ANTIMICROBIAL METABOLITES FROM *Lentinus crinitus*

Sir:

Lentinus crinitus (L. ex Fr.) Fr. is a mushroom which is widespread in Eastern Africa[1,2], but so far it has not been studied for its secondary metabolites. The strain was collected in Kaffa, Ethiopia, where it grows on dead wood. Culture extract of the fungus showed antimicrobial activity, and the new metabolite 1-desoxy-hypnophilin **1** and the known hypnophilin **2** were isolated as the most active compounds. In addition to these ketones, their corresponding alcohols **3** and **4** were isolated as well. The antimicrobial activity spectra of the new sesquiterpenes are shown in Table 1.

Lentinus crinitus DR-5 was grown in submerged culture on a rotatory shaker (150 rpm) in 1-liter Erlenmeyer flasks filled with 200 ml of the medium containing maltose (20 g), glucose (10 g), peptone (2 g), yeast extract (1 g), $MgSO_4 \cdot H_2O$ (1 g), KH_2PO_4 (0.5 g), $CaCl_2$ (0.005 g), $FeCl_3$ (0.01 g) and $ZnSO_4$ (0.012 g) in 1 liter distilled water. After 14 days, the culture broth was filtered and exgracted three times with ethyl acetate. The crude extract was separated on a Si-60 column with a dichloromethane - MeOH gradient (changing from 1:0 to 7:3). When necessary, the collected fractions were purified further by preparative TLC on silica gel.

The least polar metabolite was an UV-active compound revealing a composition of $C_{15}H_{20}O_2$ in the HR-MS, which means that it contains six equivalents of unsaturation. The ^{13}C NMR spectrum showed a carboxylic group, an exomethylene double bond and an ether, with its resonances at δ_C 61.1 and δ_C 76.6, requiring three rings to fit the molecular formula (Table 2). The ^1H NMR spectrum showed three singlets of methyl groups and an exomethylene moiety (Table 3). From the shifts of 15- and 15'-H in the ^1H and C-5 in the ^{13}C NMR, it could be deduced that the compound contains an α,β-unsaturated ketone, while this ketone must be part of a five-membered ring as required by the absorbtion at 1729 cm^{-1} in the IR spectrum. $^1H\{^1H\}$ homonuclear shift-correlation spectroscopy (COSY-45) revealed the connectivities of the protons at rings B and C. In ring A, 6-H displayed long range couplings to 8- and 15'-H which together with the ^{13}C NMR data led to the structure of 1-desoxyhypnophilin **1** (colorless oil; Rf 0.96 (dichloromethane - acetone 8:2); MS (*m/z*): 232.1470 (5%), 217 (4%), 214 (8%), 199 (5%), 175 (100%), 119 (30%). $[\alpha]^{27}$ ($CHCl_3$, *c* 1.00): 589 nm: −128.5°, 578 nm: −138.2°, 546 nm: −173.2°, 436 nm: − 600.5°), a sesquiterpene with the hirsutane skeleton.

The second, more polar, antimicrobial compound displayed very similar spectra. Instead of the resonances of 1-H, a doublet at δ_H 3.88 was seen. The ^{13}C NMR was in agreement with the 1α-hydroxy-derivative of **1** which is known as hypnophilin **2**. Two more sesquiterpenes were isolated, and they turned out to be the alcohols of **1** and **2**, *i.e.* compound **3** and **4** (Fig. 1). (2*R*,3*R*,5*R*,6*R*,7*R*,9*R*)-6,7-Epoxy-4(15)-hirsutene-5-ol **3** (colorless oil; Rf 0.90 (dichloromethane - acetone 8:2); IR ($CHCl_3$): 3439, 1718, 1665, 1464 cm^{-1}; MS (*m/z*): 234.1621 (17%), 219 (19%), 205 (46%), 197 (28%), 138 (100%), 95 (66%). $[\alpha]^{27}$ ($CHCl_3$, *c* 1.00): 589 nm: +43.5°, 578 nm: +45.9°,

Table 1. Antimicrobial activity spectrum of desoxyhypnophilin **1** and 6,7-epoxy-4(15)-hirsutene-5-ol **3** determined by a serial dilution assay.

Test organisms	MIC (μg/ml)	
	1	**3**
Bacillus cereus DSM 318	2~5	>100
Staphylococcus aureus ATCC 13709	10~25	>100
Escherichia coli ATCC 9637	>100	>100
Salmonella gallinarum ATCC 9184	50~100	50~100
Mycobacterium smegmatis ATCC 607	25~50	50~100
Candida albicans ATCC 10231	50~100	>100
C. tropicalis DSM 1346	>100	>100
Rhodotorula glutinis DSM 70398	>100	>100
Aspergillus niger (spores) DSM 737	1~2	25~50
A. flavus (spores) BD 27	2~5	25~50
Mucor rouxii (spores) DSM 1691	2~5	25~50

Table 2. ^{13}C NMR data of **1**~**4** (75.5 MHz, $CDCl_3$).

	1	2	3	4
C-1	40.1 −[a]	81.2 +	39.8 −	81.0 +
C-2	49.8 +	56.4 +	48.7 +	55.2 +
C-3	46.5 0	45.4 0	48.7 0	47.6 0
C-4	153.4 0	153.8 0	159.3 0	159.5 0
C-5	198.1 0	n.d.	74.2 +	74.2 +
C-6	61.1 +	61.1 +	63.6 +	63.8 +
C-7	76.6 0	n.d.	75.4 0	75.1 0
C-8	30.1 −	30.8 −	30.3 −	30.7 −
C-9	39.2 +	34.5 +	39.1 +	34.8 +
C-10	49.5 −	46.1 −	49.6 −	46.4 −
C-11	42.5 0	44.1 0	42.4 0	44.2 0
C-12	17.5 +	17.7 +	17.1 +	17.5 +
C-13	28.9 +	19.7 +	28.9 +	19.8 +
C-14	27.3 +	26.4 +	27.4 +	26.5 +
C-15	119.9 −	121.6 −	111.3 −	112.5 −

[a] Amplitude of signals in DEPT-135 spectrum (CH_3 or CH = +; CH_2 = −; quat. C = 0).

Table 3. ^1H NMR data of **1**, **3** and **4** (400 MHz, $CDCl_3$).

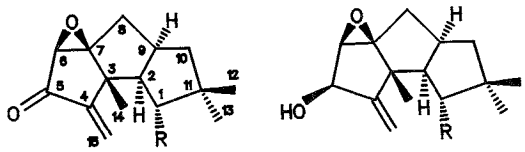
	1	3	4
1-H	1.54 dd	1.42 d	3.81 d
1′-H	1.48 ddd		—
2-H	2.40 dt	2.27 dt	2.03 dd
5-H	—	4.59 dddd	4.63 m
6-H	3.44 s	3.45 d	3.48 d
8-H	2.00 d	1.84 d	1.83 d
9-H	2.73 ddtd	2.65 ddtd	2.62 ddtd
10-H	1.80 ddd	1.72 dd	1.84 dd
10′-H	1.17 dd	1.10 dd	1.14 dd
12-H	0.92 s	0.89 s	0.89 s
13-H	1.12 s	1.07 s	1.05 s
14-H	1.16 s	1.01 s	1.18 s
15-H	6.05 s	5.23 d	5.33 d
15′-H	5.27 s	4.96 d	5.16 d

J (Hz): **1**: 1,1′ = 13; 1,2 = 1,2′ = 9; 1′,10 = 1; 2,9 = 12; 6,8 = 6,15′ > 0; 8,9 = 9; 9,10 = 8; 9,10′ = 12; 10,10′ = 12.
3: 1,2 = 9; 2,9 = 11; 5,6 = 5,15 = 5,15′ = 2; 5,OH = 10.8; 8,9 = 8.5; 9,10 = 7.5; 9,10′ = 11; 10,10′ = 12.
4: 1,2 = 8.5; 2,9 = 11; 5,6 = 5,15 = 5,15′ = 2; 8,9 = 9; 9,10 = 8; 9,10′ = 11; 10,10′ = 12.

546 nm: +50.5°, 436 nm: +61.2°), showed only weak antimicrobial activities as can be seen from Table 1. From 2.21 of culture broth, 28 mg of **1**, 36 mg of **2**, 41 mg of **3** and 11 mg of **4** were isolated.

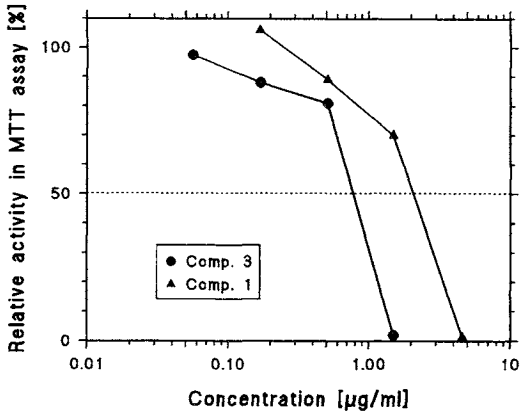
While desoxyhypnophilin **1** and 6,7-epoxy-4(15)-hirsutene-5-ol **3** are new compounds, hypnophylin **2** was first described from the fungus *Pleurotellus hypnophilus* (Berk.) Sacc. (Basidiomycotina, Agaricales)[3]. The diol **4** was formed by reduction of hypnophilin **2** by STEGLICH and coworkers, it had not been reported previously to be a natural product (**4**: Colorless oil; Rf 0.66; IR: 3398, 1716, 1668,

Fig. 1. Hirsutanes from *lentinus crinitus*.



1: R = H
2: R = OH
3: R = H
4: R = OH

Fig. 2. Toxicity assay with L929 mouse fibroblasts. Cells were grown in titerplates (6,000/180 µl) for 5 days.



1463 cm^{-1}; MS (*m/z*): 250 (23), 232 (35), 217 (50), 203 (73), 138 (100); $[\alpha]^{27}$ ($CHCl_3$, *c* 0.50): 589 nm: +91.6°, 578 nm: +95.6°, 546 nm: +107.6°, 436 nm: +178.8°).

All four metabolites, **1**~**4**, possess a hirsutane skeleton. They are structurally related to hirsutic acid C isolated from the cultures of *Stereum hirsutum*[4], complicatic acid from *Stereum complicatum* (Fr.) Fr.[5], *Galerina cephalotricha*[6], phellodonic acid from *Phellodon melaleucus* (Sw. ap. Fr. ex Fr.) P. Karst[7] and velutinol (not to be confused with the pregnane bearing the same name from *Mandevilla velutina*![8]) from *Lentinus velutinus*[9].

The antimicrobial activity of desoxyhypnophilin **1** is also similar to hypnophilin **2** and velutinol. The α-methylene ketone moiety is common to all of them. However, 6,7-epoxy-4(15)-hirsutene-5-ol **3** which possesses the α-methylene alcohol instead of the ketone, has only weak antimicrobial activity, *i.e.* the reduction of the ketone to the alcohol renders the compound to be less active. Toxicity assays were done with L929 mouse fibroblasts cells. Fig. 2 shows concentration-dependent growth inhibition curves, measured by an MTT assay[10]. The IC_{50} was 2.4 µg/ml for **1** and 0.9 µg/ml for **3**. First results show

that the relation of the ketones **1** and **2** to the alcohols **3** and **4** can be influenced by the fermentation conditions.

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