ANTIBIOTICS FROM BASIDIOMYCETES. X[1]
SCORODONIN, A NEW ANTIBACTERIAL AND ANTIFUNGAL METABOLITE FROM *MARASMIUS SCORODONIUS* (FR.) FR.

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Scorodonin (**1**), a novel biologically active metabolite, was isolated from submerged cultures of the mushroom *Marasmius scorodonius* (FR.) FR. Its structure has been determined by chemical and physical methods. The antibiotic inhibits the growth of bacteria, yeasts, and filamentous fungi. In cells of the ascitic form of EHRLICH carcinoma the incorporation of thymidine and uridine into DNA and RNA is strongly inhibited by scorodonin whereas the incorporation of leucine into protein is not affected.

Within the family *Tricholomataceae* species of the genus *Marasmius* and closely related genera have long been known to produce interesting secondary metabolites. From cultures of *Marasmiellus ramealis* marasin, an antibiotic polyacetylene, and 8-hydroxy-3-methylisocoumarin, have been isolated by BENDZ in 1959[2,3]. The same species produces 3,7-bis(hydroxymethyl)-1-benzoxepin-5(2H)-one, a metabolite with a hitherto unknown ring system as published by HOLROYDE *et al.* in 1976[4]. From fruiting bodies of *Micromphale perforans* HÖFLE *et al.*[5] isolated lentinic acid and determined its structure. Alliacolide, a new sesquiterpene hydroxy-epoxy-lactone has been described as a metabolite of *Marasmius alliaceus* by KING *et al.* in 1977[6]. During their general survey of antibacterial constituents of basidiomycetes KAVANAGH *et al.*[7] reported marasmic acid as an antibiotic from cultures of *Strobilurus* (*Marasmius*) *conigenus*. Its sesquiterpenoid structure was determined by DUGAN *et al.* in 1966[8]. From our group the antibiotics strobilurins A and B[9,10], oudemansin[1], and crinipellin[11] have been isolated from mycelia and fermentation broths of *Strobilurus tenacellus*, *Oudemansiella mucida*, and *Crinipellis stipitaria* respectively. In the following paper we describe a new antibiotically active metabolite from *Marasmius scorodonius*.

Results and Discussion

In the course of a screening of several species of *Marasmius* and some related species for the production of new antibiotics, we found that *Marasmius scorodonius*, when grown in submerged culture, produces an antibacterial and antifungal metabolite. Under the same conditions this antibiotic, scorodonin (**1**), could not be detected in cultures of *Marasmius androsaceus*, *Marasmius bulliardii*, *Marasmius cohaerens*, *Marasmius epiphyllus*, *Marasmius graminum*, *Marasmius oreades*, *Marasmius rotula*, *Micromphale foetidum*, *Micromphale perforans*, and *Xeromphalina campanella*.

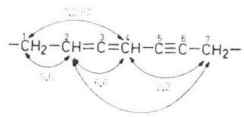
Scorodonin was obtained as a slightly yellow oil of pungent smell, which easily undergoes decomposition on storage at room temperature. It is soluble in methanol, acetone, and chloroform, and shows Rf 0.38 on silica plates (Merck 5554; cyclohexane - ethylacetate - formic acid, 120: 40: 5).

The antibiotic is optically active, $[\alpha]_D^{22} -163°$ (c 1, EtOH), and shows UV maxima at 282 (log ε 2.97), 264 (3.26), 230 (4.03), and 223 nm (4.04) in EtOH. The IR spectrum indicates the presence of OH (3650), C≡C (2250), and CH=C=CH functions (1960, 872 cm^{-1}).

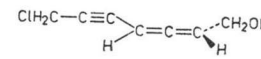
Scorodonin gives a positive BEILSTEIN test for halogen, and from elemental analysis the molecular formula C_7H_7ClO may be calculated. The mass spectrum does not show a molecular ion. However, on acetylation the compound yields a monoacetate, which exhibits an ion m/e 142/144 (C_7H_7ClO) corresponding to loss of ketene from the molecular ion. Further loss of OH or Cl leads to prominent fragments at m/e 124/126 (18.9) and 107 (82.5).

On catalytic hydrogenation with Pd-charcoal scorodonin is converted into *n*-heptanol.

The ^{1}H-NMR spectrum (Table 1) indicates the presence of two methylene groups appearing as two doublets of doublets at δ 4.22 and 4.27 ppm ($CDCl_3$). Both methylene groups are coupled independently with the two protons of the allene system, and from the different magnitude of the coupling constants the presence of part structure **I** may be concluded:



I



1, R=H
2, R=CH_3CO

Table 1. NMR spectra of scorodonin (**1**) and scorodonin acetate (**2**) (δ values, TMS as internal standard).

	^{1}H-NMR (90 MHz)			^{13}C-NMR (20.1 MHz)		
	1[a]	**1**[b]	**2**[a]		**1**[c]	
1-H	4.22 dd	4.00 dd	4.60 dd	C-1	58.1 td	J=144+4 Hz
2-H	5.69 dtt	5.68 dtt	5.63 dtt	C-2	95.5 ddt	166+7+4 Hz
4-H	5.61 dtt	5.75 dtt	5.58 dtt	C-3	210.0 s	
7-H	4.27 dd	4.51 dd	4.26 dd	C-4	76.0 dd	175+7 Hz
OH	4.2	—	—	C-5[d]	85.8 br.s	
				C-6[d]	79.6 br.s	
				C-7	31.5 t	159 Hz

$J_{1,2}$=5.6 Hz; $J_{1,4}$=3.2; $J_{2,7}$=1.2; $J_{4,7}$=2.2; $J_{2,4}$=6.6 (**1** in $CDCl_3$).
a) In $CDCl_3$; b) in DMSO-d_6+3 drops D_2O; c) in acetone-d_6; d) assignments may be interchanged.

The position of the OH and Cl substituents follows unequivocally from the ^{13}C-NMR spectrum (Table 1). The signal of the CH_2Cl-carbon appears at high field (δ=31.5 ppm) due to the shielding effect of the neighboring *sp* carbon[12]. This assignment is confirmed by the large value of $^1J_{C,H}$= 159 Hz and the lack of coupling to other protons. The signal of the CH_2OH-carbon at δ 58.1 ppm is split into a triplet of doublets with $^1J_{C,H}$=144 and $^2J_{C,H}$=4 Hz.

This leads to structure **1** for scorodonin, further supported by the downfield shift of the hydroxymethylene protons on formation of the acetate **2** (Table 1).

LOWE[13] has found a reliable dependence of the sign of optical rotation on the absolute configuration of diyneallenes. Using his rule for **1**, its strongly negative rotation is in accord with the *R*-configuration given in the formula.

Scorodonin is a member of the rare group of C_7-acetylenes isolated so far only from basidiomycetes[14]. Chlorinated acetylenes have been found in several genera of the *Compositae*[15~19] and

Table 2. Antimicrobial spectrum of scorodonin. (serial dilution test)

Test organism	MIC (μg/ml)
Aerobacter aerogenes	25~50
Arthrobacter citreus	>50
Bacillus brevis	50
Bacillus subtilis	5~ 8
Corynebacterium insidiosum	25~50
Escherichia coli K12	>50
Escherichia coli K183	>50
Micrococcus roseus	10~50
Micrococcus luteus	>50
Mycobacterium phlei	5~10
Proteus vulgaris	10~25
Staphylococcus aureus	>50
Streptomyces sp. ATCC 23836	>50
Streptomyces viridochromogenes	>50
Candida albicans	10~25
Nadsonia fulvescens	25~50
Rhodotorula glutinis	>50
Saccharomyces cerevisiae αS288C	25~50
Saccharomyces cerevisiae FL 200	25~50
Saccharomyces cerevisiae is 1	8~10

Fig. 1. Effect of scorodonin on the growth of *Rhizoctonia solani*.
(1) Control without antibiotic; (2) growth on agar plates containing 3.3 μg/ml; (3) 13.3 μg/ml; (4) 33 μg/ml.

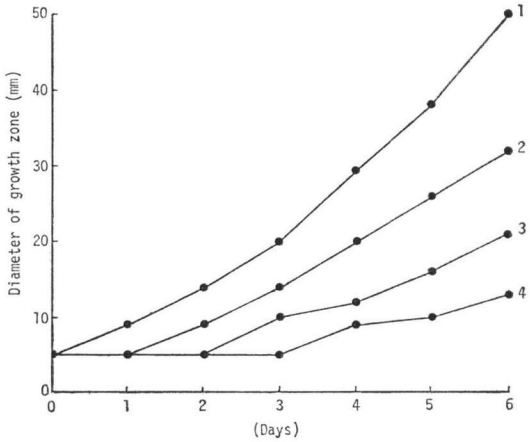
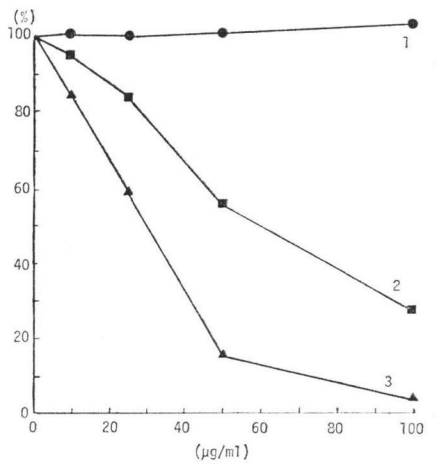


Fig. 2. Effect of scorodonin on macromolecular syntheses in EHRLICH carcinoma ascites cells.
(1) protein synthesis
(2) RNA synthesis
(3) DNA synthesis
Controls without antibiotic (=100%), incorporation per ml cell suspension: (^{14}C)-leucine 10,640 cpm, (^{14}C)-uridine 10,500 cpm, (^{14}C)-thymidine 3,520 cpm.



more recently in algae[19].

Table 2 shows the antimicrobial spectrum of scorodonin. Scorodonin inhibits Gram-negative and Gram-positive bacteria as well as yeasts at rather high concentrations. The effect of scorodonin on the growth of *Rhizoctonia solani* is shown in Fig. 1. At the highest concentration the growth of the colony is only retarded for 3 days. This is due to decomposition of the compound which occurs rapidly at room temperature under the influence of light. The effect of scorodonin on the macromolecular syntheses in cells of the ascitic form of EHRLICH carcinoma is shown in Fig. 2. The incorporation of thymidine and uridine into the 5% trichloroacetic acid insoluble fraction of cells (DNA, RNA) is strongly inhibited whereas the incorporation of leucine into protein is not affected. When tested in a similar way in *Bacillus brevis* cells the incorporation of uracil and leucine into trichloroacetic acid-precipitable material was inhibited 40% at 50 μg/ml whereas the incorporation of thymidine was not affected. *In vitro* a 50% inhibition of DNA dependent RNA polymerase from *Escherichia coli*, tested according to FUCHS *et al.*[20], was found at 25 μg/ml of scorodonin.

Experimental

Fermentation and Isolation Procedures

Mycelial cultures of *Marasmius scorodonius* strain Nr. 7750 and the other species which were tested for the production of antimicrobial activities were obtained from spore prints. For submerged cultivation and maintenance on agar slants the strains were grown in a yeast extract-malt extract-glucose (YMG) medium composed of (g/liter): yeast extract 4, malt extract 10, glucose 4.

For the production of scorodonin *Marasmius scorodonius* was grown in 20 liters of YMG medium in a Biolafitte fermentation apparatus (3.3 liters air/min, 150 rpm, 22°C) for 2 weeks. After extraction with ethyl acetate (5 liters) of the culture fluid (18 liters) the crude product (3 g) was chromatographed on silica gel (elution with $CHCl_3$-EtOH, 99: 1) and Sephadex LH-20 in methanol. The pure product (0.2 g) was obtained as a homogeneous yellowish oil.

Scorodonin (1)

IR ($CHCl_3$): 3650 (st), 3030 (m), 2980 (m), 2900 (m), 2250 (w), 1960 (m), 1735 (m), 1260 (sst), 1120 (sst), 1040 (sst), 872 (m), 705 cm^{-1} (st). MS (AEI MS 50): *m/e* 107 (11%), 91 (20.8), 77 (23.5), 38 (31), 36 (100).

Anal. Calcd. for C_7H_7ClO: C, 58.97; H, 4.95; Cl, 24.86
Found: C, 58.54; H, 5.02; Cl, 21.97

Hydrogenation of 1

A solution of **1** (23 mg) in methanol (30 ml) was hydrogenated with Pd/charcoal for 4 hours. The reaction mixture was filtered and the filtrate was evaporated. The resulting oil was in every respect (GC, MS, and ^{1}H-NMR) identical with authentic *n*-heptanol.

Scorodonin acetate (2)

To a solution of **1** (8 mg) in acetic anhydride (1 ml) a trace of conc. H_2SO_4 in acetic anhydride was added. After 3 minutes the mixture was distributed between water and chloroform, and the organic layer was dried ($MgSO_4$) and evaporated to give **2** (9 mg) as slightly yellow oil. $[\alpha]_{578}^{22}-30°$ (*c* 0.02, EtOH); IR ($CHCl_3$): 2240 (w), 1960 (w), 1742 (sst), 1372 (st), 1259 (sst), 1110 (m), 1036 (m), 982 (m), 861 (w), 696 cm^{-1} (m). MS: *m/e* 142.0188 (28.8%, calcd. $C_7H_7{}^{35}ClO$ 142.0185), 124 (18.9), 107 (82.5).

Biological assays

The antimicrobial spectrum and the effect of scorodonin on the growth of *Rhizoctonia solani* were determined as described earlier[9]. Macromolecular syntheses in cells of the ascitic form of EHRLICH carcinoma (ECA) were measured as described previously[11]. The effect of scorodonin on protein, RNA, and DNA syntheses in cells of *Bacillus brevis* was tested in a similar way as described for ECA cells: 1.8×10^8 cells in 3 ml of nutrient broth (Difco) were preincubated with or without the antibiotic for 10 minutes. Then the radioactive precursors—0.1 μCi L-(1-^{14}C)-leucine (59 mCi/mmol), 0.1 μCi (2-^{14}C)-thymidine (61 mCi/mmol), or 0.1 μCi (2-^{14}C)-uracil (59 mCi/mmol)—were added to the cell suspension and after 30 minutes the incorporation into protein, DNA, and RNA was determined by collecting the 5% TCA insoluble fraction of cells on membrane filters and measuring the radioactivity by liquid scintillation counting. DNA-dependent RNA polymerase from *Escherichia coli* was kindly provided by W. ZILLIG; the assay is described by FUCHS *et al.*[20]

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