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Inhibition of 3α-hydroxysteroid dehydrogenase by polyene macrolides

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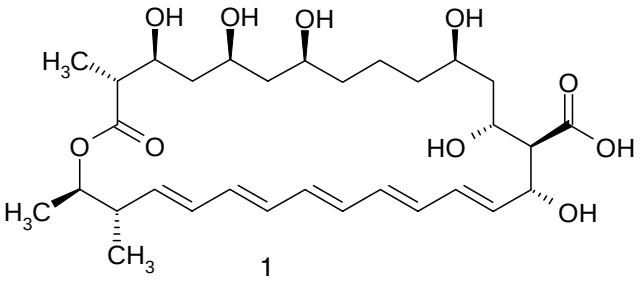
In a screening for inhibitors of 3α-hydroxysteroid dehydrogenase as one of the key enzymes involved in inflammatory events [1] we disclosed an *Actinomyces* strain, HKI 0242, as the producer of an active principle. The strain HKI 0242 was isolated from a soil sample obtained from the rhizosphere of *Putterlickia verrucosa* (E. Mey. ex Sond) Szyszyl. occurring in South Africa and it was identified as *Streptomyces olivoreticuli* [2, 3]. Submerged cultivation of *Streptomyces olivoreticuli* HKI 0242, extraction of the culture broth with ethyl acetate and several subsequent chromatographic steps of purification afforded **1** as a strong inhibitor of the above enzyme. The structure of **1** was identified as the known strevertene A [4] by optical spectroscopy, MS, 1D and 2D NMR measurements. It is a 28-membered pentaene macrolide antibiotic possessing a carboxylic acid substituent in addition to hydroxyl and methyl groups [4]. This finding suggested that other polyene macrolide antibiotics could exert a similar effect on 3α-hydroxysteroid dehydrogenase. Shown in the Table are the IC_{50} values determined for hexafungin [5], fungicidin [5], levorin [5], roflamycoin [5], nystatin [5], amphothericin B [5], pimaricin [5], streverten (**1**) [4] and the standard compound indomethacin. Amphothericin B and levorin displayed an inhibitory effect comparable to streverten exceeding that of standard compounds such as indomethacin. The other polyene macrolides inhibited the enyzme markedly, too, but with lower efficiency than indomethacin.



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The results suggest that polyene macrolide antibiotics such as the therapeutically used amphothericin B exert antiinflammatory effects due to the inhibition of glucocorticoid turnover.

Table: Inhibition of 3α-hydroxysteroid dehydrogenase by polyene macrolide antibiotics

Comp.	IC_{50} (μg/ml)
Strevertene A (1)	15
Nystatin	51
Levorin	11
Pimaricin	76
Roflamycoin	37
Hexafungin	76
Amphotericin B	9
Fungicidin	80
Indomethacin	24

Experimental

The strain *Streptomyces olivoreticuli* HKI 0242 was isolated from a soil sample obtained from the rhizospere of the African plant *Putterlickia verrucosa* and was identified on the basis of its morphological properties [2]. Cultivation was carried out on a rotary shaker (180 ppm, 28 °C) for 96 h in Erlenmeyer bottles with 100 ml of the liquid medium composed as follows (g/l): dextrose 20, soybean meal 20, NaCl 5, $CaCO_3$ 3, final pH 6,5–6,8.

Thereafter, the culture broth (10 l) was extracted twice by 2 volumes of ethyl acetate. The residue of the dried and evaporated extracts was chromatographed on silica gel 60 (TLC plates Merck 0.063–0.1 mm; $CHCl_3$/MeOH 9 : 1; v/v). Fractions containing **1** were detected due to their antifungal activity against *Sporobolomyces salmonicolor* as test organism.

Nystatin and amphothericin B were purchased from Sigma. Roflamycoin, hexafungin, levorin, fungicidin and pimaricin were obtained from the natural product sample collection of the Hans-Knöll-Institute, Jena.

Inhibition of 3α-hydroxysteroid dehydrogenase was determined by spectroscopic measurement of 5β-dihydrocortison reduction by NADPH using rat liver cytosol [1].

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