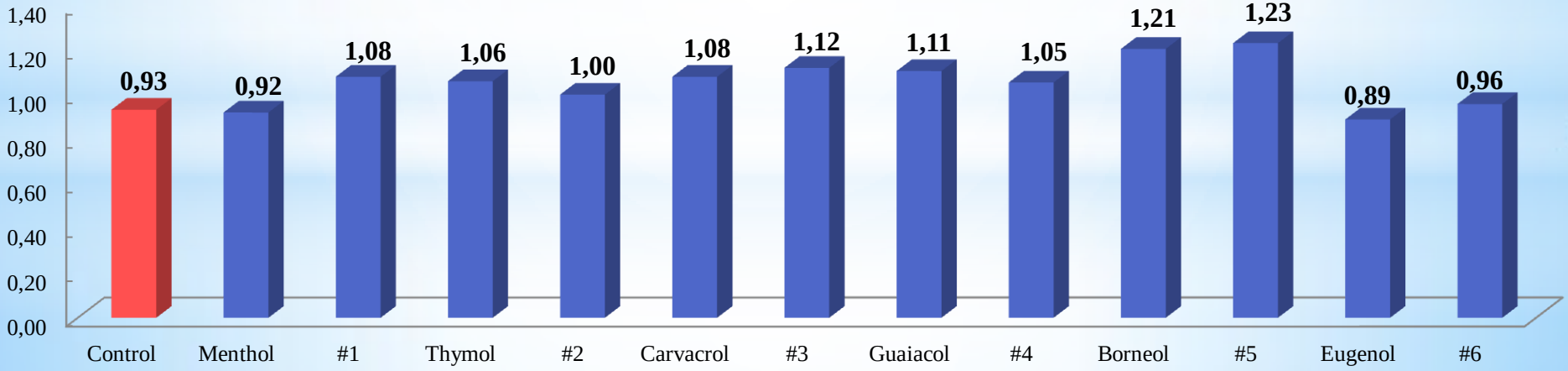


The influence of terpenoids and their esters on membrane microviscosity and polarity

Excimer to monomer ratio : $I_E/I_M = I_{475}/I_{394}$



Ratio of the first to third vibronic band : I_{373}/I_{384}

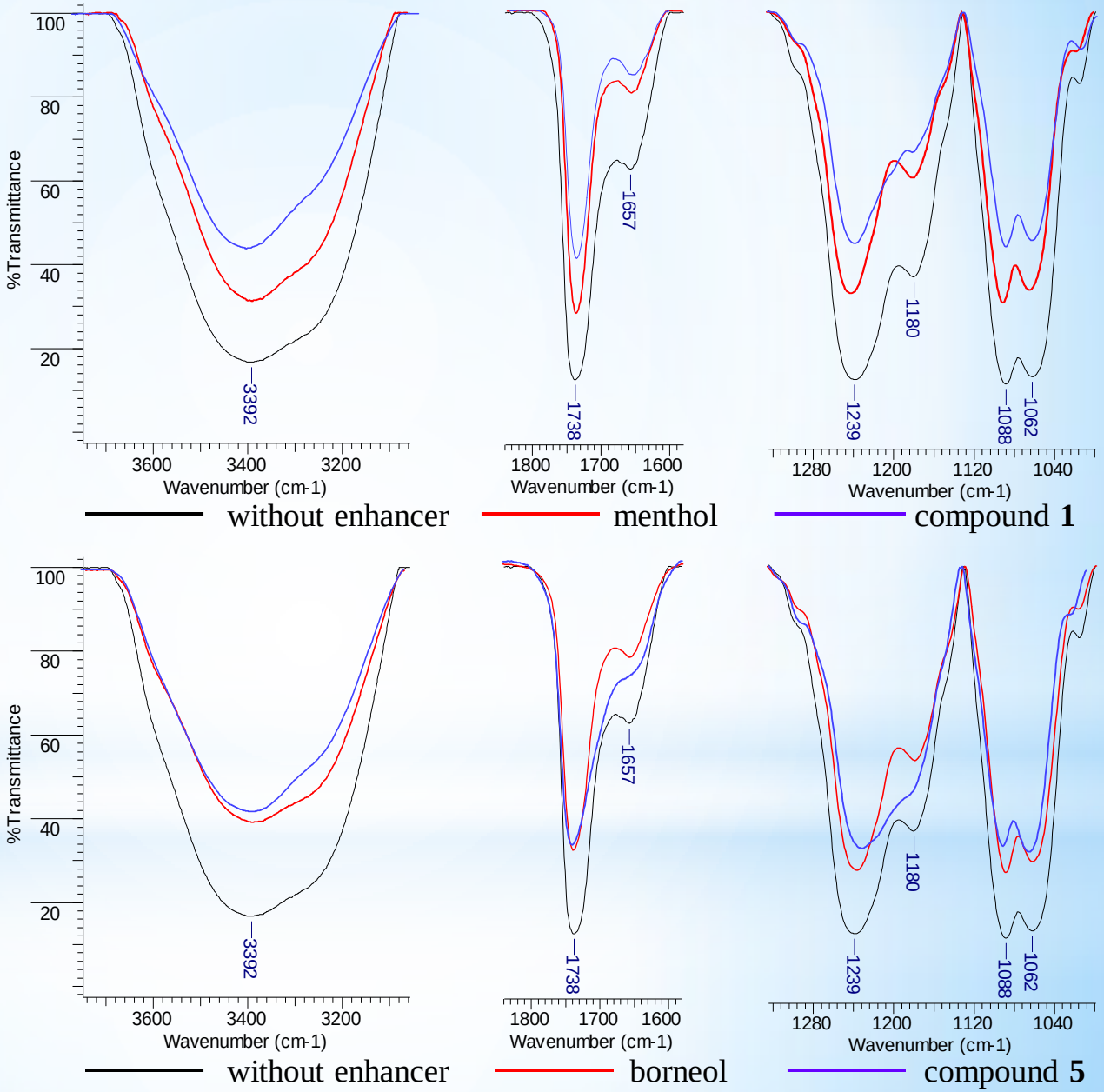


Investigation of esters' influence on phospholipid membrane using FT-IR spectroscopy

FT-IR spectra were measured with a Frontier FT-IR spectrometer (Perkin-Elmer, Hopkinton, MA, USA).

The samples for FT-IR study have been prepared by dissolving the obtained lipids in carbon tetrachloride (CCl_4) with subsequent addition of terpenoid esters (10% relative to lipids' mass).

FT-IR spectra were recorded for films obtained using a method of slow evaporation of solvent directly from undercover under a nitrogen atmosphere.



Investigation of esters' influence on lipids of stratum corneum using FT-IR spectroscopy

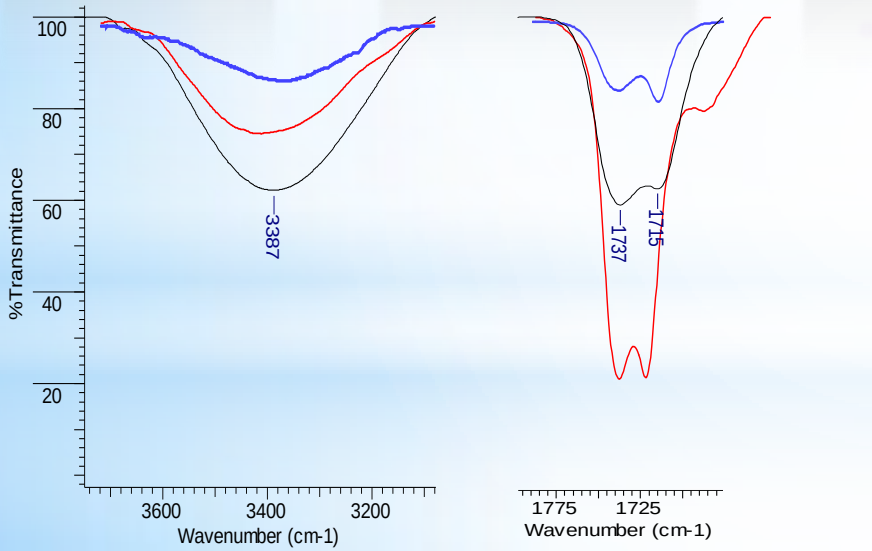
Preparation of stratum corneum

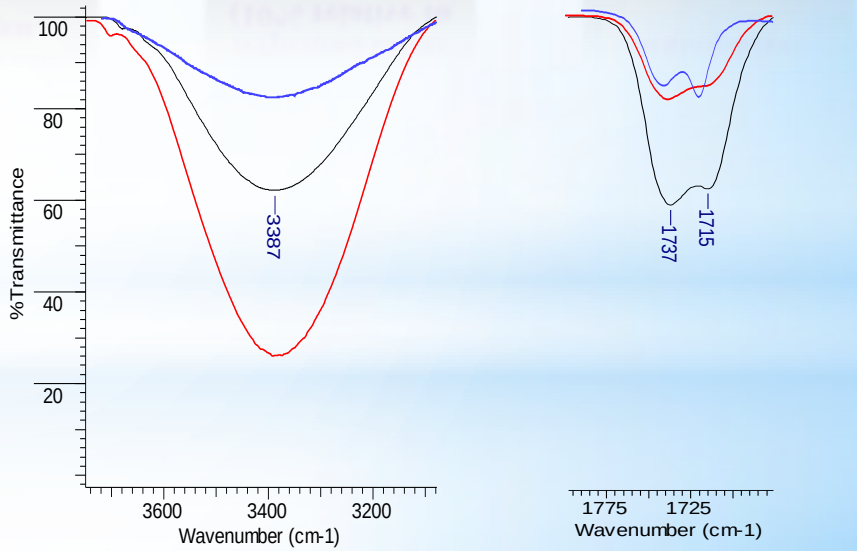
The SC was dipped into chloroform: methanol (2:1) solution and kept in the dark for 72 h

Then the extract was washed twice with distilled water and the lower organic layer was evaporated under vacuum below 40 °C under a stream of nitrogen.

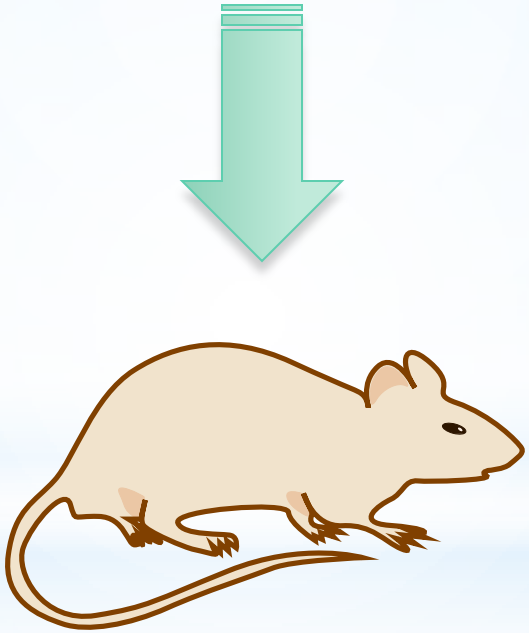
The samples for FT-IR study have been prepared by dissolving the obtained lipids in carbon tetrachloride (CCl_4) with subsequent addition of terpenoid esters (10% relative to lipids' mass).

FT-IR spectra were recorded for films obtained using a method of slow evaporation of solvent directly from undercover under a nitrogen atmosphere.





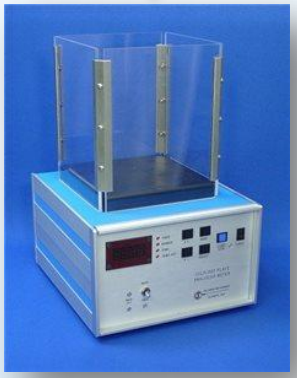
Thus, the influence of terpenoid esters on molecular organization of the lipid matrix was confirmed by method of fluorescence probe and FT-IR spectroscopy. These data substantiate the feasibility of esters' use after their transdermal delivery *in vivo*. In the present study analgesic and anti-inflammatory activity of terpenoid esters has been shown after transdermal delivery.



Experimental methods of pain induction

Thermal methods of induction

✓**“Hot plate” test**



The mice were placed on a hot plate maintained at 55°C one at a time. In this experiment, latency to respond to the heat stimulus was determined by the amount of time (in seconds) it takes for mouse to lick one of its paws. Cut-off time was fixed at 60 sec to minimize the tissue damage that occurs during prolonged contact with heated surface.

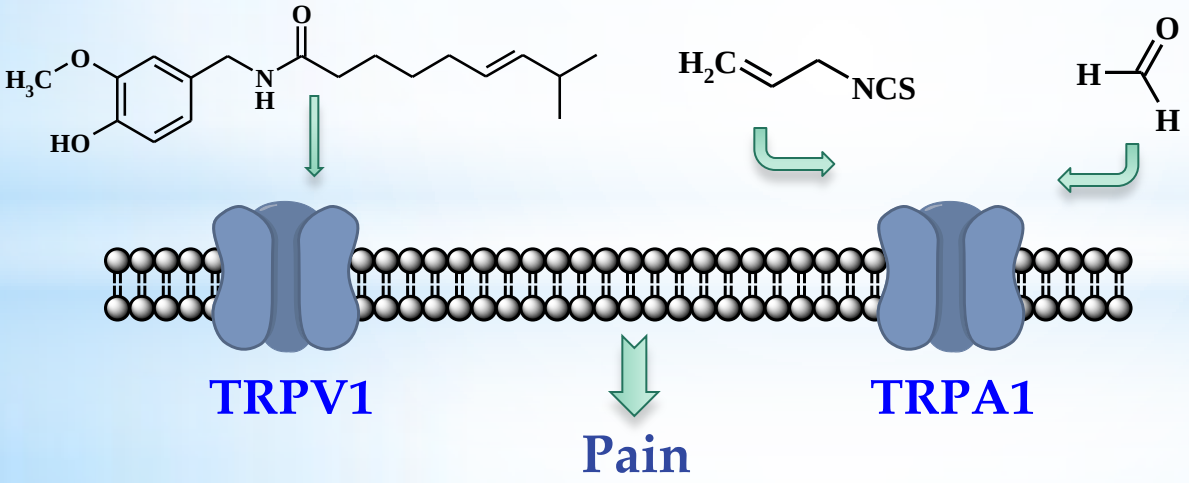
Chemical methods of induction

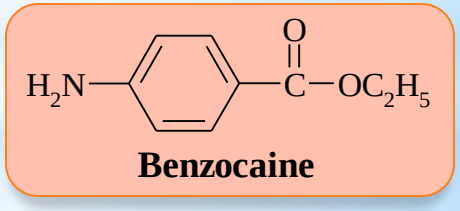
- ✓ **Capsaicin-induced licking** 20 µl (6 µg/paw) of solution
- ✓ **Formalin-induced licking** 20 µl of 2% solution
- ✓ **AITC-induced licking** 20 µl of 0,5% solution

The animal then was placed in an individual plexiglass cage. The time spent licking the injected paw was measured from 0 to 5 min after formalin/capsaicin/AITC administration and was considered as an indicator of pain response.

Analgesic properties of terpenoid esters investigated by «hot plate» test

Compound	Latency, sec	Compound	Latency, sec
Menthol	24 ± 3,9	**1**	30 ± 2,6
Thymol	15 ± 0,3	**2**	21 ± 0,9
Carvacrol	19 ± 1,9	**3**	19 ± 3,8
Guaiacol	20 ± 0,5	**4**	17 ± 3,7
Borneol	27 ± 2,8	**5**	46 ± 2,2
Eugenol	21 ± 3,1	**6**	26 ± 2,9
Benzocaine	18 ± 0,9	Control	10 ± 0,6





Analgesic properties of terpenoid esters investigated on formalin-induced model of pain

Compound	Reaction time, sec	Compound	Reaction time, sec
Menthol	24 ± 5,6	**1**	30 ± 7,4
Thymol	50 ± 5,2	**2**	52 ± 6,6
Carvacrol	38 ± 3,8	**3**	31 ± 8,3
Guaiacol	54 ± 6,8	**4**	47 ± 9,8
Borneol	26 ± 4,7	**5**	23 ± 2,0
Eugenol	40 ± 3,3	**6**	32 ± 4,8
Benzocaine	36 ± 2,4	Control	103 ± 8,5

***Dosage form*: 2% ointment**

***Base*: PEG – PEO – 1,2-Propyleneglycol**

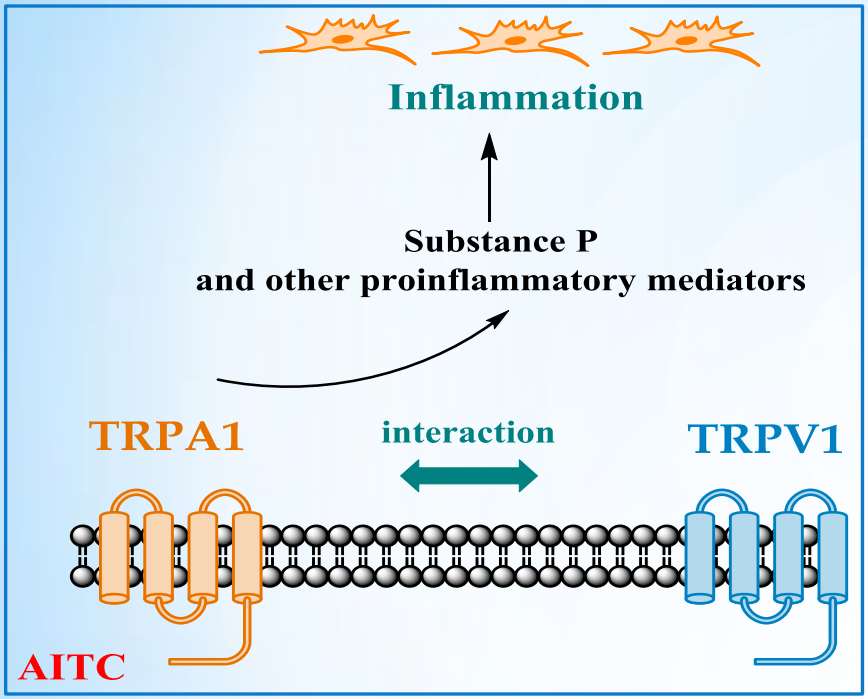
Analgesic properties of terpenoid esters investigated on capsaicin-induced model of pain

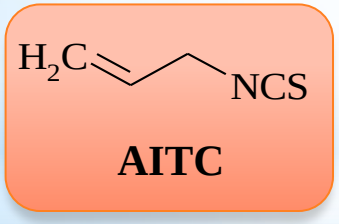
Compound	Reaction time, sec	Compound	Reaction time, sec
Menthol	11 ± 2,7	**1**	4 ± 0,9
Thymol	35 ± 4,1	**2**	20 ± 2,6
Carvacrol	23 ± 6,4	**3**	12 ± 1,2
Guaiacol	23 ± 2,7	**4**	24 ± 3,3
Borneol	12 ± 5,0	**5**	15 ± 3,0
Eugenol	20 ± 3,4	**6**	17 ± 2,2
Benzocaine	29 ± 6,6	Control	46 ± 1,8

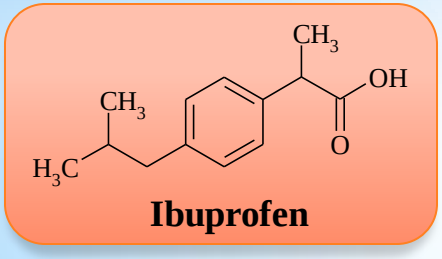
Analgesic properties of terpenoid esters investigated on AITC-induced model of pain

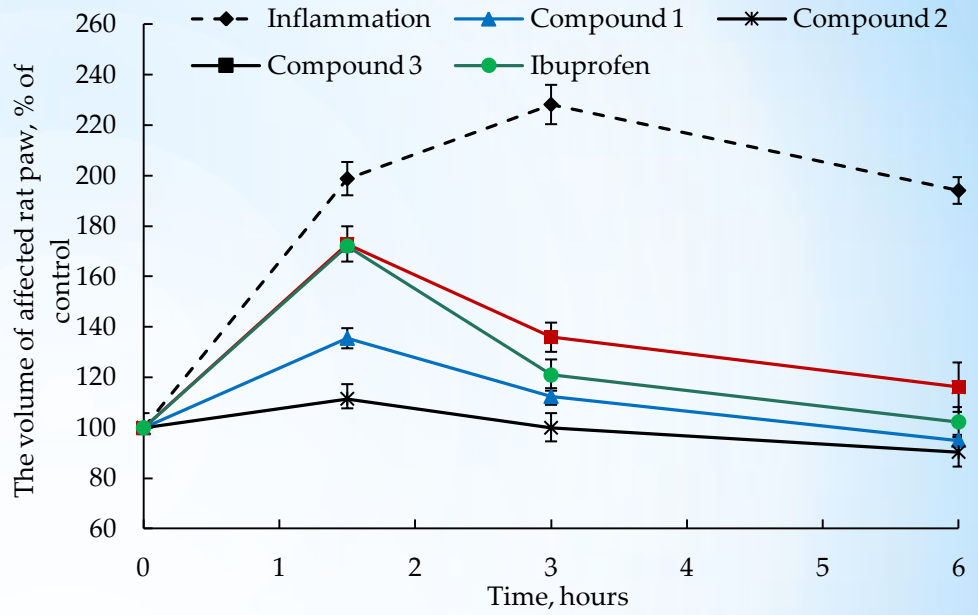
Compound	Reaction time, sec	Compound	Reaction time, sec
Menthol	7 ± 1,2	**1**	3 ± 0,3
Thymol	25 ± 3,8	**2**	20 ± 5,8
Carvacrol	35 ± 2,2	**3**	23 ± 4,3
Guaiacol	21 ± 2,8	**4**	25 ± 1,5
Borneol	8 ± 3,5	**5**	23 ± 3,0
Eugenol	30 ± 2,2	**6**	22 ± 3,1
Benzocaine	48 ± 2,0	Control	71 ± 1,8

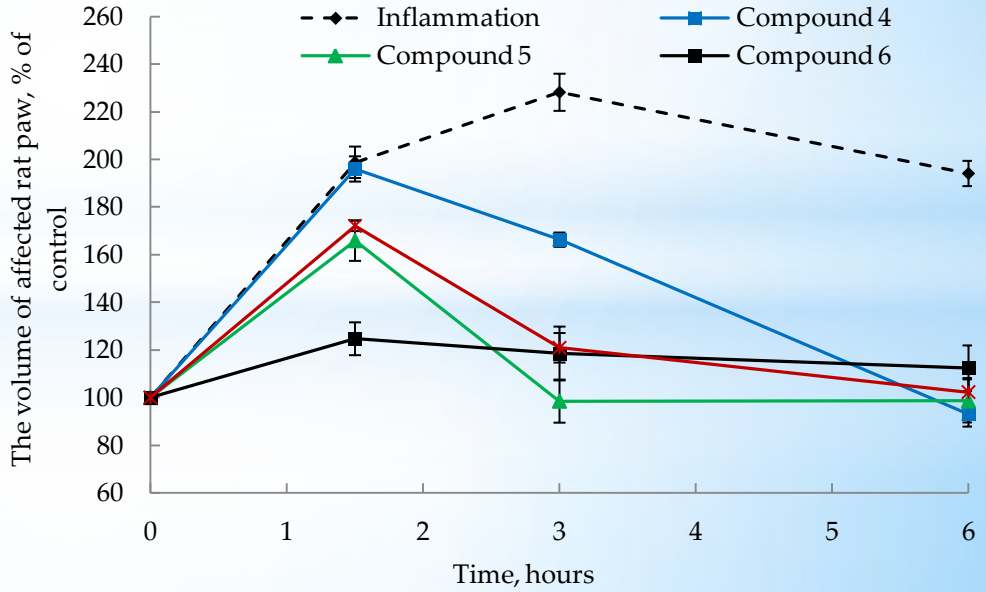
Anti-inflammatory activity of terpenoid esters











Conclusions

In this study, the interaction of terpenoid esters with artificial membranes and lipids isolated from rat SC was investigated with fluorescence and FT-IR spectroscopy.

According to the obtained results, the incorporation of monocyclic terpenoid esters into membranes increased the fluidity of lecithin phospholipids. Interestingly, bicyclic terpenoid borneol and its ester when inserted into liposomes do not affect I_E/I_M fluorescence ratio; in turn, these compounds were shown to increase the membrane polarity. The disruption of hydrogen-bonded network formed by polar lipid groups was suggested as mechanism of terpenoid esters action confirmed by FT-IR analysis.

Thus, the influence of terpenoid esters on molecular organization of the lipid matrix substantiates the feasibility of their use after transdermal delivery *in vivo*.