which had been derived by three different methodologies. Finally, the detected variation between the PSA_{CWM} and the PSA_d, the PSA_s or the TPSA values is about $\pm 10\%$ which, for low molecular weight molecules, is not considered critical [1, 8] for making first estimates (e.g. BBB uptake).

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Alteration of membrane polarization by (−)-terpestacin, a biologically active fungal metabolite

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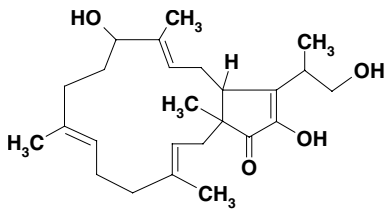
Previously we isolated (−)-terpestacin (**1**) from the fermentation broth of the hyphomycete *Ulocladium* sp. HKI 0226 [1] as a naturally occurring stereoisomer of (+)-terpestacin [2–4]. Compound **1** was found to inhibit syncytium formation by cells infected with respiratory syncytial virus (RSV) and to stimulate aerial mycelium formation by surface cultures of the fungus *Fusarium culmorum* JP15 [1]. Due to the interesting biological activities of (−)-terpestacin we investigated its effect on ion permeability of an artificial black-lipid bilayer membrane (BLM). (−)-Terpestacin (**1**) [1] and its stereoisomer [2] are lipophilic, moderately polar compounds which should well dissolve in biological membranes. Due to the presence of a β-hydroxyketone structure under physiological pH conditions the formation of a negatively charged ion can be suggested. Insertion of this anion into the membrane could increase the negative surface charge of the lipid bilayer due to the orientation of the polar negatively charged part of the molecule towards the water phase. In order to evaluate this suggestion we investigated the influence of **1** on an artificial membrane (BLM, see Experimental).



In concentrations up to 5 μg/ml of **1** given to one or two sides of the BLM made from soybean lecithin no change of membrane permeability was observed. This finding suggested that (−)-terpestacin (**1**) neither destabilized the membrane as a dipole-type tenside nor formed ion-penetrable pores or channels. In another series of experiments we added amphothericin B and ergosterol to the membrane. Under this condition ion-penetrable pores were formed in the BLM as was indicated by the decrease of electric resistance. Increasing concentrations of **1** (0.1–4 μg/ml) given to the membrane site with negative sign of the applied voltage caused a selective increase of potassium ion penetration across the membrane pores formed by amphotericin B and ergosterol. Thus we plotted the zero-current potentials which were necessary to stop the membrane current which appeared in presence of different KCl concentrations at both sides of the membrane. Thereby the KCl concentration was increased in the cis compartment and electrical potential of this compartment was measured in reference to the other compartment. Due to the higher penetrability of the potassium ions we had to apply a negative voltage to stop the current and increase in conductance caused by the flux of this ions (Fig). Amphotericin B is known to owe its therapeutical use as a systemic antifungal agent to the formation of ion-penetrable pores in presence of ergosterol [5, 6]. The constitut-

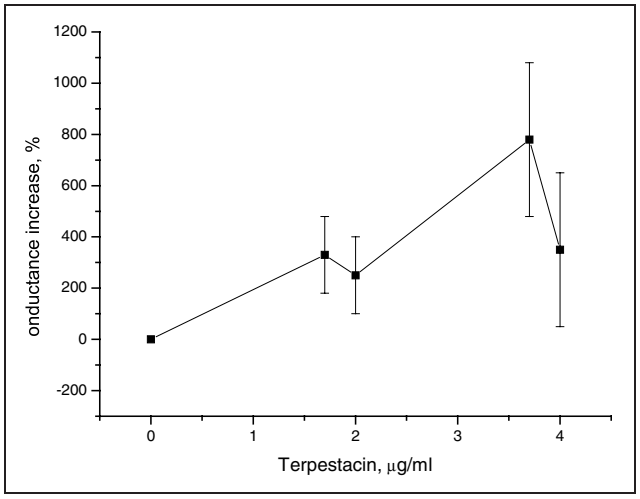


Fig.: Increase of conductance of an artificial membrane made from soyabean phosphatidylcholine and ergosterol in ratio 20 : 1, and amphothericin B (see Experimental) in presence of (−)-Terpestacin (**1**). Conditions: 25 mM KCl, pH 6.4. Starting conductance of the membrane was 480 nS/cm^2. (−)-Terpestacin (**1**) was added to the chamber with negative sign of the applied voltage.

ing aminosugar moiety of amphothericin is positioned at the membrane surface but the lipophilic heptaene lactone ring and ergosterol moieties are integrated into the bilayer. Due to protonation of the amino group at pH < 7.0 the surface charge of the amphotericin B channel is also positive. Hence penetration of cations such as potassium is hampered in comparison with anions and neutral solutes [5]. We suggest that (−)-terpestacin (**1**), as a negatively charged lipophilic compound, compensates for the positive surface charge of amphotericin B channels. Due to this effect the diffusion of positively charged ions such as K^+ to the membrane surface will be enhanced. In accord with this contention we observed a strong increase of membrane conductance for potassium by factor $3-5 \cdot 10^2$ in presence of 3–4 μg/ml of **1** (Fig.).

These findings suggest that increase of negative surface charge of the bio-membranes could play a role in the observed inhibition by **1** of syncytium formation by virus-infected cells and stimulation of aerial mycelium formation by *Fusarium culmorum* [1]. The change in membrane polarity could improve the uptake of positively charged ions and nutrients by transporting systems but could hamper the transport of negatively charged nutrients and ions. As a consequence in the fungal culture earlier onset of cytodifferentiation may occur as was indicated by stimulation of aerial mycelium formation.

Experimental

1. Materials

(−)-Terpestacin was isolated as a single compound on HPLC from *Ulocladium* sp. HKI 0226 as was described previously [1]. The physico-chemical properties (MS, NMR) were fully consistent with the reported data [1].

2. Measurements employing lipid bilayer membranes

Planar bilayer lipid membranes (BLM) were prepared from soya bean phosphatidylcholine (Sigma, P. 5638, 20 mg/ml n-heptane). In other experiments 1 mg/ml ergosterol and 0.3 μg/ml amphotericin B were admixed to the above solution of phosphatidylcholine to form amphotericin B channels.

The measuring glass cell (25 ml of total volume) was equipped with a teflon cylinder (1 cm diameter) which contained a hole of 0.5 mm diameter to harbour BLM. Formation of the BLM was controlled by use of a binocular microsope [6, 7]. Both the measuring cell (10 ml outside cis-volume) and the inner side of the teflon cylinder (transvolume; 1 ml) were filled with a solution of potassium chloride (25 mM).

Membrane current was measured by the voltage-clamp method [7, 8]. The current measuring device consisted of an operational amplifier model Keithly 301 (USA). Amplitude current noise of the amplifier was less than 10^{-13} A in the frequency range 0.1–20 Hz. Output of the operational amplifier was connected to the input of device analog-digital changing device of National Instrument, USA. Data recording and processing was done by use of recording software of National Instruments, USA.

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