Notes

Sch 31828, A NOVEL ANTIBIOTIC FROM A *MICROBISPORA* SP.: TAXONOMY, FERMENTATION, ISOLATION AND BIOLOGICAL PROPERTIES

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In the course of screening for novel antifungal antibiotics, a new solvent extractable antifungal, Sch 31828 (antibiotic EV22) was isolated from an unusual *Microbispora* sp. SCC 1438. Sch 31828 exhibited good *in vitro* activity against *Candida albicans* and dermatophytic fungi, and moderate activity against *Staphylococcus aureus*[1],†.

Microorganism

The producing culture, SCC 1438, was isolated from a mixture of soils plated on yeast-starch agar (yeast extract 0.5%, soluble starch 1.0%, Difco agar 1.5%, pH 7.0) containing 25 μg/ml everninomicin. On the descriptive media of SHIRLING and GOTTLIEB[5], and WAKSMAN[6], after 21 days at 30°C, the culture forms beige to tan vegetative mycelia pigments, gray-white to pink aerial mycelia and a faint yellow-brown diffusible pigment. Upon incubation for an additional 7 to 14 days, the aerial mycelia turn gray-green. Microscopically the substrate mycelia are long, irregularly branched, do not fragment into short elements, or form spores. The aerial hyphae branch monopodially. Elliptical spores are born in longitudinal pairs closely arranged along the aerial hyphae. The culture grows well from 27 to 40°C on yeast-glucose agar (yeast extract 1.0%, glucose 1.0%, Difco agar 1.5%, pH 7.0). Growth does not occur at 45°C. By the methods of LECHEVALIER[7], whole cells contain *meso*-diaminopimelic acid and madurose.

Based on morphological and whole cell analysis, the producing culture is identified as a mesophilic species of *Microbispora*. The culture has been deposited in The American Type Culture Collection (U.S.A.) and has been assigned accession number ATCC 53620.

Fermentation

The inoculum for antibiotic production was prepared in a medium containing beef extract 0.3%, Tryptone 0.5%, yeast extract 0.5%, Cerelose 0.1%, potato starch 2.4% and $CaCO_3$ 0.2%, in tap water. A 200-ml Erlenmeyer flask containing 70 ml of this medium was inoculated with 3 ml of a frozen whole broth of SCC 1438. The flask was incubated at 30°C on a rotary shaker at 300 rpm for 72 hours. Twenty five ml of this seed culture was transferred to 500 ml of the same medium (in 2-liter flasks) and incubated as above for 48 hours. The entire contents of the second germination were used to inoculate a 14-liter fermentor (New Brunswick Scientific) containing 10 liters of the following medium: Hy-Soy 0.6%, Nutritone 0.2%, Cerelose 0.5%, soluble starch 2%, $CaCO_3$ 0.2%, in tap water. The fermentation was carried out for 48 hours at 32°C with an air flow of 0.25 v/v/m and an agitation rate of 200 rpm. Antibiotic production was monitored by bioassay using *C. albicans* Wisconsin and *S. aureus* 209P. Maximum activity was observed at 48 hours.

Isolation

The whole broth adjusted to pH 2 from twenty, 10-liter fermentations (200 liters) was extracted twice with equal volumes of EtOAc. The extract was concentrated to an oil, dissolved in small volumes of CH_3CN and precipitated with hexane. The precipitate was filtered and dried *in vacuo*. The resulting crude complex (75 g) was dissolved in CH_3CN (100 ml), applied to a column of Sephadex LH-20 (2.7 liters) and eluted with CH_3CN (100-ml fractions). Silica

† Recently KEMPF *et al.*[2], ONISHI *et al.*[3], and LEWIS and MENES[4] reported the isolation of a compound, L-660,631, from the culture broth of two unknown Actinomycetes species, the structure of L-660,631 is identical to Sch 31828.

gel TLC of the Sephadex LH-20 fractions in a $CHCl_3$ - MeOH (9:1) system, followed by bioautography against *C. albicans*, demonstrated that the complex consisted of at least three biologically active components. Component **I**, Sch 31828, Rf 0.20, was the major component. Sephadex LH-20 fractions were kept in solution for evaluation and derivatization since concentration caused a considerable loss of bioactivity. Sch 31828 (component **I**) is extremely unstable to light, pH and heat. In order to stabilize the compound the methyl ester derivative was synthesized.

Preparation of Methyl Ester of Sch 31828 (Component I)

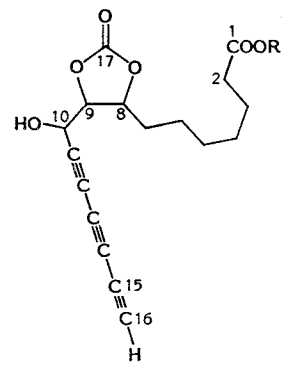
To 600 ml of the Sephadex LH-20 CH_3CN fraction (component **I**) in a 2-liter round bottom flask, 3 ml of anhydrous MeOH (saturated with HCl gas) was added. The mixture was refluxed for 15 minutes, cooled and the insolubles removed by filtration. The CH_3CN - MeOH solution was evaporated to a volume of 100-ml. An additional 300-ml of $CHCl_3$ was added, and the contents filtered to remove insoluble material. The solution (400 ml) was washed with saturated sodium carbonate, followed by distilled water and dried over magnesium sulfate. The solution was concentrated *in vacuo* to yield 525 mg of crude methyl ester.

The purification of Sch 31828 methyl ester was accomplished by preparative HPLC using a Waters PREP 500 LC system with a single C-18 cartridge. The column was eluted with an CH_3CN - H_2O mixture (50:50). The resulting fractions were monitored by reverse phase HPLC (μBondapak C-18 (10 μm) 30 cm × 3.9 mm) using CH_3CN - H_2O (50:50) as a mobile phase. The fractions containing Sch 31828 methyl ester were combined, concentrated to dryness, and 190 mg of pure Sch 31828 methyl ester was isolated. This compound was at least ten times more stable than the parent acid. Self induced polymerization caused by the acetylene groups were markedly inhibited by esterification of the carboxyl functional group in the molecule.

Physico-chemical Properties

Sch 31828 and its methyl ester (Fig. 1) are yellow oily compounds, unstable to light and heat, and are soluble in MeOH, EtOH, EtOAc and Me_2CO, but insoluble in ethyl ether, petroleum ether and water. Sch 31828 methyl ester has the following spectroscopic properties: UV λ_{max}^{MeOH} nm 255, 270, 287, 305; IR (KBr) cm^{-1} 2250, 1810, 1755, 1725. Additional spectroscopic data from Sch 31828 methyl ester and several other derivatives will be reported elsewhere[8]. Sch 31828 (EV22) is similar to other naturally occurring acetylenic compounds which are produced by various plants and fungi among the basidiomycetes[9~11]. Sch 31828 differs from all other compounds in the class by the presence of the novel dioxolone ring system.

Fig. 1. Structures of Sch 31828 acid and methyl ester.



Sch 31828 R=H
Sch 31828 methyl ester R=CH_3

Biological Properties

In vitro broth dilution tests for fungi (Sabouraud dextrose broth, pH 5.7 with and without 10% horse serum), or microtiter dilution tests for bacteria (Mueller-Hinton broth, pH 7.4), were performed to determine MICs. The results, shown in Table 1, indicated that Sch 31828 methyl ester had broad spectrum antifungal activity *in vitro*, with geometric mean MICs against *Candida* of 0.034 μg/ml, and against dermatophytes of 1 μg/ml. The addition of serum significantly reduced the activity (MICs 3.6 μg/ml), indicating high protein binding. The geometric mean MICs against 22 strains of *Staphylococcus*, 4 strains of *Streptococcus* and 78 strains of Gram-negative bacteria were 29.1, $\geq$76.1, and $\geq$128 μg/ml, respectively.

The *in vivo* activity of Sch 31828 methyl ester was determined in hamsters and guinea pigs. Sch 31828 methyl ester was applied once daily for 8 days, as a 1%-solution (10% EtOH - 45% glycerol - 45% polyethelyene glycol 400) to hamsters infected intravaginally, with *C.*

Table 1. *In vitro* activity of Sch 31828 methyl ester.

Strains	MICs (μg/ml)		
	SDB[a]		SDB+10% HS[b], 24 hours
	24 hours	72 hours	
Candida albicans C40	0.031		2.0
C. albicans C41	9.031		4.0
C. albicans C42	0.031		4.0
C. albicans C43	0.015		4.0
C. tropicalis C44	0.015		4.0
C. stellatoidea C45	0.015		2.0
C. parapsilosis C53	0.50		8.0
Geometric mean	0.034		3.6
Trichophyton mentagrophytes D23		1	
T. rubrum D61		1	
Microsporum canis D18		1	
M. gypseum D16		1	
Geometric mean		1	

[a] SDB: Sabouraud dextrose broth, pH 5.7, 28°C.
[b] HS: Horse serum.

albicans; and applied twice daily, for 10 days, as a 2%-solution (as above), to male guinea pigs infected topically, with *Trichophyton mentagrophytes*[12]. Intravaginal, skin and hair samples obtained from the animals, were cultured at various times during and after treatment. No improvement was noted in either hamsters or guinea pigs treated with Sch 31828 methyl ester.

The intravenous LD_{50} of Sch 31828 methyl ester in male mice was 35 mg/kg.

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