New Bioactive Sesquiterpenes from *Ripartites metrodii* and *R. tricholoma*

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The metabolites of two different *Ripartites* species, *R. tricholoma* (A. & S. ex Fr.) Karst. and *R. metrodii* Huijsm. were investigated. Three new sesquiterpenes were isolated from three different strains. In addition, the strains produced 13-oxo-9(*Z*),11(*E*)-octadecadienoic acid, psathyrellon A, 5-desoxyilludosin, an illudane (previously isolated from a *Bovista* sp.) 96042 and demethylovalicin, five known compounds.

Key words: Ripartites, Metabolites, Antimicrobial and Cytotoxic Activities

Introduction

The Basidiomycotina provide a rich and varied source of terpenoids, especially sesquiterpenoids (Ayer and Browne, 1981). Many of these are derived from the protoilludane skeleton, which is transformed and rearranged to a large number of compounds (Abraham, 2001). For some sesquiterpenes interesting biological activities have been described (Lorenzen and Anke, 1998).

During a screening of fungal extracts for new metabolites with antimicrobial and cytotoxic activities, extracts of the basidiomycetes *Ripartites tricholoma* (A. & S. ex Fr.) Karst. (strain 76048) and *Ripartites metrodii* Huijsm. (strains 82136 and 93109) exhibited antibiotic and cytotoxic activities. As to our knowledge so far no secondary compounds have been described from the genus *Ripartites*. Fermentations of our *Ripartites* strains yielded eight compounds. Besides the known 13-oxo-9(*Z*),11(*E*)-octadecadienoic acid (**4**), psathyrellon A (Bastian, 1985) (**5**), 5-desoxyilludosin (Rasser *et al.*, 2002) (**6**), the illudane of *Bovista* sp. (Rasser *et al.*, 2002) (**7**), and demethylovalicin (Ito *et al.*, 1999) (**8**), the illudane riparol A (**1**), the illudalane riparol B (**2**), and the protoilludane riparol C (**3**) were obtained. The riparols are new compounds and this paper describes the isolation, the structure elucidation and the biological activities of the compounds.

Materials and Methods

Producing organisms

Mycelial cultures of *Ripartites tricholoma* (A. & S. ex Fr.) Karst. (strain 76048) and *Ripartites metrodii* Huijsm. (strains 82136 and 93109) were obtained from spores or tissue plugs derived from fruiting bodies. The species were identified by macroscopic and microscopic characteristics. Voucher specimen and mycelial cultures are deposited in the culture collection of IBWF e. V., Kaiserslautern, Germany. For maintenance on agar slants the fungi were grown on YMG medium (g/l): yeast extract (4), malt extract (10), glucose (4) and agar (1.4%) for solid media. The pH was adjusted to 5.5.

Fermentation

Fungi were grown in YMG medium. Fermentations were carried out in a Biolafitte C6 fermentor containing 20 l of medium with aeration (3 l air/min) and stirring (120 rpm) at room temperature. A well-grown culture (in 250 ml YMG medium) in a 500 ml Erlenmeyer flask (grown at 22 °C and 120 rpm) was used as inoculum. When the antibiotic activity in the culture fluid had reached the maximal value (approx. 15 d), the culture fluid was separated from the mycelia by filtration. The culture broth was extracted with an equal volume of

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EtOAc, the organic phase dried over Na_2SO_4 and concentrated *in vacuo*.

Isolation of the compounds

The crude extracts were applied onto a column containing silica gel (Merck 60, 0.063–0.2 mm) and eluted with cyclohexane, cyclohexane/ethylacetate (9:1, 3:1, 1:1 v/v), ethylacetate, ethylacetate/methanol (3:1, 1:1 v/v) and methanol. For preparative HPLC a Jasco Model PU-1586 instrument with a multiwavelength-detector MD-910 was used [column: Merck RP 18, 7 μm; 250 × 25 mm; gradients: H_2O/MeCN; flow: 15 ml/min].

*Fermentation of Ripartites metrodii 82136 and isolation of **1**, **2**, **3**, and **7***

The fermentation was stopped after 17 d and the culture fluid extracted with EtOAc. The crude product (1.3 g) was separated by silica gel chromatography (silica gel 60, Merck; column 2.5 × 25 cm). An enriched product (246 mg) was obtained after elution with cyclohexane/ethylacetate (1:1 v /v). Preparative HPLC (see above) yielded 5 mg of **1** (Fig. 1) eluting at 44% MeCN, 9 mg of **7** (Fig. 2) eluting at 50% MeCN, 7 mg of **2** (Fig. 1) eluting at 58% MeCN, and 39 mg of **3** (Fig. 1) eluting at 68% MeCN.

*Fermentation of Ripartites tricholoma 76048 and isolation of **4***

After 14 d of fermentation the mycelia were separated from the culture broth and the culture fluid was extracted with EtOAc yielding 336 mg of crude extract. This was applied onto a column (2.5 × 10 cm) containing silica gel 60 (Merck). An enriched product (33 mg) was obtained after elution with cyclohexane/ethylacetate (3:1 v/v). Preparative HPLC (see above) yielded 8 mg of **4** (Fig. 2) eluting at 80% MeCN.

*Fermentation of Ripartites metrodii 93109 and isolation of **5**, **6**, and **8***

After 15 d, when glucose had been completely used up, the fermentation was stopped and the culture filtrate extracted with an equal volume of EtOAc. After evaporation of the organic solvent 891 mg of crude extract were obtained. An enriched product (132 mg) was obtained by silica gel chromatography (see above) after elution with cyclohexane/ethylacetate (3:1 v/v). Preparative HPLC yielded 47 mg of **8** (Fig. 2) eluting at 56% MeCN, 7 mg of **5** (Fig. 1 v/v) eluting at 60% MeCN, and 18 mg of **6** (Fig. 2) eluting at 65% MeCN.

*Psathyrellon A (**5**), 5-desoxyilludosin (**6**), illudane (compound of Bovista sp.) (**7**)*

5, **6** and **7** were detected and identified by HPLC-DAD-MS (HP-LC/MSD-System Series 1100, Hewlett Packard, Waldbronn; APCI, positive and negative mode) with a LiChroCART Supersphere 100 RP-18 column (125 × 2 mm; 4 μm particle size). A gradient H_2O/acetonitrile 0–100% in 20 min at a flow rate of 0.8 ml/min was applied. Retention times [min]: Psathyrellon A, 12.5; 5-desoxyilludosin, 13.4; illudane, 10.5.

Spectroscopic characterization and structure elucidation

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded at room temperature using a Bruker DRX500 spectrometer with an inverse multinuclear 5 mm probe head equipped with a shielded gradient coil. The spectra were recorded in $CDCl_3$, and the solvent signals (7.26 and 77.0 ppm, respectively) were used as reference. The chemical shifts (δ) are given in ppm, and the coupling constants (J) in Hz. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses. For the 2D heteronuclear correlation spectroscopy the refocusing delays were optimized for $^1J_{CH}$ = 145 Hz and $^nJ_{CH}$ = 10 Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker XWIN-NMR software (rev. 010101). Mass spectra were recorded with a LC-MS (HP 1100; APCI, positive/negative mode) and a Micromass Q-TOF MICRO instrument (HR electrospray spectra), while the UV and IR spectra were recorded with a Perkin-Elmer λ 16 and a Bruker IFS 48 spectrometer. The optical rotation was measured with a Perkin-Elmer 141 polarimeter at 22 °C.

Riparol A (**1**) (2,3,14-trihydroxy-7,9-illudadien) was obtained as a colourless oil; $[\alpha]_D$ + 1.8° (*c* 0.6, $CHCl_3$). – UV (MeOH): λ_{max} (log ε) = 250 nm (4.26). – IR (KBr): ν = 3413, 2951, 1646, 1376, 1152, 1092, 1219, 887 cm^{-1}. – ^{1}H and ^{13}C NMR data: see Tables I and II. – HRESIMS: found *m*/*z* 273.1434, $C_{15}H_{22}O_3Na$ requires 273.1467.

Riparol B (**2**) was obtained as a colourless oil. – IR (KBr): ν = 3363, 2953, 2922, 1635, 1434, 1377, 1065, 1038 cm^{-1}. – ^1H and ^{13}C NMR data: see Tables I and II. – HRESIMS: found *m/z* 257.1538, $C_{15}H_{22}O_2Na$ requires 257.1517.

Riparol C (**3**) (8,14-dihydroxy-6-protoilludene) was obtained as a colourless oil; $[\alpha]_D$ – 32° (*c* 0.7, $CHCl_3$). – IR (KBr): ν = 3404, 2951, 1464, 1109, 1019 cm^{-1}. – ^1H and ^{13}C NMR data: see Tables I and II. – HRESIMS: found *m/z* 259.1658, $C_{15}H_{24}O_2Na$ requires 259.1674.

Table I. ^1H (500 MHz) NMR data (δ; multiplicity; *J*) for riparol A (**1**), riparol B (**2**) and riparol C (**3**). The spectra were recorded in $CDCl_3$ and the solvent signal (7.26 ppm) was used as reference. The coupling constants *J* are given in Hz.

H	**1**	**2**	**3**
1a	2.02; d; 13.9	2.68; s	1.40; dd; 7.6, 12.7
1b	1.75; d; 13.9	2.68; s	1.31; dd; 10.7, 12.7
2	–	–	2.38; ddd; 7.6, 10.7, 11.6
4a	1.21; m	3.86; t; 5.9	1.85; m
4b	0.71; m	3.86; t; 5.9	1.85; m
5a	0.92; m	3.03; t; 5.9	2.78; m
5b	0.81; m	3.03; t; 5.9	2.65; m
8	6.26; s	7.01; s	4.15; d; 8.4
9	–	–	2.31; m
10a	5.61; s	2.73; s	1.78; dd; 7.5, 12.3
10b	–	2.73; s	1.13; dd; 10.0, 12.3
12	1.27; s	1.16; s	0.96; s
13	1.15; s	1.16; s	1.09; s
14	3.85; s	4.62; s	4.22; s
15	1.11; s	2.21; s	1.05; s

Table II. ^{13}C (125 MHz) NMR data (δ; multiplicity) for riparol A (**1**), riparol B (**2**) and riparol C (**3**). The spectra were recorded in $CDCl_3$ and the solvent signal (77.0 ppm) was used as reference. The multiplicities of the carbon signals were determined indirectly from HMQC experiments.

C	**1**	**2**	**3**
1	46.3; t	47.3; t	41.2; t
2	87.6; s	143.4; s	46.1; d
3	73.0; s	133.1; s	45.6; s
4	10.9; t	61.7; t	36.1; t
5	4.6; t	31.6; t	24.8; t
6	27.4; s	133.3; s	145.9; s
7	142.2; s	137.9; s	128.9; s
8	118.6; d	124.0; d	74.8; d
9	138.4; s	141.6; s	51.0; d
10	140.6; d	47.8; t	46.3; t
11	44.0; s	39.3; s	39.8; s
12	30.0; q	29.2; q	27.0; q
13	29.4; q	29.2; q	29.5; q
14	62.8; t	64.3; t	59.0; t
15	22.1; q	16.0; q	20.2; q

Biological assays

Antimicrobial activities were determined in the agar plate diffusion assay as described previously (Anke *et al.*, 1989). Nematicidal activity was measured as described by Stadler *et al.* (1994).

Colo-320 cells (DSMZ ACC 144, human colon adenocarcinoma), Jurkat cells (ATCC TIB 152, human acute T cell leukemia), L1210 (ATCC CCI 219, lymphocytic leukemia, mouse), and HL-60 cells (DSMZ ACC 3, human promyelocytic leukemia) were grown in RPMI 1640 medium (GIBCO, BRL), MDA-MB-231 cells (ATCC HTB26, human breast adenocarcinoma) and MCF-7 cells (DSMZ ACC 115, human breast adenocarcinoma) in D-MEM medium (GIBCO, BRL). The medium was supplemented with 10% fetal calf serum (FCS) (GIBCO, BRL), 65 μg/ml of penicillin G and 100 μg/ml of streptomycin sulfate. The cells were maintained in a humidified atmosphere containing 5% of CO_2 at 37 °C.

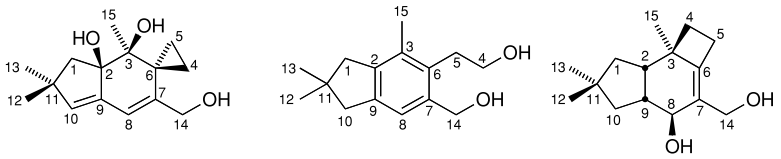
Cytotoxicity was measured in microtiter plates with $1-5\times10^5$ cells/ml medium. Cells were incubated with and without test compounds. In addition to a microscopic examination after 24, 48, and 72 h, the effect on the growth of monolayer cell lines was measured by Giemsa staining. The viability of suspension cells was measured in a test based on XTT [2,3-bis(2-methoxy-4-nitro-5-sulfo-phenyl)-2H-tetrazolium-5-carboxanilide].

Results and Discussion

Isolation and structure determination

Riparol A (**1**), riparol B (**2**), and riparol C (**3**)

The three sesquiterpenes **1**, **2** and **3** (Fig. 1) were isolated from *R. metrodii* 82136 as described above, and their structures determined by spectroscopic techniques. For riparol A (**1**), high-resolution MS experiments showed that the elemental composition is $C_{15}H_{22}O_3$, which is in agreement with the 1D NMR data. This gives the unsaturation index five, and with four signals for unsaturated carbon atoms in the ^{13}C NMR spectrum riparol A should be tricyclic. It has three methyl groups, all appearing as singlets in the ^1H NMR spectrum, of which 12-H_3 and 13-H_3 are geminal as they give HMBC correlations to each other carbon atoms. Both are connected to the quaternary carbon atom C-11, which in turn is connected to C-1 and C-10 according to the HMBC correlations from the methyl protons. 1-H_2 give HMBC correlations to C-2, C-3 and C-9, besides to C-10, C-11,



1 **2** **3**

Fig. 1. Structures of riparol A (**1**), riparol B (**2**) and riparol C (**3**).

C-12 and C-13, while 10-H correlates with C-2, C-8 and C-9, besides C-1 and C-11, showing that C-2 is connected to C-9 and closing the first ring. The protons of the third methyl group correlate with the three quaternary carbon atoms C-2, C-3 and C-6, of which the two former are oxygenated according to the ^{13}C chemical shifts, and this methyl group must be positioned at C-3. 8-H gives HMBC correlations to C-2, C-9 and C-10 in the five-membered ring, as well as to C-6 and C-14. As 14-H_2 give HMBC correlations to C-6, C-7 and C-8, the second ring, which is six-membered, can be closed. C-4 and C-5 remaining, which according to the COSY spectrum constitute a $-CH_2-CH_2-$ fragment. All four protons give HMBC correlations to C-3, C-6 and C-7, besides to each other carbon atoms, and must be part of a cyclopropyl ring (together with C-6). This is also supported by the short range $^1H-^{13}C$ coupling constants for 4/5, which are approx. 160 Hz. The relative configuration of **1** was indicated by the correlations observed in the NOESY spectrum. 14-H_2 correlate approx. equally strong to 4-Hb and 5-Ha, while 15-H_3 correlate to 1-Ha (strongly) and 5-Hb but not to 1-Hb and 5-Ha. The inspection of a Dreiding model of the proposed structure shows that C-15 thereby should be pseudoaxial and on the opposite side of the C-2 hydroxy group.

The exact molecular weight of riparol B (**2**) corresponds with the composition $C_{15}H_{22}O_2$ and, consequently, an unsaturation index of five. With six unsaturated carbon atoms the possibility of a benzene ring comes to mind, which would give **2** one additional ring. The ^1H NMR spectrum contains only singlets, except for the two triplets integrating for two protons each caused by the $-CH_2-CH_2-$ fragment 4/5. Besides to C-5, 4-H_2 give only a HMBC correlation to C-6, while 5-H_2 give HMBC correlations to C-3, C-6 and C-7. 14-H_2 give HMBC correlations to C-6, C-7 and C-8, 10-H_2 correlate to C-2, C-8 and C-9, 1-H_2 correlate to C-2, C-3 and C-9, while 15-H_3 correlate to C-2, C-3 and C-6. This establishes the benzene ring, which is confirmed by the HMBC correlations from 8-H to C-2, C-6, C-10 and C-14. The second ring is determined by the HMBC correlations from 12-H_3/13-H_3 (appearing as one signal integrating for 6 protons) to C-1, C-10 and C-11. The core of **2** is therefore flat, explaining why all proton signals (except 4-H_2 and 5-H_2) appear as singlets and why 12-H_3 and 13-H_3 have the same chemical shift. The NOESY correlations observed confirm the structure suggested.

Riparol C (**3**) has, according to HRESIMS, the elemental composition $C_{15}H_{24}O_2$, corresponding to the unsaturation index four. With only one double bond, which is tetrasubstituted, **3** should be tricyclic. The first ring is easily assembled by the HMBC correlations from the protons 12-H_3 and 13-H_3 of the geminal methyl groups to C-1, C-10 and C-11, and by the COSY correlations from 1-H_2 *via* 2-H and 9-H to 10-H_2. 9-H is strongly coupled with 8-H, and together with the HMBC correlations from 14-H_2 to C-6, C-7 and C-8 as well as from 15-H_3 to C-2, C-3 and C-6, the second ring is in place. 15-H_3 also give a HMBC correlation to C-4, which forms a $-CH_2-CH_2-$ fragment with C-5. The latter can only be connected to C-6, and HMBC correlations between 5-H_2 and C-3, C-6 and C-7 confirm this. The relative configuration was suggested by the NOESY correlations between 12-H_3 and 2-H, 1-Ha, 9-H as well as 10-Ha, between 8-H and 10-Hb, and between 15-H_3 and 1-Hb, 5-Ha as well as 8-H.

The three new compounds isolated in this investigation are sesquiterpenes, with different skeleta. The illudane **1** is very similar to a sesquiterpene isolated from *Agrocybe aegerita*, which is lacking a hydroxy group (Stransky *et al.*, 1992). Compound **2** shares the illudalane skeleton with pholiotic acid isolated from the basidiomycete *Pholiota destruens* (Becker *et al.*, 1994) and a sesquiterpene isolated from *Fomitopsis insularis* (Nozoe *et al.*, 1977). Pholiotic acid was isolated in a screening for metabolites inducing morphological and physiological differentiation of human promyelotic leukemia cells. Compared to pholiotic acid compound **2** bears an additional hydroxy group at C-14 and

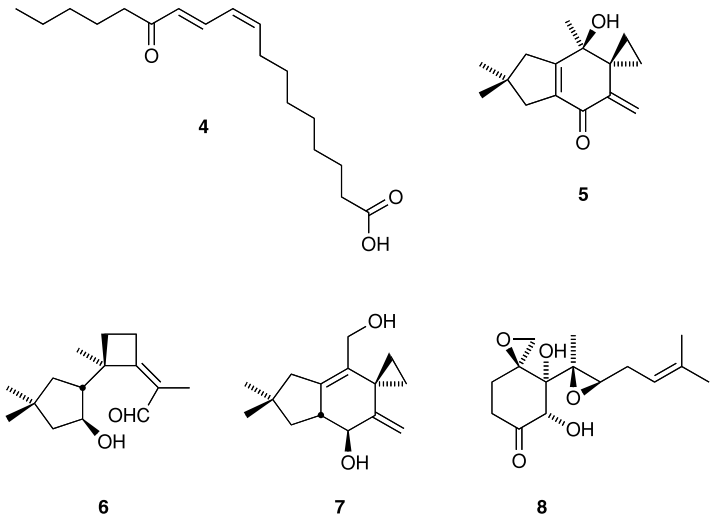


Fig. 2. Structures of the known compounds 13-oxo-9(*Z*),11(*E*)-octadecadienoic acid (**4**), psathyrellon A (**5**), 5-desoxyilludosin (**6**), illudane (**7**), and demethylovalicin (**8**).

the carboxy group of the sidechain is reduced to the alcohol. The sesquiterpene isolated from *F. insularis* lacks the hydroxy group at C-14 of compound **2**. Compound **3** is a protoilludane sesquiterpene. The tsugicolines and armillol are similar compounds isolated from the basidiomycetes *Laurilia tsugicola* and *Laurilia sulcata* (Arnone *et al.*, 1995, 1992), with oxidized cyclobutane rings. Armillol exhibits antifungal activity, whereas no antimicrobial activities have been described for tsugicoline.

Illudane (**7**)

7 is a known compound which was previously isolated from *Bovista* sp. 96042 (Rasser *et al.*, 2002). For **7** moderate cytotoxic activity against HeLa cells has been described, but it does not exhibit antimicrobial activities. Here, **7** was identified by HPLC-MS and comparison with an authentic sample (Fig. 2).

13-Oxo-9(*Z*),11(*E*)-octadecadienoic acid (**4**)

4 was isolated from the culture fluid of *Ripartites tricholoma* 76048 as described above. The metabolite was identified as 13-oxo-9(*Z*),11(*E*)-octadecadienoic acid by spectroscopy (Fig. 2). **4** was previously isolated from cotyledons of sunflowers (*Helianthus annuus* L.) during germination (Gerhardt *et al.*, 2005). Coriolic acid [13-hydroxy-9(*Z*),11(*E*)-octadecadienoic acid] was isolated in a screening for compounds with nematicidal activities from the basidiomycete *Pleurotus pulmonarius* (Stadler *et al.*, 1994). It showed nematicidal, antibacterial, and weak cytototoxic activities.

Psathyrellon A (**5**), 5-desoxyilludosin (**6**), and demethylovalicin (**8**)

5, **6** and **8** were isolated from the culture fluid of *Ripartites metrodii* 93109 as described above. **5** and **6** were identified by HPLC-MS, whereas the structure of **8** was elucidated by NMR spectroscopy (Fig. 2). Psathyrellon A (**5**) was first isolated from *Psathyrella pseudogracilis* (Bastian, 1985). It exhibited strong antimicrobial, cytotoxic, and phytotoxic activites. Arnone and coworkers isolated **5** from *Clitocybe illudens* (now *Omphalotus olearius*) and named it illudin C (Arnone *et al.*, 1991). *Omphalotus* and *Ripartites* both belong to the family Paxillaceae (Singer, 1986). **6** is a know compound which was previously isolated from *Bovista* sp. 96042 (Rasser *et al.*, 2002). For **6** moderate cytotoxic activity against HeLa cells has been described. The compound lacks antimicrobial activities. Demethylovalicin (**8**) was first described by Ito *et al.* (1999). Its production by *Chrysosporium luchnowense* has been reported by Son *et al.* (2002). **8** is an inhibitor of the human methionine aminopeptidase-2 and inhibits the growth of human endothelial cells. Ovalicin was isolated from *Pseudeurotium ovalis* (Sigg and Weber, 1968) and exhibits immunosuppressive activity. Biosynthetic studies showed that ovalicin is a sesquiterpene because its synthesis is consistent with the *cis*-farne-

Table III. Antimicrobial activities (diameter of inhibition zones, mm) of compounds **1**–**5** and **8** in the plate diffusion assay. 100 μg where applied to the paper discs (diameter 6 mm).

Microorganism	**1**	**2**	**3**	**4**	**5**	**8**
Penicillium notatum	–	–	8	8i	19/30i	14i
Paecilomyces variotii	–	–	–	8i	15/30i	17i
Mucor miehei	–	–	10	9i	9i	–
Nematospora coryli	–	–	10	–	26	–
Bacillus brevis	–	–	9	9	15	–
Bacillus subtilis	–	–	9	10	12	–
Micrococcus luteus	–	–	10	–	15	–
Enterobacter dissolvens	–	–	–	–	–	–

i, incomplete inhibition zone; –, no inhibition.

Table IV. Cytotoxic activities of compounds **1**–**5** and **8**.

Cell line	IC_{50} [μg/ml]					
	1	**2**	**3**	**4**	**5**	**8**
COLO-320	20	>50	50	50	0.1	20
L1210	20	20	>50	20	0.5	20
HL-60	20	20	>50	50	0.1	20
Jurkat	20	50	>50	20	0.1	20
MDA-MB-231	1	20	50	>50	0.1	20
MCF-7	1	50	50	50	0.1	20

sylpyrophosphat route (Tanabe and Suzuki, 1974). Up to now ovalicin and its derivates have been isolated from the ascomycetes *Pseudoeurotium ovalis* (Sigg and Weber, 1968), a *Metarrhizium* species (Kuboki *et al.*, 1999), a *Chrysosporium* species (Son *et al.*, 2002), and a *Sporothrix* species (Hayashi *et al.*, 1996). 5-Demethylovalicin is the first ovalicin derivate reported from basidiomycetes.

Biological properties

The antimicrobial activities of the new compounds **1**–**3** as well as of **4**, **5** and **8** are shown in Table III. Cytotoxic activities were tested up to a concentration of 50 μg/ml and the results are shown in Table IV. For all compounds no nematicidal activities were observed at concentrations up to 100 μg/ml against *Meloidogyne incognita* and *Caenorhabditis elegans* (data not shown).

Since the two illudane sesquiterpenes **1** and **7** are not as active as psathyrellon A, it can be assumed that the keto group in the cyclohexane ring is important for the biological activities. **1** and **7** exhibit cytotoxic and no antimicrobial activities. Compound **2** was active against some of the cell lines and compound **4** exhibited both antimicrobial and cytotoxic activities. 13-Oxo-9(*Z*),11(*E*)-octadecadienoic acid (**4**) showed weak antimicrobial and cytotoxic effects, but in contrast to coriolic acid it was not nematicidal.

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Abraham W. R. (2001), Bioactive sesquiterpenes produced by fungi: are they useful for humans as well? Curr. Med. Chem. **8**, 583–606.

Anke H., Bergendorff O., and Sterner O. (1989), Assay of the biological activities of guaiane sesquiterpenoids isolated from the fruit bodies of edible *Lactarius* species. Food. Chem. Toxicol. **27**, 393–398.

Arnone A., Cardillo R., di Modugno V., and Nasini G. (1991), Secondary mould metabolites. XXXIII. Structure elucidation of illudins C–E, novel illudane sesquiterpenoids isolated from *Clitocybe illudens*, using one- and two-dimensional NMR spectroscopy. Gazz. Chim. Ital. **121**, 345–348.

Arnone A., Nasini G., Assante G., and van Eijk G. W. (1992), Three sesquiterpenes produced by the fungus *Laurilia sulcata*. Phytochemistry **31**, 2047–2050.

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 tsugicola*. Tetrahedron **51**, 13357–13364.

Ayer W. A. and Browne L. M. (1981), Terpenoid metabolites of mushrooms and related basidiomycetes. Tetrahedron **37**, 2199–2248.

Bastian W. (1985), Vergleichende Untersuchung zum Sekundärstoffwechsel von coprophilen und erd- oder holzbewohnenden Basidiomyceten. PhD Thesis, Universität Kaiserslautern.

Becker U., Anke T., and Sterner O. (1994), A novel bioactive illudalane sesquiterpene from the fungus *Pholiota destruens*. Nat. Prod. Lett. **5**, 171–174.

Gerhardt B., Fischer K., Balkenhohl T., Pohnert G., Kuehn H., Wasternack C., and Feussner I. (2005), Lipoxygenase-mediated metabolism of storage lipids in germinating sunflower cotyledons and β-oxidation of (9*Z*,11*E*,13*S*)-13-hydroxy-octadeca-9–11-dienoic acid by the cotyledonary glyoxysomes. Planta **220**, 919–930.

Hayashi M., Kim Y., Takamatsu S., Preeprame S., Komiya T., Masuma R., Tanaka H., Komiyama K., and Omura S. (1996), Chlovalicin, a new cytocidal antibiotic produced by *Sporothrix* sp. FO-4649. I. Taxonomy, fermentation, isolation and biological activities. J. Antibiot. **49**, 631–634.

Ito C., Abe N., and Hirota A. (1999), Isolation and structure of a novel indophenol-reducing and 1,1-diphenyl-2-picrylhydrazyl radical-scavenging compound from fungus. Biosci. Biotechnol. Biochem. **63**, 1993–1996.

Kuboki H., Tsuchida T., Wakazono K., Isshiki K., Kumagai H., and Yoshioka T. (1999), Mer-f3, 12-hydroxyovalicin, produced by *Metarrhizium* sp. f3. J. Antibiot. **52**, 590–593.

Lorenzen K. and Anke T. (1998), Biologically active metabolites from basidiomycetes. Curr. Org. Chem. **2**, 329–364.

Nozoe S., Kobayashi H., Urano S., and Furukawa J. (1977), Isolation of ^{6}-protoilludene and the related alcohols. Tetrahedron Lett. **18**, 1381–1384.

Rasser F., Anke T., and Sterner O. (2002), Terpenoids from *Bovista* sp. 96042. Tetrahedron **58**, 7785–7789.

Sigg H. P. and Weber H. P. (1968), Isolierung und Strukturaufklärung von Ovalicin. Helv. Chim. Acta **51**, 1395–1408.

Singer R., (1986), The Agaricales in Modern Taxonomy. Koeltz Scientific Books, Koenigstein.

Son K. H., Kwon J. Y., Jeong H. W., Kim H. K., Kim C. J., Chang Y. H., Chio J. D., and Kwon B. M. (2002), 5-Demethylovalicin, as a methionine aminopeptidase-2 inhibitor produced by *Chrysosporium*. Bioorg. Med. Chem. **10**, 185–189.

Stadler M., Mayer A., Anke H., and Sterner O. (1994), Fatty acids and other compounds with nematicidal activity from cultures of basidiomycetes. Planta Med. **60**, 128–132.

Stransky K., Semerdzieva M., Otmar M., Prochazka Z., Budesinsky M., Ubik K., Kohoutova J., and Streinz L. (1992), Antifungal antibiotic from the mushroom *Agrocybe aegerita* (Brig.) Sing. Collect. Czech. Chem. Commun. **57**, 590–603.

Tanabe M. and Suzuki K. T. (1974), Biosynthetic studies with carbon-13: Incorporation pattern of 1,2-^{13}C-acetate into the fungal sesquiterpene ovalicin. Tetrahedron Lett. **15**, 4417–4420.