Lactarane Type Sesquiterpenoids as Inhibitors of Leukotriene Biosynthesis and Other, New Metabolites from Submerged Cultures of *Lentinellus cochleatus* (Pers. ex Fr.) Karst.

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Basidiomycetes, *Lentinellus cochleatus*, Inhibitors of Leukotriene Biosynthesis, Lactaranes, Protoilludanes

Three known sesquiterpenoids of the lactarane and secolactarane type, deoxylactarorufin A (**1**), blennin A (**2**) and blennin C (**3**), have been obtained from cultures of *Lentinellus cochleatus* (Basidiomycetes) together with the new metabolites (*Z*)-2-chloro-3-(4-methoxyphenyl)-2-propen-1-ol (**4**) and lentinellone (**5**), a protoilludane derivative. The structures were determined by spectroscopic investigations. **1, 2** and **3** are potent inhibitors of leukotriene biosynthesis in rat basophilic leukemia (RBL-1) cells and human peripheral blood leukocytes (PBL).

Introduction

The leukotrienes are potent biological mediators derived from arachidonic metabolism generated via the 5-lipoxygenase pathway. Leukotriene B_4, a dihydroxy derivative, stimulates leukocyte functions (e.g. chemotactic movement, adhesion, and degranulation) whereas the sulfidopeptide leukotrienes C_4, D_4 and E_4, known as "slow reacting substance of anaphylaxis" (SRS-A), primarily affect smooth muscle (Samuelsson *et al.*, 1987). In the respiratory tracts the latter induce bronchoconstriction, stimulate mucus production and increase vascular permeability (Samuelsson *et al.*, 1987). Due to these biological effects the leukotrienes participate in host defense mechanisms but under pathophysiological conditions they also mediate immediate hypersensitivity reactions and inflammatory processes in diseases like asthma, psoriasis and inflammatory bowel disease (Wasserman *et al.*, 1991).

During a screening for inhibitors of leukotriene biosynthesis in intact rat basophilic leukemia (RBL-1) cells, cultures of *Lentinellus cochleatus* were found to produce active metabolites. Five compounds were isolated. Three compounds, identified as deoxylactarorufin A (**1**) (Daniewski *et al.*, 1977, 1981), blennin A (**2**) (Vidari *et al.*, 1976) and blennin C (**3**) (De Bernardi *et al.*, 1976), were quite effective inhibitors of leukotriene biosynthesis in RBL-1 cells and human peripheral blood leukocytes (PBL). The structure elucidation of two additional metabolites revealed (*Z*)-2-chloro-3-(4-methoxyphenyl)-2-propen-1-ol (**4**) and lentinellone (**5**), a sesquiterpene of the protoilludane type, both new natural compounds.

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Material and Methods

General

For TLC aluminum foils coated with silica gel Merck 60 F_{254} were used. Analytical HPLC was done with a Hewlett-Packard 1090 series II instrument. Melting points were determined on a Reichert Thermovar hot stage apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 Polarimeter. Spectral data were recorded on the following instruments: ^1H and ^{13}C NMR, Bruker ARX-300 and Bruker AMX-600; EI-MS, Finnigan MAT 95 Q; FT-IR, Bruker IFS 48; UV, Perkin-Elmer Lambda 16; CD, Jobin Yvon CD 6.

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Producing Organism

The *Lentinellus cochleatus* (Pers. ex Fr.) Karst. strain 90321 was isolated from a spore print of a fruiting body collected near Lake Baker (USA). The specimen showed all characteristics of the genus and the species (Watling, 1989). The fungus was cultivated and maintained on YMG agar (yeast extract 0.4%, malt extract 1.0%, glucose 0.4%, and agar 1.5%, pH 5.5). The strain is deposited in the culture collection of the LB Biotechnologie, University of Kaiserslautern.

Fermentation and isolation of deoxylactarorufin A (**1**), *blennin A* (**2**), *blennin C* (**3**), *(Z)-2-chloro-3-(4-methoxyphenyl)-2-propen-1-ol* (**4**), *and lentinellone* (**5**)

Fermentations were carried out in 5 liter-Erlenmeyer flasks containing 2 l of YMG medium at 24 °C on a rotary shaker (130 rpm). The pH was adjusted to 5.5 prior to sterilization. A culture grown 14 -21 days in 200 ml of the same medium was used as inoculum. During fermentations the content of **1**, **2** and **4** could be monitored by analytical HPLC (LiChrosorb RP-18, 5 µm, Merck, column size 4 x 125 mm; 1.5 ml/min, 40 °C; H_2O : MeCN 0 → 60% in 15 minutes).

After 4 to 5 weeks of fermentation , the culture fluid (2 liters) was separated from the mycelia and the culture broth was extracted two times with 1.5 l of ethyl acetate (EtOAc). The crude extract (300 mg) was applied onto a silica gel column (Merck 60; 60–200 µm; column size: 2.5 x 20 cm). Elution with cyclohexane-EtOAc (7:3) yielded 60 mg of an enriched product. Final purification was achieved by preparative HPLC (Merck LiChrosorb DIOL 7 µm; column size: 2.5 x 25 cm; flow rate 5 ml/min) using a cyclohexane – *tert*-butyl methyl ether (*t*-BME) gradient: 20% → 40% *t*-BME (150 min) and yields 8.4 mg **1,** 11.3 mg **2,** 2.8 mg **3,** 2.6 mg **4** and 8.5 mg **5.**

Deoxylactarorufin A (**1**)

Colorless crystals. R_f = 0.37 (toluene:acetone 7:3, silica gel). m.p. 115–116°C. $[\alpha]_D^{23}$ = +53° (*c* = 0.16 in $CHCl_3$). CD (MeCN, *c* = 0.32 mg/ml) λ_{max} 245 nm (Δε +1.21), 220 (-2.42). The spectroscopic data were in agreement with those reported in the literature (Daniewski *et al.*, 1977). ^{13}C NMR data (not described in literature, $CDCl_3$, 75.5 MHz) δ: 174.93 (C-5), 162.85 (C-7), 125.20 (C-6), 70.48 (C-8), 70.34 (C-13), 48.42 (C-9), 47.72 (C-1), 47.18 (C-2), 45.70 (C-10), 37.06 (C-11), 33.96 (C-4), 33.17 (C-3), 29.56 (C-15), 26.97 (C-14), 22.05 (C-12).

Blennin A (**2**)

Colorless oil. R_f = 0.35 (toluene:acetone 7:3, silica gel). $[\alpha]_D^{23}$ = +67° (*c* = 0.61 in $CHCl_3$). CD (MeCN, *c* = 0.30 mg/ml) λ_{max} 247 nm (Δε +2.93), 221 (-2.78), 217 (-2.54). The other spectroscopic data were identical with those given in the literature (Vidari *et al.*, 1976).

Blennin C (**3**)

Colorless oil. R_f = 0.51 (toluene:acetone 7:3, silica gel). $[\alpha]_D^{23}$ = + 27.5° (*c* = 0.17 in $CHCl_3$). CD (MeCN, *c* = 0.53 mg/ml) λ_{max} 316 nm (Δε + 0.37), 267 (+0.01), 210 (+1.16). The other spectroscopic data were similar to those reported in the literature (De Bernardi *et al.*, 1976).

(Z)-2-Chloro-3-(4-methoxyphenyl)-2-propen-1-ol (**4**)

Colorless oil. R_f = 0.62 (toluene:acetone 7:3, silica gel). UV (MeOH) λ_{max} (lg ε) 263 nm (3.868). IR (KBr) 3416, 2929, 2858, 1608, 1512, 1150, 1033, 890 and 820 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.60 (d, *J* = 8.5 Hz, 2-H und 6-H), 6.88 (d, *J* = 8.5 Hz, 3-H und 5-H), 6.69 (s, 1′-H), 4.18 (s, 3′-C$\underline{H}_2$), 3.81 (s, 1″-C$\underline{H}_3$). HREI-MS (70 eV, DI 60°C) *m/z* (relative intensity %) 200 (33), 198.0445 (100, M^+, calcd. for $C_{10}H_{11}ClO_2$ 198.0448), 163 (76), 145 (27), 131 (11), 121 (20), 108 (15), 103 (11), 91 (14), 77 (11), 55 (30).

Lentinellone (**5**)

Colorless oil. R_f = 0.45 (toluene:acetone 7:3, silica gel). $[\alpha]_D^{23}$ = +286° (*c* = 0.36 in $CHCl_3$). UV (MeOH) λ_{max} (lg ε) 310 nm (2.290), 208 (3.556). CD (MeCN, *c* = 0.54 mg/ml) λ_{max} 312 nm (Δε +7.66), 227 (-2.57), 202 (+1.91). IR (KBr) 3380, 2951, 2932, 2866, 1774, 1450, 1384, 1365, 1167, 1108, 1065 and 1012 cm^{-1}. ^1H and ^{13}C NMR see Table I. HREI-MS (70 eV, DI 180 °C) *m/z* (relative intensity %) 250.1562 (2, M^+, calcd. for $C_{15}H_{22}O_3$ 250.1569), 232 (10), 208 (100), 190 (68),

175 (45), 151 (20), 134 (68), 133 (43), 121 (33), 107 (23), 91 (35), 77 (12), 69 (10), 55 (13).

Tests for biological activities

Inhibition of Ca^{2+} induced leukotriene C_4 synthesis in RBL-1 cells (rat basophilic leukemia, ATCC CRL 1378): The cells were grown in Dulbecco's modified Eagle Medium containing 10% fetal calf serum at 37 °C in a 5% CO_2 atmosphere. Cells were harvested by centrifugation (400×*g*, 10 min, washed once with PBS, and resuspended in PBS buffer with Ca^{2+} (0.9 mM) and Mg^{2+} (0.5 mM) and 0.1% glucose at 2×10^6 cells per ml. 225 µl of the cell suspension were incubated with the test compounds dissolved in DMSO or ethanol (final concentration <0.2% in a final volume of 250 µl) for 15 min. Then 2 µM of the calcium ionophore A23187 (Calbiochem) was added. After 10 min the reaction was terminated by addition of 250 µl ice-cold PBS and centrifugation (1000×*g*, 10 min). The concentration of leukotriene C_4 in the supernatant was determined by a radioimmunoassay according to the manufacturer's instructions (Amersham-Buchler, Braunschweig).

Inhibition of Ca^{2+} induced leukotriene C_4 and B_4 and prostaglandin E_2 synthesis in human peripheral leukocytes (PBL): Anticoagulated human venous blood was diluted with an equal volume of PBS buffer and centrifugated (800×*g*, 25 °C) for 10 min.The cell pellet was resuspended to the original volume and the PBL were isolated by density gradient centrifugation layering the diluted blood onto lymphocyte separation medium (Boehringer Mannheim). After centrifugation the PBL at the interface were collected and washed once. The cell pellet was resuspended in PBS buffer with Ca^{2+} (0.9 mM) and Mg^{2+} (0.5 mM) to a titre of 1×10^7 cells/ml. 200 µl of the cell suspension were incubated with the test compounds dissolved in DMSO (final concentration <0.2%) for 25 min at 25 °C. After 5 min at 37 °C 25 µl of A23187 solution (final concentration 0.25 µM) were added and the incubation continued for 10 min at 37 °C. The reaction was terminated as described above. The concentrations of leukotriene C_4, B_4 and prostaglandin E_2 were determined in the supernatant by radioimmunoassay according to the manufacturer's instructions. (LTC_4, LTB_4 Du Pont; PG_E Advanced Magnetics).

5-Lipoxygenase-assay: The 5-lipoxygenase activity was determined in the cytosolic 13.000×*g* fraction of broken RBL-1 cells according to Hook *et al.* (1990) with modifications. The content of 5-hydroxyeicosatetraenoic acid (5-HETE) was quantified by reversed phase HPLC using a 0.1% H_3PO_4 in H_2O/MeCN gradient (0 → 100% in 20 minutes).

12-Lipoxygenase-assay: The 12-lipoxygenase was prepared from bovine platelets by $(NH_4)_2SO_4$ fractionation according to the method of Nugteren (1982). The reaction was followed UV-spectrometrically at 246 nm.

15-Lipoxygenase (soybean)-assay: The assay was performed with 500 U/ml soybean 15-lipoxygenase type I-S (Sigma, Deisenhofen) and 30 µM linoleic acid as substrate in 0.2 M borate buffer (pH 9). The production of 15-hydroperoxyeicosatetraenoic acid (15-HPETE) was followed UV-spectrometrically at 234 nm.

PLA_2 (synovial): Synovial PLA_2 was obtained from Boehringer Mannheim and tested according to Scheuer (1989).

PLA_2 (cytosolic): The enzyme was obtained from Boehringer Mannheim and the assay performed according to Rodewald *et al.* (1994).

Other biological assays: Cytotoxicity, antifungal and antibacterial activity, incorporation of ^{14}C-labelled precursors in macromolecules (DNA, RNA, proteins), phytotoxic and hemolytic activity, and platelet aggregation were determined as described previously (Anke *et al.*, 1989; Kuschel *et al.*, 1994; Zapf *et al.*, 1995).

Results and Discussion

Fermentation and isolation

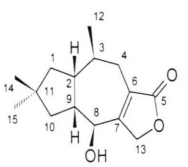
In submerged cultures *Lentinellus cochleatus* 90321 showed only a slow mycelial growth yielding 1–2 g/l (dry weight). After 4–5 weeks, when the fungus had consumed both glucose and maltose, the cultures were harvested. The metabolites were isolated from the culture filtrate following the procedure described above. The major components were deoxylactarorufin A (**1**), blennin A (**2**) and lentinellone (**5**) with about 4 to 5 mg/l culture filtrate. Only minor amounts (1 to 2 mg/l) of blennin C (**3**) and (*Z*)-2-chloro-3-(4-methoxyphenyl)-2-propen-1-ol (**4**) were obtained.

Table I. ^{1}H and ^{13}C NMR data of lentinellone (**5**) (600 and 150.9 MHz, respectively; $CDCl_3$ as solvent and internal standard).

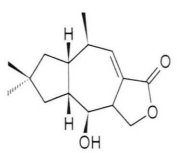
H-atom	δ	Multiplicity	*J* [Hz]	C-atom	δ	Multiplicity	*J* [Hz]
1α	1.39	dd	12.5/12.5	1	42.53	Tm	126.9
1β	1.50	dd	12.5/7.0	2	42.30	Dm	130.8
2	2.41	ddd	12.5/7.7/7.0	3	37.12	m	
4α	2.76	dq	16.3/0.8	4	51.19	Tq	137.8
4β	2.30	d	16.3	5	206.90	t	5.8
8	5.76	ddd	2.2/0.9/0.9	6	87.69	m	
9	2.72	dddddd	8.8/7.7/3×2.2/0.9	7	128.65	m	
10α	1.93	dd	13.3/8.8	8	135.55	Ddddd	155.0/4×7.0
10β	1.54	dd	13.3/2.2	9	39.16	Dm	130.7
12	1.17	d	0.8	10	47.11	Tm	128.7
13a	4.28	ddd	12.1/2.2/0.9	11	38.57	m	
13b	4.16	ddd	12.1/0.9/0.9	12	20.32	Qt	126.7/5.9
14	1.01	s		13	65.43	Td	144.0/6.0
15	1.03	s		14	31.54	Qm	126.5
				15	31.84	Qm	126.5

Structural elucidation

Deoxylactarorufin A (**1**), blennin A (**2**) and blennin C (**3**) were identified by their physical and spectroscopic data, which were in close agreement with those reported in the literature. **1** has been found before in *Lactarius necator* (Daniewski *et al.*, 1977, 1981), whereas **2** and **3** occur in fruit bodies of *Lactarius blennius* (Vidari *et al.*, 1976; De Bernardi *et al.*, 1980).



1



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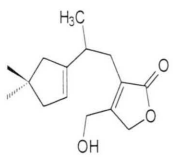
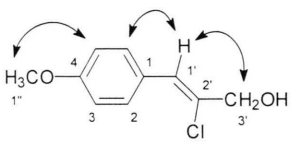


3

The mass spectrum of metabolite **4** showed a 3:1 isotopic ratio of the molecular ions at *m/z* 198 and 200 which indicates the presence of a chlorine atom. High resolution mass spectrometry led to the molecular formula $C_{10}H_{11}ClO_2$. From the ^{1}H NMR spectrum the presence of a 1,4-disubstituted phenyl ring (AA'BB'-system at δ 6.88 and 7.60), an isolated olefinic proton at δ 6.69, a hydroxymethylene group at δ 4.18 and a methoxy group at δ 3.81 can be deduced. This leads to the structure of (*Z*)-2-chloro-3-(4-methoxyphenyl)-2-propen-ol (**4**) for this compound which is supported by the NOESY correlations shown in the formula.



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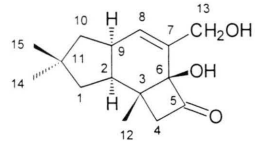


5

Lentinellone (**5**) exhibited a molecular ion at *m/z* 250 in the EI mass spectrum which corresponds to the molecular formula $C_{15}H_{22}O_3$. The compound showed an intense IR band (KBr) at 1774 cm^{-1} and a ^{13}C NMR signal at δ 206.9 which disclose a cyclobutanone moiety.

The ^{13}C NMR spectrum and DEPT experiments indicate the presence of three methyl groups at δ 20.3, 31.5 and 31.8, four methylene groups at δ 42.3, 51.2, 65.4 and 74.1, three methine groups at δ 39.2, 42.3 and 135.6 and four quarternary carbons at δ 37.1, 38.6, 87.7 and 128.7. From an analysis of the ^{1}H-^{1}H and ^{1}H-^{13}C coupling patterns given in Table I and the ^{1}H-^{1}H-COSY and NOESY correlations shown in Fig. 1 structure **5** can be assigned to lentinellone.

The NMR data of lentinellone are in excellent agreement with those given for melleolide F (**6**) (Arnone *et al.*, 1988). **5** is closely related to lentinellic acid (**8**), which has been isolated from a *Lentinellus* species before (Stärk *et al.*, 1988). The absolute configuration for **5** is arbitrarily assigned by comparison with that of other protoilludane

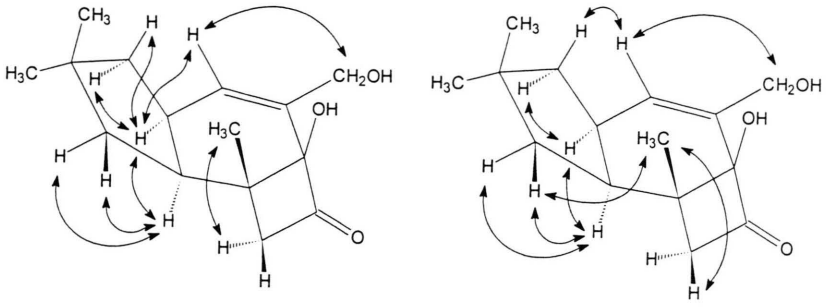
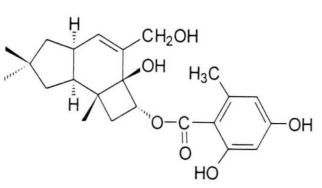


Fig. 1. Selected $^1H-^1H$-COSY (left) and) and NOESY correlations (right) of lentinellone.

sesquiterpenoids from basidomycetes. Very recently a group of related protoilludane sesquiterpenes, named tsugiculines, was isolated from *Laurilia tsugicula* (Arnone *et al.*, 1995).



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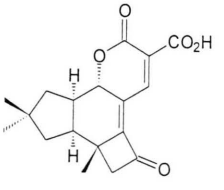

7

Biological activities

The cytotoxicity of the isolated compounds was tested against several cell lines. The metabolites exhibited cytotoxic effects at concentrations of between 25 and 100 μg/ml (Table II). Deoxylactarorufin A and blennin A (**1, 2**) showed cytostatic activities at concentrations of 25–50 μg/ml.

The effects of the compounds **1-4** on LTC_4 biosynthesis in RBL-1 cells are shown in Table III. **1** had the strongest inhibitory effect while **2** and **3** were less potent. **4** is only a weak inhibitor and **5** was inactive. Since IC_{50} values for the inhibition of macromolecule biosynthesis for **1** and **2** at concentrations of 25–50 μg/ml were 5 to 10 times higher than for LTC_4 formation, an inhibition of the leukotriene synthesis due to unspecific toxic effects seemed unlikely.

In an human system with peripheral blood leukocytes the results obtained for the inhibition of LTC_4 formation in RBL-1 cells could be confirmed (Table IV).

In order to get some information about the cellular target of the inhibitors the effects of **1** and **2**

Table II. Cytotoxic activities of deoxylactarorufin A (**1**), blennin A (**2**), blennin C (**3**), *Z*-2-chloro-3-(4-methoxyphenyl)-2-propen-1-ol (**4**), and lentinellone (**5**) on different cell lines.

Cell line	IC_{50} [μg/ml]				
	1	**2**	**3**	**4**	**5**
RBL-1	25–50[a]	25–50[a]	> 50	> 50	50–100
HL 60	25	25	50	50	20
HeLa S_3	25–50[a]	50[a]	>100	>100	>100
BHK	25–50[a]	50[a]	50–100[a]	100	>100
L 1210	50[a]	50[a]	100	100	100

[a] Arrest of growth.

Table III. C_{50} values of deoxylactarorufin A (**1**), blennin A (**2**), blennin C (**3**), and *Z*-2-chloro-3-(4-methoxyphenyl)-2-propen-1-ol (**4**) for the inhibition of leukotriene C_4 and macromolecule (DNA, RNA, protein) biosynthesis in rat basephilic leukemia (RBL-1) cells.

Compound	IC_{50} [μg/ml, (μM)]	
	LTC_4	DNA, RNA, protein
1	1–2 (4–8)	25–50 (100–200)
2	5 (20)	25–50 (100–200)
3	4–5 (16–20)	n.t.
4	15 (75)	n.t.

n.t. = not tested.

Table IV. Influence of deoxylactarorufin A (**1**), blennin A (**2**), blennin C (**3**), and *Z*-2-chloro-3-(4-methoxyphenyl)-2-propen-1-ol (**4**) on the eicosanoid (LTC_4, LTB_4, and PGE_2) biosynthesis in human peripheral leucocytes (PBL).

Compound	IC_{50} [μg/ml, (μM)]		
	LTC_4	LTB_4	PGE_2[a]
1	3–4 (7.5–8)	4–5 (8–12.5)	–12
2	3–4 (7.5–8)	4–5 (8–12.5)	–39
3	5 (12.5)	n.t.	3
4	15 (75)	n.t.	n.t.

n.t. = not tested; [a] inhibition at 10 μg/ml in [%]; – = stimulation.

Table V. Minimal inhibitory concentrations (MIC) of **4** in the serial dilution assay.

Organism	MIC [μg/ml] of compound **4**
Acinetobacter calcoaceticus	50–100
Arthrobacter citreus	>100
Bacillus brevis	50
Bacillus subtilis	50–100
Corynebacterium insidiosum	>100
Escherichia coli	>100
Micrococcus luteus	50–100
Mycobacterium phlei	>100
Salmonella thyphimurium	50
Streptomyces sp.	>100
Fusarium oxysporum	>100
Mucor miehei	>100
Nadsonia fulvescens	>100
Nematospora coryli	>100
Paecilomyces variotii	>100
Rhodotorula glutinis	>100
Saccharomyces cerevisiae is 1	>100
Saccharomyces cerevisiae S 288 c	>100
Ustilago nuda	100

on the biosynthesis of different eicosanoids (LTB_4, LTC_4 and PGE_2) in human PBL were investigated (Table III). For the lactarane derivatives (**1, 2, 3**) no inhibition of the cyclooxygenase pathway (PGE_2 synthesis) could be detected at 10 μg/ml (40 μM). At this concentration the LTC_4 and LTB_4 synthesis were significantly inhibited. The increased PGE_2 formation observed for **1** and **2** could be caused by a shift of arachidonic acid from the lipoxygenase to the cyclooxygenase pathway, a kwown phenomenon for inhibitors of leukotriene biosynthesis (Mong, 1991).

Since only the 5-lipoxygenase pathway was affected, an inhibition of phospholipase A_2 seemed unlikely. The assays with synovial and cytosolic PLA_2 confirmed that **1**, **2** and **3** had no effect on the activity of both enzymes at concentrations up to 20 μg/ml (80 μM) (data not shown). The simultaneous inhibition of LTB_4 and LTC_4 synthesis pointed to 5-lipoxygenase and its activation mechanisms as a target of the inhibitors. However, up to concentrations of 50 μg/ml (200 μM) neither a direct effect on 5-lipoxygenase in an cell-free extract of RBL-1 cells nor on 12- and 15-lipoxygenases from bovine platelets and soy bean could be observed (data not shown). Therefore a reaction involved in the activation of 5-lipoxygenase (translocation from the cytosol to the membrane of the nuclear envelope or transfer of arachidonic acid from five-lipoxygenase-activating protein (FLAP) to the enzyme is assumed as possible target.

The antibacterial and antifungal profile was determined in the serial dilution assay. **1, 2, 3,** and **5** showed no antimicrobial activities against the tested organisms (Table V). **4** inhibited the growth of *Acinetobacter calcoaceticus, Bacillus brevis, Bacillus subtilis, Micrococcus luteus, Salmonella typhimurium* and *Ustilago nuda* at concentrations of 50–100 μg/ml (Table V). At concentrations of 60–330 μg/ml for **1** and **2** a phytotoxic activity against *Lepidium sativum* seedlings could be detected. All isolated metabolites showed neither a hemolytic activity nor affected platelet aggregation at concentrations up to 100 μg/ml.

Acknowledgements

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Anke H., Bergendorff O. and Sterner O. (1989), Assays of the biological activities of guaiane sesquiterpenoids isolated from the fruit bodies of edible *Lactarius* species. Food Chem. Toxicol. **37,** 393–398.

Arnone A., Cardillo R., Di Modugno V. and Nasini G. (1988), Isolation and structure elucidation of Melledonals D and E and Melleolides E–H, novel sesquiterpenoid aryl esters from *Clitocybe elegans* and *Armillaria mellea.* Gazz. Chim. Ital. **118,** 317–521.

Arnone A., Brambilla U., Nasini G. and Vajna de Pava O (1995), Isolation and structure elucidation of Tsugicolines A–D, novel protoilludane sesquiterpenes from *Laurilia tsugicula.* Tetrahedron **51,** 13357–13364.

Daniewski W. M., Kocor M., and Krol J. (1977), Constituents of higher fungi. Pol. J. Chem. (Rocz. Chem.) **51,** 1395–1398.

Daniewski W. M., and Krol J. (1981), Constituents of higher fungi. Part XII. Pol. J. Chem. (Rocz. Chem.) **55,** 1247–1252.

De Bernardi M., Fronza G., Vidari G., and Vita-Finzi P. (1976), Fungal metabolites II: new sesquiterpenes from *Lactarius scrobiculatus* Scop. (Russulaceae). Chim. Ind **58,** 177–178.

De Bernardi M., Fronza G., Mellerio G., Vidari G., and Vita-Finzi P. (1980), Stereochemistry of blennin A and blennin D from *Lactarius blennius.* Phytochemistry **19,** 99–101.

Hook D. J., Yacobucci J. J., O'Connor S., Lee M., Kerns E., Krishnan B., Matson J. and Hesler G. (1990), Identification of the inhibitory activity of carbazomycins B and C against 5-lipoxygenase, a new activity for these compounds. J. Antibiot. **43,** 1347–1348.

Kuschel A., Anke T., Velten R., Klostermeyer D., Steglich W. and König B (1994), The mniopetals, new inhibitors of reverse transcriptases from a *Mniopetalum* species (basidiomycetes). J. Antibiot. **47,** 733–739.

Mong S (1991), Receptors and receptor antagonists for mammalian 5-lipoxygenase products. In: Lipoxygenases and their Products. (Crooke, S. T. and Wong A., eds), Academic Press, San Diego, pp. 185–206.

Nugteren D. H. (1982), Arachidonic acid 12-lipoxygenase. Meth. Enzymol. **86,** 49–54.

Samuelsson B., Dahlen S.-E., Lindgren J. A., Rouzer C. A. and Serhan C. N. (1987), Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. Science **237,** 1171–1176.

Rodewald E., Tibes U., Maass G. and Scheuer W. (1994), Induction of cytosolic phospholipase A_2 in human leukocytes by lipopolysaccharide. Eur. J. Biochem. **223,** 743–749.

Scheuer W. (1989), Phospholipase A_2-regulation and inhibition. Klin. Wochenschr., **67,** 153–159.

Stärk A., Anke T., Mocek U., Steglich W., Kirfel A. and Will G. (1988), Lentinellic acid, a biologically active protoilludane derivative from *Lentinellus* species. Z. Naturforsch. **43c,** 177–183.

Vidari G., De Bernardi M., Vita-Finzi P., and Fronza G. (1976), Sesquiterpenes from *Lactarius blennius.* Phytochemistry **15,** 1953–1955.

Wassermann M. A., Smith III E. F., Underwood D. C., and Barnette M. A. (1991). In: Lipoxygenases and their Products. (Crooke S. T. and Wong A., eds), Acedemic Press, San Diego, pp. 1–50.

Watling R. and Gregory N. M. (1989), Crepidotaceae, Pleurotaceae and other pleurotid agarics. In: British Fungus Flora. Agarics and Boleti. Royal Botanic Garden, Edinburgh, 72–79.

Zapf S., Hoßfeld M., Anke H., Velten R. and Steglich W. (1995), Darlucin A and B, new isocyanide antibiotics from *Sphaerellopsis filum (Darluca filum).* J. Antibiot. **48,** 36–41.