NOTES

Favolon, a New Antifungal Triterpenoid from a *Favolaschia* Species

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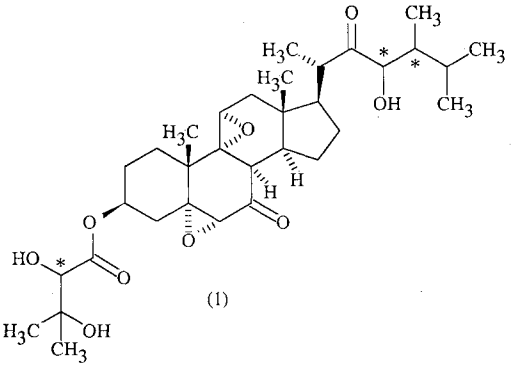
In our ongoing search for novel biologically active metabolites from basidiomycetes an ethiopian *Favolaschia* species was detected as a new producer of a series of strongly antifungal compounds. Besides the strobilurins A and F, and oudemansin A, two new 9-methoxy derivatives of strobilurin A, 9-methoxystrobilurin A and K, were isolated and characterized previously[1]. In the following we wish to describe the fermentation, isolation, and biological characterization of favolon (**1**), a new antifungal sterol from cultures of the same fungus.

The small bright yellow fruiting bodies of *Favolaschia* sp. 87129 were found growing on wood in a forest close to Kolobo, Ethiopia. The specimen showed all characteristics of the genus[2], the species, however, could not be unequivocally identified. Mycelial cultures were derived from spore prints of fruiting bodies. Voucher specimen and cultures are deposited in the collection of the Lehrbereich Biotechnologie, University of Kaiserslautern.

For maintenance *Favolaschia* sp. 87129 was cultivated in YMG medium composed of: Yeast extract 0.4%, malt extract 1%, glucose 0.4% and agar 1.5%, pH 5.5. For the production of favolon a well grown seed culture (150 ml) in YMG was used to inoculate 10 liters of YMG in a Biostat V fermentation apparatus. The fermenter was incubated at 22°C with an aeration of 1.5 liters air/minutes and agitation (150 rpm). After one week this culture was used as inoculum for 100 liters of the same medium in a 150-liter stainless steel vessel (Deutsche Metrohm, temperature: 22°C, aeration: 15 liters/minute, stirrer speed: 150 rpm). Antifungal activity in fermentations and in fractions after chromatography were measured in the agar plate-paper disc diffusion assay using *Mucor miehei* as test organism.



(1)

Favolon was detected only in the mycelia. 2.5 kg wet weight from a 100-liter batch were extracted with 7.5 liters of methanol. Evaporation of the solvent yielded 1.5 liters of an aqueous phase from which favolon was extracted with 6 liters of ethyl acetate. Evaporation of the organic phase yielded 3.2 g of crude product which was further purified by chromatography on silica gel (Merck 60; elution with cyclohexane-ethyl acetate 1:1) resulting in a red fraction from which favolon readily crystallized. Recrystallization from methanol yielded 130 mg of white needles (mp 188~190°C, Rf 0.46, silica gel Merck 60, toluene-acetone-acetic acid 70:30:1). The physico-chemical characterization and structure elucidation of favolon[3] (**1**) will be described elsewhere[4]. Favolon exhibits an unusual *cis* connection of the rings B and C. Stereochemistry of the marked carbon atoms

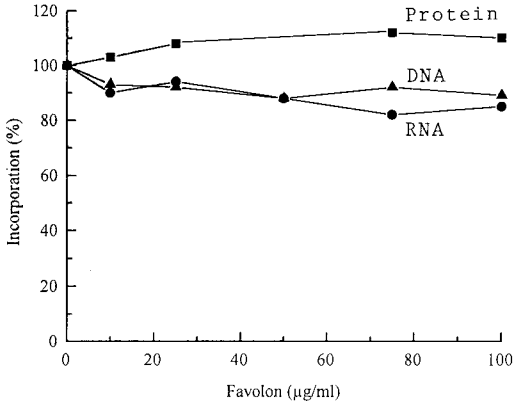
Table 1. Antifungal activity of favolon in the agar diffusion assay.

Organism	Diameter of inhibition zone (mm)		
	μg/disc*		
	0.1	1	10
Absidia glauca (+)	–	–	–
A. glauca (–)	–	–	–
Alternaria porri	19i	23i	26i
Aspergillus ochraceus	11i	19i	20i
Botrytis cinerea	16i	20i	26i
Cladosporium cladosporioides	16i	22i	26i
Epicoccum purpurascens	–	15i	19i
Fusarium fujikuroi	19i	22i	22i
Fusarium oxysporum	15	25	25
Mucor miehei	24	30	30
Nematospora coryli	–	–	–
Neurospora crassa	+	+	+
Paecilomyces varioti	20	30	30
Penicillium islandicum	25	34	34
P. notatum	12	23	25
Pythium ultimum	+	+	+
Rhodotorula glutinis	–	–	–
Saccharomyces cerevisiae is 1**	–	–	–
Ustilago nuda	–	9	11
Zygorhynchus moelleri	15i	22i	25i

–: no inhibition zone, +: inhibition zone just visible, *: diameter = 6 mm, **: Gift of Prof. LACROUTE, STRASBOURG, F, i: inhibition incomplete.

Dedicated to Prof. Dr. S. ŌMURA on occasion of his 60th birthday.

Fig. 1. Effect of favolon on the synthesis of protein, RNA, and DNA in L 1210 cells.



Controls without antibiotic (100%): ^{14}C-leucine (9251 cpm), ^{14}C-uridine (19949 cpm), ^{14}C-thymidine (10363 cpm).

has not been determined.

Favolon exhibits potent antifungal activities against ascomycetes, basidiomycetes, oomycetes, and zygomycetes (Table 1). In the plate diffusion assay[5] no antibacterial activity could be detected using *Bacillus brevis*, *B. subtilis*, *Micrococcus luteus*, and *Enterobacter dissolvens* at 100 μg/disc.

No cytotoxic activities on L 1210 cells (mouse lymphocytic leukemia ATCC CCL 219) grown in F 12 medium containing 20% of horse serum as described previously[6] could be detected at concentrations up to 100 μg/ml. In addition, no effect on the incorporation of ^{14}C-thymidine, ^{14}C-uridine, and ^{14}C-leucine into DNA, RNA, and proteins in L 1210 cells could be observed (Fig. 1, for assay conditions see ref[5]). These findings together with the lack of antibacterial activity are indicative that favolon interferes with a pathway or structure which is essential for fungi but may not be present in bacterial or animal cells.

Acknowledgments

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