Chromanones from *Lentinus crinitus* (Basidiomycetes)

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The fungus *Lentinus crinitus* DR-5 (Basidiomycotina) produces in submerse culture the new natural products 2,2-dimethyl-6-methoxy-4-chromanone **1**, its alcohol **3** and (S)-2,2-dimethyl-3-hydroxy-6-methoxy-4-chromanone **2**. Beside these merohemiterpenes the antibiotically active hirsutane sesquiterpenes **5** – **8** were isolated. The formation of these metabolites displayed strong dependence from the carbon source in the medium. While lactose favoured the formation of the antibiotic sesquiterpene ketones **5** and **6**, they were suppressed with mannose where the hemiterpenes **1** and **4** and the hirsutane-diol **8** were the dominating metabolites.

Introduction

The mushroom *Lentinus crinitus* (L. ex Fr.) Fr. (Basidiomycotina) grows on dead wood and is a common species of the genus *Lentinus* in East Africa (Pegler, 1977, Pegler 1983). We reported recently on the sesquiterpene antibiotics 1-desoxyhypnophilin **5**, hypnophilin **6** and its dihydro-derivatives **7** and **8** from the strain DR-5 of this species from Ethiopia (Abate and Abraham 1994). In this communication we report on the isolation and structure elucidation of three hemiterpenes from the same strain and the results of our fermentation experiments using different carbon sources.

Results and Discussion

The least polar compound of the extract exhibited a strong fluorescence at the TLC plate when viewed at 366 nm. The mass spectrum revealed a molecular ion at *m/z* 206 of the composition $C_{12}H_{14}O_3$. Its ^{1}H NMR spectrum showed an 1,2,4-trisubstituted aromatic ring with a methoxy group being one of the substituents (Table I). The ^{13}C NMR spectra and other NMR data led to the structure of 2,2-dimethyl-6-methoxy-4-chromanone (**1**) (Fig. 1). To our knowledge this is the first report of this compound from nature. It is known for its strong fluorescence and this behaviour was discussed in detail by Matsushima *et al.* (1988). Its alcohol **3** was also isolated in much lower amount. Again this metabolite was hitherto unknown from natural sources but known as an intermediate in chemical syntheses (Ohta and Bowers, 1977).

Table I. ^{13}C NMR data of **1, 2** and **4** ($CDCl_3$).

	1		**2**		**4**	
C-1	153.7	0[a]	154.0	0	194.4	0
C-2	120.0	0	118.4	0	138.2	0[b]
C-3	119.6	+	119.8	+	137.7	+
C-4	154.6	0	154.5	0	63.2	+
C-5	125.3	+	126.0	+	57.7	+
C-6	107.1	+	107.3	+	53.9	+
C-7	192.5		194.5	0	65.4	+
C-8	48.8	–	77.3	+	123.8	8
C-9	79.0	0	83.5	0	139.1	[b]
C-10	26.5	+	26.9	+	19.8	+
C-11	26.5	+	17.1	+	25.8	+
C-12	55.8	+	55.9	+	–	

[a] Amplitude of signals in DEPT-135 spectrum (CH_3 or CH = +; CH_2 = –; quat. C = 0); [b] assignments may be interchanged.

Using a minimal medium with lactose as sole source of carbon 2,2-dimethyl-6-methoxy-4-chromanone (**1**) could not be detected. Instead of this metabolite a slightly more polar compound was found which also showed a strong fluorescence. ^{1}H and ^{13}C NMR spectra (Table II) revealed its structure as the hydroxylated derivative of **1** so it was identified as 2,2-dimethyl-3-hydroxy-6-methoxy-4-

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Table II. ^{1}H NMR data of **1–4** (400 MHz, $CDCl_3$).

	1	**2**	**3**	**4**
3-H	7.30 d	7.24 d	7.01 d	6.73 ddd
4-H	–	–	–	4.74 ddd
5-H	7.09 dd	7.13 dd	6.78 dd	3.82 ddd
6-H	6.87 d	6.88 d	6.73 d	3.50 dd
7-H	–		4.83 dd	5.32 d br
8-H	2.71 s	4.42 s	2.18 dd	5.07 dqq
8′-H		–	1.85 dd	–
10-H	1.45 s	1.64 s	1.43 s	1.74 d
11-H	1.45 s	1.20 s	1.30 s	1.74 d
12-H	3.81 s	3.80 s	3.78 s	–

J [Hz]: **1, 2:** 3,5 = 2; 5,6 = 8. **3:** 3,5 = 2; 5,6 = 8; 7,8 = 6, 7,8′ = 9; 8,8′ = 13. **4:** 3,4 = 5; 3,5 = 1; 3,7 = 2; 4,5 = 2; 4,6 = 1; 5,6 = 4; 7,8 = 9; 8,10 = 8, 11 = 1.

chromanone (**2**). Again it is a novel metabolite which was synthesised already by Snatzke *et al.* in 1970 (Kis *et al.* 1970). Using CD Snatzke and coworkers determined the absolute configuration of **2** to (3*S*). Because their compound had a positive α_D as our metabolite has, *Lentinus crinitus* produces (S)-2,2-dimethyl-3-hydroxy-6-methoxy-4-chromanone (**2**) in the lactose medium.

The most polar compound found in the fermentation broth also absorbs UV at 254 nm. Using ^{1}H and ^{13}C NMR it could be identified as panepoxydone (**4**). This metabolite was found in *Panus rudis* and *P. conchatus* by Snatzke *et al.* Its occurrence in *Lentinus crinitus* gives a chemotaxonomical argument towards the close relation between the fungal genera *Panus* and *Lentinus* (Hibbett *et al.*, 1993; Hibbett and Vilgalys, 1993). Panepoxydone inhibits the proliferation of mouse cell tumours

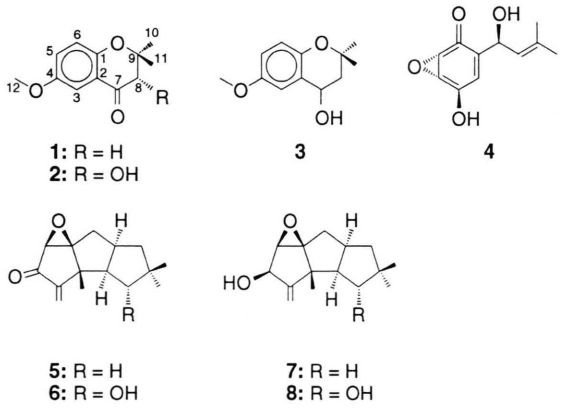

Fig. 1. Metabolites isolated from the culture broth of *Lentinus lepideus* DR-5.

with an ED_{50} of 1mg/l (Kis *et al.*, 1970). The related epoxydone which bears at C-2 a hydroxymethyl group instead of the isopentenyl side chain of the panepoxydone is known from a number of sources (Turner and Aldridge 1983). Beside this we found epoxydone in the culture broth of *Aspergillus giganteus* DSM 1146. Its NMR data are in accordance with the data observed for panepoxydone. Very recently derivatives bearing a farnesyl side chain instead of the isoprenyl group of panepoxydone have been described from *Arthrobotrys spp.*, which also display cytotoxic activity (Stadler *et al.*, 1993).

The yield in different media varies considerably. The fermentation in a complex medium containing maltose, glucose, peptone, yeast extract and salts yields mainly sesquiterpenes. This is also seen in the fermentation in a defined medium with lactose as the sole source of carbon (Fig. 2). With lactose however the formation of the ketones **4** and **5** is much more favoured than their alcohols. Using mannose instead of lactose in this minimal medium the situation changed drastically and the compounds **1** and **4** are here the main metabolites (Fig. 2). Sesquiterpenes are also formed but contrary to the lactose medium the formation of ketones is extremely reduced, instead their alcohols **7** and **8** are dominating. So changes of the medium composition caused a dramatical change in the product spectrum leaving much room for optimi-

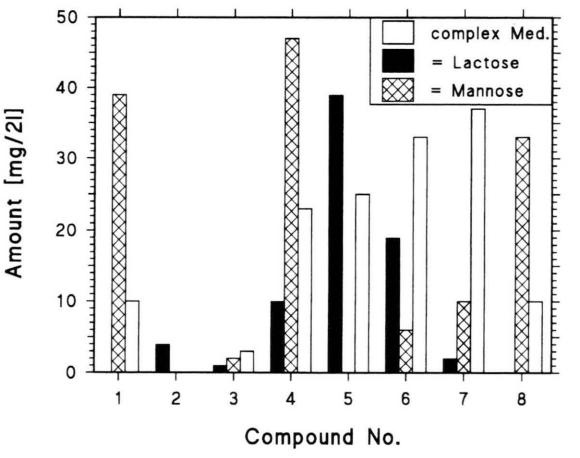

Fig. 2. Amount of the individual metabolites in two minimal media differing only in the carbon source (10 g/l). For comparison the yield in a complex medium is also given.

zation. Depending on the medium used in the fermentation the production of either the cytotoxic sesquiterpene ketones, their alcohols or the chromenes can be enhanced.

Experimental

The mushroom, *Lentinus crinitus* (L. ex Fr.) Fr., was collected in Kaffa, Ethiopia growing on dead wood. The culture (DR-5) made from fresh specimen of the mushroom is maintained on 2% malt extract agar.

Lentinus crinitus DR-5 was grown in 1 litre Erlenmeyer flasks filled with 200 ml of the defined medium containing the carbon source (10g), sodium nitrate (3g), yeast extract (1g), dipotassiumhydrogene phosphate trihydrate (1.3g), potassium chloride (0.5g), magnesium sulphate hepathydrate (0.5g), iron(II)sulphate heptahydrate (0.05g) and citric acid monohydrate (0.7g) in 1 litre deionized water. The medium was adjusted to p_H 4.5 prior to the sterilisation, carbon sources were mannose or lactose. After 14 days the culture broth was filtered and extracted three times with EtOAc. After drying with sodium sulphate the solvent was evaporated and the crude extract was separated on a Si-60 column with a n-hexane/ethyl acetate gradient (changing from 19:1 to 0:1). When necessary the collected fractions were purified further by preparative TLC.

The ^1H and ^{13}C NMR spectra were obtained at 400 and 75.5 MHz, respectively. Deuterochloroform was the solvent and tetramethylsilane the internal standard. Mass spectra were recorded with 70 eV. IR spectra were measured on potassium bromide, UV spectra in methanol, optical rotations in chloroform.

From the fermentation with lactose as the source of carbon in the defined medium and 2 litres of culture broth **2** (4mg), **3** (1mg), **4** (10mg), **5** (39mg), **6** (19mg) and **7** (2mg) were isolated. The mannose medium brought **1** (39mg), **3** (2mg), **4** (47mg) **6** (6mg), **7** (10mg) and **8** (33mg) per 2 litres. Fermentation in a complex medium (containing maltose (20g), glucose (10g), peptone (2g), yeast extract (1g), magnesium sulphate hepathydrate (1g), dipotassiumhydrogene phosphate trihydrate (0.5g), calcium chloride (0.005 g), iron(III)chloride (0.01 g) and zinc sulphate (0.012 g) in 1 litre distilled water) yielded **1** (11 mg), **3** (3 mg), **4** (28 mg)(α_D -24.8°, c=0.25, $CHCl_3$), **5** (25 mg), **6** (36 mg), **7** (41 mg) and **8** (11 mg).

(3S)-2,2-Dimethyl-3-hydroxy-6-methoxy-4-chromanone **2**: Yellowish oil, strong fluorescence at 366 nm. R_f 0.56 (n-hexane/ethyl acetate 6:4, v/v). ms (*m/z*): 222 (M^+)(40%), 204 (9), 193(25), 151 (100).

$$[\alpha]^{27} = \frac{589\text{ nm} \quad 578\text{ nm} \quad 546\text{ nm} \quad 436\text{ nm}}{+32.0° \quad +32.8° \quad +37.0° \quad +68.5°} \; (c = 0.50).$$

2,2-Dimethyl-6-methoxy-4-chromanol **3:** Yellowish oil, R_f 0.88 (dichloromethane/acetone 8:2, v/v). ms (*m/z*): 208 (M^+)(12), 190(100), 175 (76).

$$[\alpha]^{27} = \frac{589\text{ nm} \quad 578\text{ nm} \quad 546\text{ nm} \quad 436\text{ nm}}{+16.0° \quad +16.5° \quad +15.5° \quad +18.5°} \; (c = 0.20).$$

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