Two new antifungal metabolites produced by *Sparassis crispa* in culture and in decayed trees

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The basidiomycete fungus *Sparassis crispa* produced three antifungal compounds during submerged culture in 2% malt broth. One compound appeared to be sparassol (methyl-2-hydroxy-4-methoxy-6-methylbenzoate), first characterized in 1924. The other two, termed ScI and ScII, exhibited considerably greater antifungal activity than did sparassol against *Cladosporium cucumerinum*, and were characterized as methyl-2,4-dihydroxy-6-methylbenzoate (methyl orsellinate) and an incompletely determined methyl-dihydroxy-methoxy-methylbenzoate, respectively. Both compounds were found in the decayed wood of trees, where their presence was diagnostic of *S. crispa* infection. The possible ecological role of these compounds is discussed.

Introduction

Although largely neglected, the first reported isolation and characterization of a microbially produced antibiotic, antedating comparable work on penicillin (Hütterman, 1987), was from the wood-rotting basidiomycete *Sparassis crispa* (formerly *S. ramosa*). Falck (1923) observed that, unlike cultures of other species, *S. crispa* did not become contaminated with other fungi, and isolated a compound that occurred as crystals in and on the medium of old cultures. This compound exhibited mycocidal activity, although full data on its biological action remained unpublished (cf. Cwielong, 1986; Hütterman, 1987). The compound, given the trivial name sparassol, was shown to be methyl-2-hydroxy-4-methoxy-6-methylbenzoate (Wedekind & Fleischer, 1923, 1924). In subsequent screenings for antibiotic production, *S. crispa* has been noted as moderately active (Wilkins & Harris, 1944; Robbins *et al.*, 1945; Hervey, 1947), and suppression of growth of *Bacillus subtilis* on agar media pre-inoculated with *S. crispa* has been attributed to sparassol (Siepmann, 1987).

S. crispa causes a brown root- and butt-rot in living conifers, closely similar to damage caused by *Phaeolus schweinitzii*. Both fungi can cause significant losses in managed conifer forest, but without basidiocarps or cultures, it is difficult to determine which species is responsible for the damage (Phillips & Burdekin, 1982). Although *S. crispa* has been less intensively studied than *P. schweinitzii*, it appears to resemble the latter in having little or no ability to invade the living tissues of its host (Rayner & Boddy, 1988), and is generally restricted to the older, dead wood of standing trees.

Antibacterial and antifungal compounds have been detected in fruit bodies and cultures of many wood-inhabiting fungi (Wilkins & Harris, 1944; Wilkins, 1945, 1946; Robbins *et al.*, 1945; Mathieson, 1946; Hervey, 1947; Brian, 1951; Broadbent, 1968). However, the significance of these compounds in natural environments is uncertain. Uncharacterized antifungal activity from the aquatic (non-basidiomycete) fungus *Massarina aquatica* has been reported in colonized oak wood blocks, and in culture filtrates (Fisher & Anson, 1983). The colonized blocks inhibited the growth of several other fungi which are found in the same habitat, suggesting that production of the antifungal substances may confer an ecological advantage on *M. aquatica* under natural conditions. The root- and butt-rot pathogen *Heterobasidion annosum* produces inhibitory compounds in the presence of plant cells or antagonistic fungi (Sonnenbichler *et al.*, 1989), but the inference that these are 'ecological metabolites' was based only on cultural studies.

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This paper describes the characterization of two further antifungal metabolites from *S. crispa*, and their production both in culture and in naturally decayed wood.

Methods

Organisms. *Sparassis crispa* isolates 5B and 5C were from laboratory stocks, obtained from decayed conifer trees some years previous to this study, and maintained by repeated subculture on 2% malt agar (2%, w/v, Oxoid malt; 1.5%, w/v, Oxoid agar no. 3), with storage at 4 °C. Additional isolates, 5E–5I, were isolated from decayed wood of European larch (*Larix decidua*) trees from Bagley Wood, Oxfordshire, UK, by plating small pieces (about 5 mm²) of wood removed aseptically from near the margin of brown rot lesions on 3% malt agar. Decay fungi isolated in this way were identified on the basis of their characteristics in culture (Stalpers, 1978).

The wood-decaying basidiomycetes *Armillaria gallica*, *A. mellea*, *A. ostoyae*, *A. tabescens*, *Coriolus versicolor*, *Heterobasidion annosum*, *Hypholoma fasciculare*, *Inonotus dryadeus*, *Phaeolus schweinitzii*, *Rigidoporus ulmarius* and *Stereum sanguinolentum*, and the ascomycete root pathogen *Rhizina undulata*, were from laboratory stocks, maintained and grown as above. *Botrytis cinerea* and *Cladosporium cucumerinum*, also laboratory stock isolates, were maintained and sporulated on *Botrytis* medium X (Last & Hanley, 1956).

Antibiotic production in culture. *S. crispa*, strains 5B and 5C, were grown as stationary submerged cultures in 2% malt extract (Oxoid) broth (50 ml) in 250 ml Erlenmeyer flasks, at 25 °C in darkness.

Samples (0.8 ml) were withdrawn from culture flasks, mixed with methanol (1.2 ml) and stored at –20 °C prior to HPLC analysis. Three replicate flasks were used at each sampling time, and the mycelial dry weight of these cultures was determined. Fungal mycelium was collected by filtration through muslin, rinsed with water and dried to constant weight at 80 °C.

Isolation of antibiotic compounds produced by *S. crispa*. Culture filtrates from 28-d-old cultures of *S. crispa* 5B, grown as above, were partitioned twice against 50 ml vols of redistilled chloroform. Combined chloroform phases were evaporated to dryness under reduced pressure at 30 °C, and the residue redissolved in a small volume of ethyl acetate.

Antifungal compounds were detected in these extracts by direct bioassay with *Cladosporium cucumerinum* on developed TLC plates (Homans & Fuchs, 1970). Aluminium-backed TLC plates (silica gel 60F₂₅₄, 0.2 mm thick) were loaded with the ethyl acetate extract (0.5–2.5 ml culture volume equivalent) and developed. Two alternative solvent systems were used: A, chloroform/methanol (96:4, v/v); B, cyclohexane/ethyl acetate (1:1, v/v). Air-dried plates were sprayed with dense conidial suspensions of *C. cucumerinum* in half-strength Czapek Dox salts containing 15 g sucrose l⁻¹.

The two most active antifungal compounds detected on bioassays were purified by sequential preparative TLC on silica gel 60F₂₅₄ plates (0.5 mm thick), using the two solvent systems. Compounds were eluted from the silica gel with methanol and crystallized by evaporation under reduced pressure.

Characterization of antibiotic compounds. UV absorption spectra were obtained for the purified antifungal compounds. These compounds were further characterized by mass spectrometry and ¹H nuclear magnetic resonance.

Antifungal activity of pure compounds. Spore germination tests against *Botrytis cinerea* were done as described by Rossall & Mansfield (1978), using the active compounds at a concentration of 100 µg ml⁻¹ in the assay solution. Assays against mycelial growth of *Heterobasidion annosum* were done in flat-bottomed glass tubes (25 mm diam.)

containing 2.5 ml 2% malt extract (Oxoid) broth (Woodward & Pearce, 1988), with 100 µg of active compound incorporated.

Interactions of *S. crispa* with other fungi. Interactions between *S. crispa* and other wood-inhabiting fungi were examined in dual culture on Malt Extract Agar (Difco). Petri dishes (90 mm diam.) containing approximately 25 ml of medium were inoculated eccentrically with *S. crispa* and, except with *Armillaria* spp., incubated for 14 d (22 °C, in darkness) before inoculation with other fungi 60 mm from the *Sparassis* inoculation site. Effects on the growth of these fungi were assessed after further incubation for 14 d. In tests with *A. gallica*, *A. mellea*, *A. ostoyae* and *A. tabescens*, the fungi were inoculated simultaneously and examined after 28 d.

Estimation of antibiotics from decayed wood. Wood from a wind-snapped Sitka spruce (*Picea sitchensis*) tree from Bagley Wood, exhibiting an advanced brown cubical rot attributable to *S. crispa* (identified by cultural characteristics after isolation, and from basidiomes produced on the stump), was extracted in methanol (5 vols). Extracts were concentrated by evaporation under reduced pressure, and antifungal activity was detected on TLC plates by bioautography with *C. cucumerinum*. Samples equivalent to 1 g fresh wt decayed wood were loaded, and developed in solvent system B.

The compounds in wood extracts were quantified spectrophotometrically (*A*₂₆₆) after methanol elution from preparative TLC plates.

Quantification of antibiotics in culture filtrates using HPLC. Total antibiotics in culture filtrate samples were determined by reverse phase HPLC. Aliquots (20 µl) were separated isocratically on a NovaPak-C18 Radial-Pak cartridge column, 100 mm × 8 mm i.d., protected by a µ-Bondapak C18 Guard-Pak cartridge pre-column (Waters). The solvent mixture, 60% (v/v) methanol in water, was pumped at a flow rate of 1.5 ml min⁻¹, and eluting compounds were detected by absorbance at 266 nm. Samples were injected at intervals of 10 min: the column was washed (100% methanol, 10 min) after every 10 determinations. The two antibiotic compounds, which eluted as a single, shouldered, peak, were quantified on a peak area basis against an external standard of the compounds mixed in their naturally occurring proportions.

Detection of antibiotics in decayed wood using HPLC. Samples of brown-rotted wood from felled coniferous trees [mainly, but not exclusively, European larch (*Larix decidua*)] from Bagley Wood were extracted in 2 vols (w/v) methanol for 24 h at 4 °C. Samples (20 µl) of the resulting extract were separated using reverse-phase HPLC as previously, but with gradient elution. The initial solvent was 30% (v/v) aqueous methanol, increasing linearly to 100% methanol over 20 min. This solvent composition was maintained for 5 min before returning to the starting conditions (via 100% water) over a total cycle (including re-equilibration) of 40 min. Purified samples of the antibiotic compounds were used as external standards.

Isolation of sparassol. For use as a chromatography standard for comparison with the antibiotics isolated in this study, a sample of sparassol was obtained from 6–9-month-old cultures of *S. crispa* 5G on 2% malt agar slopes, grown initially at 22 °C for about 1 month, then maintained at 4–8 °C. Crystals, produced abundantly on the surface of these cultures, were harvested mechanically and dissolved in methanol.

Results

Antifungal antibiotics produced by Sparassis crispa in culture

A large zone of inhibition was observed at *R_F* 0.50, and a minor inhibition zone at *R_F* 0.67 on TLC plates loaded

with organic phase extracts from submerged cultures of *S. crispa* strain 5B, developed in solvent system A and bioassayed with *Cladosporium cucumerinum*. Solvent system B resolved the large inhibition zone into two major active compounds, with R_F values of 0.44 and 0.53. The minor zone had an R_F of 0.66 in this system. All antifungal zones corresponded to areas of extinction under UV light. Sparassol did not give a strong inhibition zone on bioassay plates at the (undetermined) concentration used. The R_F of sparassol was 0.64 and 0.70 in solvent systems A and B, respectively. The two major antifungal compounds, neither of which could be equated with sparassol, were termed ScI (R_F in B = 0.44) and ScII (R_F in B = 0.53).

When purified from TLC plates, these compounds were obtained as pale yellow crystals. Yields (per litre of culture medium) of 26.5 mg (ScI) and 35.5 mg (ScII) were obtained.

ScI and ScII had HPLC retention times of 13.8 and 14.2 min, respectively, when eluted under gradient conditions. Sparassol had a retention time of 18.2 min. All these compounds were present in 6-week-old culture filtrates of *S. crispa*.

Chemical and physical properties of antifungal compounds

ScI. Methyl-2,4-dihydroxy-6-methylbenzoate (methyl orsellinate). Fig. 1(b). $C_9H_{10}O_4$; m.p. 137–139 °C; UV λ MeOH_{max} nm (log ϵ) 301 (3.51), 264 (3.94), 229 (3.43); precise mass measurement M^+ 182.0551; MS (electron impact 40 eV) m/z 182 (M^+ , 46%), 181 (14), 152 (28), 151 (36), 150 (100), 131 (23), 122 (34); ^1H NMR (200 MHz, $CDCl_3$, TMS internal standard) δ 2.49 (3H, s, Me), 3.92 (3H, s, MeO–), 6.23 (1H, δ , Ar–H, $J = 2.9$ Hz), 6.28 (1H, δ , Ar–H, $J = 2.9$ Hz), 11.73 (1H, s, chelated Ar–OH).

ScII. Methyl-dihydroxy-methoxy-methylbenzoate (substitution pattern not determined except for hydroxy– in the 2-position), Fig. 1(c). $C_{10}H_{12}O_5$; UV λ MeOH_{max} nm (log ϵ) 302 (3.36), 268 (3.96), 224 (3.95); precise mass measurement M^+ 212.0671; MS (electron impact 40 eV) m/z 212 (M^+ 49%), 181 (35), 180 (95), 165 (25), 152 (100), 151 (82), 150 (62), 137 (48), 122 (26); ^1H NMR (200 MHz, $CDCl_3$, TMS internal standard): δ 2.46 (3H, s, Me), 3.94 (6H, s, 2* MeO– degenerate), 6.36 (1H, s, Ar–H), 11.93 (1H, s, chelated OH).

Sparassol (for comparison). Methyl-2-hydroxy-4-methoxy-6-methylbenzoate. Fig. 1(a). $C_{10}H_{12}O_4$. (Wedekind & Fleischer, 1924.)

Antifungal activity of ScI and ScII

The effects of these compounds on germ-tube growth in

(a) Sparassol
(Methyl-2-hydroxy-4-methoxy-6-methylbenzoate)

(b) ScI
(Methyl-2, 4-dihydroxy-6-methylbenzoate)

(c) ScII
(Methyl-dihydroxy-methoxy-methylbenzoate)

Fig. 1. Chemical structures of (a) sparassol, (b) ScI and (c) ScII.

Botrytis cinerea are shown in Table 1. At the concentration used (100 $\mu g\ ml^{-1}$), ScI showed greater activity than ScII against *B. cinerea*. At this concentration, the inhibitory effects of these compounds against mycelial growth of *Heterobasidion annosum* were less, but remained significant (Table 1).

Interactions of *S. crispa* with other fungi

A range of responses to pairing with *S. crispa* was observed (Fig. 2). Growth of *H. annosum* was almost completely suppressed, and that of *Ionotus dryadeus* and *Rigidoporus ulmarius* markedly inhibited. Mutual growth inhibition was evident with *Armillaria gallica*, *A. mellea*, *A. ostoyae* and *Stereum sanguinolentum*. With *A. tabescens*, *Coriolus versicolor*, *Hypholoma fasciculare*, *Phaeolus schweinitzii* and *Rhizina undulata*, no gross inhibition was observed prior to contact, although *P. schweinitzii* exhibited a strong incompatibility response along the interaction interface.

Fig. 2. Dual culture interactions between *Sparassis crispa* (right on all plates) and (a) *Stereum sanguinolentum* (mutual inhibition), (b) *Heterobasidion annosum* (growth strongly suppressed), (c) *Rhizina undulata* (no obvious inhibition) and (d) *Phaeolus schweinitzii* (interaction line formation).

Table 1. Effects of antibiotic compounds from *Sparassis crispa* on extension of *Botrytis cinerea* germ tubes and mycelial growth of *Heterobasidion annosum*

Compound (100 µg ml ⁻¹)	<i>Botrytis cinerea</i>		Effect on: <i>Heterobasidion annosum</i>	
	Percentage decrease in germ tube length*	LSD†	Percentage decrease in mycelial dry wt‡	LSD†
ScI	61.9 ± 2.7	7.0***	8.9 ± 6.3	7.0**
ScII	37.7 ± 2.7	7.4***	15.1 ± 9.6	4.8***

* Based on means of 120 measurements on three replicate slides per treatment ± SEM. 100 % = 351.75 ± 1.12 µm.

† Least significant difference between treatments and control, expressed as a percentage.

** $P = 0.01$; *** $P = 0.001$.

‡ Based on means of 10 replicates per treatment ± SEM. 100 % = 27.1 ± 1.0 mg.

Accumulation of ScI and ScII in cultures of *S. crispa*

ScI (methyl orsellinate) comprised the major component of the shouldered peak, with a retention time of

approximately 5.18 min. The ScII shoulder appeared at a retention time of 5.83 min. Throughout the incubation period, the peak height ratio ScI:ScII remained essentially constant between 3.8 and 4.1, in both isolates

Table 2. HPLC detection of antibiotics ScI and ScII in brown-rotted wood of conifers and the decay fungi isolated from these trees

+, Detected; —, not detected.

Tree	ScI + ScII	Fungus isolated
1. Sitka spruce	+	<i>Sparassis crispa</i>
2. Larch	—	Undetermined. White, rapidly growing mycelium
3. Larch	—	Undetermined. White, rapidly growing mycelium
4. Corsican pine	—	<i>Phaeolus schweinitzii</i>
5. European larch	—	<i>P. schweinitzii</i>
6. European larch	+	<i>S. crispa</i>
7. European larch	+	<i>S. crispa</i>
8. European larch	—	<i>P. schweinitzii</i>
9. European larch	—	<i>P. schweinitzii</i>
10. European larch	—	<i>P. schweinitzii</i>
11. European larch	—	<i>P. schweinitzii</i>
12. European larch	—	<i>P. schweinitzii</i>
13. European larch	—	<i>P. schweinitzii</i>
14. Corsican pine	+	<i>S. crispa</i>
15. European larch	+	<i>S. crispa</i>
16. European larch	+	<i>S. crispa</i>
17. European larch	—	<i>P. schweinitzii</i>
18. European larch	—	<i>P. schweinitzii</i>

* Very small peaks at a retention time of about 14 min in this sample.

examined. Total antibiotic levels (ScI + ScII) and fungal biomass production for *S. crispa* isolates 5B and 5C are shown in Fig. 3.

Although growth curves for the two isolates were closely similar, the onset of antibiotic production was later in 5B than 5C: mean concentrations of total antibiotics in culture filtrates after 8 weeks were 39 and 65 $\mu\text{g ml}^{-1}$, respectively. However, variation between individual replicate flasks was large.

Detection of antibiotics in decayed wood

Zones of antifungal activity were detected at R_f values of 0.44 and 0.53 in solvent system B, corresponding to ScI and ScII, but not at R_f 0.65–0.70 (sparassol). Quantitative estimations of the two active compounds were $102 \pm 44 \mu\text{g (g fresh wt)}^{-1}$ (ScI) and $90 \pm 13 \mu\text{g (g fresh wt)}^{-1}$ (ScII).

Gradient HPLC of extracts from the decayed Sitka spruce wood revealed a shouldered peak at retention time 14.1 min, corresponding to ScI and ScII. A peak at 18.4 min may relate to sparassol, although the identity was not confirmed (Fig. 4).

Double or merged peaks, eluting with retention times between 13.5 and 14.2 min, were observed in HPLC separations of 6 out of 18 brown rotted wood samples. *S. crispa* was isolated from the same 6 wood samples,

Fig. 3. Growth (□, ■) and antibiotic accumulation (○, ●; ScI plus ScII) in liquid cultures of *Sparassis crispa* (□, ○, 5B; ■, ●, 5C). Vertical bars represent standard errors.

Fig. 4. Detection of ScI, ScII and (possibly) sparassol in methanol extracts of Sitka spruce wood decayed by *Sparassis crispa*, separated by HPLC. Compounds were separated on a Waters NovaPak-C18 cartridge column (100 mm $\times$ 8 mm i.d.) eluted with 60% methanol in water at a flow rate of 1.5 ml min⁻¹. Absorbance of the eluate was monitored at 266 nm.

whereas other species of decay-causing fungi were cultured from the remaining 12 trees (Table 2).

HPLC of sparassol crystals

Sparassol eluted in the HPLC gradient at about 18.2 min

as a single peak. Only trace quantities of ScI and ScII (< 1% total A_{266}) were present in the methanol solution of these crystals.

Discussion

Three antifungal orsellinic acid derivatives were produced by *S. crispa* in culture and in wood decayed by this species. The previously described sparassol appears to be the least active, although it is possible that its high vapour pressure (Cwielong, 1986) resulted in loss during sample preparation. The identity of sparassol was inferred from its chromatographic properties, which were identical to those of crystals harvested from old cultures of *S. crispa*, the original source of sparassol (Falck, 1923).

The two more active compounds, ScI and ScII, were purified and characterized in the present study. ScI was identified as methyl-2,4-dihydroxy-6-methylbenzoate (methyl orsellinate) and ScII as a methyl-dihydroxy-methoxy-methyl benzoate. In the latter compound, one of the hydroxyl groups occupies the 2-position: the relative positions of the second hydroxy- group and the methyl- and methoxy- groups remain to be determined. It is likely that a major part of the antifungal activity that Falck (1923) observed preventing the contamination of old cultures was attributable to ScI and ScII, rather than to sparassol.

Antifungal orsellinic acid derivatives are also known in other fungi. A series of sesquiterpene orsellinates has been isolated and characterized from cultures of *Armillaria mellea* and *A. ostoyae* (Donnelly *et al.*, 1982, 1984; Medland *et al.*, 1982), and orsellinic acid itself is reported to exhibit antibacterial and antifungal activity (Lennartz, 1947; Fujikawa *et al.*, 1956; 1970; Cwielong, 1986). Although inhibition of other fungi by *S. crispa* may be due to the effects of these compounds, and growth of *Heterobasidion annosum*, which is strongly inhibited by *S. crispa* on agar plates, was reduced by purified ScI and ScII, the data available are insufficient for proof.

In an early survey, the rate of extension and antibiotic production among a range of 500 basidiomycetes appeared to be negatively correlated (Hervey, 1947). *S. crispa* is particularly slow growing in culture compared with other wood decay fungi (Stalpers, 1978). Although self-inhibition provides a possible explanation for this effect, rapid early growth of cultures before compounds ScI and ScII had begun to accumulate was not apparent (Fig. 3). Also, growth of isolates 5B and 5C was closely similar, despite their different patterns of antibiotic accumulation.

Overall, the patterns of accumulation of ScI and ScII observed parallel those of the phytotoxic fomajorins

produced by *H. annosum* (Donnelly *et al.*, 1987). Despite the phytotoxic nature of the fomajorins, the disease syndrome caused by *H. annosum* does not appear typically toxin-mediated. It has been postulated that the biological role of the fomajorins is in the infection process (Sonnenbichler *et al.*, 1989). However, as they are also produced in interactions with antagonistic fungi (Sonnenbichler *et al.*, 1989) they may be important in the long-term maintenance of the fungus in resources (cf. Rayner & Boddy, 1988) captured initially in the living tree. *H. annosum* is capable of survival for many years in buried wood (e.g. stumps) (Phillips & Burdekin, 1982).

Other root- and butt-rot fungi, including *Phaeolus schweinitzii*, also persist for prolonged periods in the decayed wood of stumps initially infected when the trees were living (Barrett, 1985). In the living tree, the endogenous defences of the host may serve to exclude other micro-organisms from competition with such decay fungi, but this protection ceases on death of the host (Gramss, 1987) and the residual tissues, comprising the stump, then become available to a wide range of colonizers (cf. Rayner & Boddy, 1988). The present study has demonstrated that ScI and ScII occur naturally in wood decayed by *S. crispa*, at concentrations sufficient to impair the growth of other fungi. It seems possible, therefore, that these compounds may contribute to the suppression of potential competitors, hence allowing *S. crispa* to persist for an extended period. The production of antifungal compounds by many wood-decay fungi, e.g. *P. schweinitzii* (S. Woodward, unpublished data) suggests that such effects may be widespread among these fungi.

Detection by HPLC of ScI and ScII in extracts of conifer wood (which in some cases had been exposed to environmental weathering) decayed by *S. crispa*, but not in wood decayed by other brown rot fungi, notably *P. schweinitzii*, may provide the basis for a rapid diagnostic method. This technique would obviate the requirements for basidiome production, which is often sporadic, or isolation of the causal fungi, which can be problematic, especially from wood in an advanced state of decay. Moreover, the widespread production of antibiotic secondary metabolites by wood-inhabiting fungi suggests that this approach may be more generally applicable.

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References

- BARRETT, D. K. (1985). Basidiospores of *Phaeolus schweinitzii*: a source of soil infestation. *European Journal of Forest Pathology* **15**, 417–425.

- BRIAN, P. W. (1951). Antibiotics produced by fungi. *Botanical Review* **17**, 357–430.
- BROADBENT, D. (1968). Antibiotics produced by fungi. *Pest Articles and News Summaries* **14**, 120–141.
- CWIELONG, P. (1986). *Mechanismen der Resistenz und der Pathogenität von fungizid wirksamen Naturstoffen gegenüber dem Erreger der Rotfäule Heterobasidion annosum (Fr.) Bref.* PhD dissertation, Universität Göttingen, Germany.
- DONNELLY, D., SANADA, S., O'REILLY, J., POLONSKY, J., PRANGÉ, T. & PASCARD, C. (1982). Isolation and structure (X-ray analysis) of the orsellinate of armillol, a new antibacterial metabolite from *Armillaria mellea*. *Journal of the Chemical Society. Chemical Communications* (1982) 135–137.
- DONNELLY, D. M. X., POLONSKY, J., PRANGÉ, T., SNATZKE, G. & WAGNER, U. (1984). The absolute configuration of the orsellinate of armillol: application of the coupled oscillator theory. *Journal of the Chemical Society. Chemical Communications* (1984) 222–223.
- DONNELLY, D. M. X., O'REILLY, J., POLONSKY, J. & SHERIDAN, M. H. (1987). *In vitro* production and biosynthesis of fomajorin D and S by *Fomes annosus* (Fr.) Cooke. *Journal of the Chemical Society. Perkin Transactions* (1987) 1869–1872.
- FALCK, R. (1923). Über ein krystallisiertes Stoffwechsel produkt von *Sparassis ramosa* Schaff. *Berichte der Deutschen Chemischen Gesellschaft (Berlin)* **56**, 2555–2556.
- FISHER, P. J. & ANSON, A. E. (1983). Antifungal effects of *Massarina aquatica* growing on oak wood. *Transactions of the British Mycological Society* **81**, 523–527.
- FUJIKAWA, F., HITOSA, Y., YAGI, Y. & YASUDA, K. (1956). Antiseptics for foodstuff. LX. *Yakugaku Zasshi* **76**, 674–678.
- FUJIKAWA, F., HIRAYAMA, T., NAKAMURA, Y., SUZUKI, M., DOI, M. & NIKI, C. (1970). Studies for antiseptics for foodstuff. LXXI. Studies on orsellinic acid ester, *p*-orcincolcarboxylic acid ester and olivetonide as a preservative for saki. *Yakugaku Zasshi* **90**, 1517–1519.
- GRAMSS, G. (1987). The influence of the concomitant microflora on establishment and dieback of decay fungi in standing timber. *Journal of Phytopathology* **120**, 205–215.
- HERVEY, A. H. (1947). A survey of 500 basidiomycetes for antibacterial activity. *Bulletin of the Torrey Botanical Club* **74**, 476–503.
- HOMANS, A. L. & FUCHS, A. (1970). Direct bioautography on thin-layer chromatograms as a method for detecting fungitoxic substances. *Journal of Chromatography* **51**, 327–329.
- HÜTTERMANN, A. (1987). History of forest botany (Forstbotanik) in Germany from the beginning in 1800 until 1940 – Science in the tension field between university and professional responsibility. *Berichte der deutschen botanische Gesellschaft* **100**, 107–141.
- LAST, F. T. & HANLEY, R. E. (1956). A local lesion technique for measuring the infectivity of conidia of *Botrytis fabae* Sardiña. *Annals of Applied Biology* **44**, 410–418.
- LENNARTZ, T. (1947). Über antituberkulöse Stoffe: Antibiosche Eigenschaften von Derivaten des Orcins. *Zeitschrift für Naturforschung* **2B**, 7–9.
- MATHIESON, J. (1946). Antibiotics from Victorian basidiomycetes. *Australian Journal of Experimental Biology and Medical Science* **24**, 57–62.
- MEDLAND, S. L., IZAK, R. R., WING, R. M., ZAKI, A. I., MUNNECKE, D. E. & SIMS, J. J. (1982). Melleolide, a new antibiotic from *Armillaria mellea*. *Tetrahedron Letters* **23**, 2515–2518.
- PHILLIPS, D. H. & BURDEKIN, D. A. (1982). *Diseases of Forest and Ornamental Trees*. London: Macmillan.
- RAYNER, A. D. M. & BODDY, L. (1988). *Fungal Decomposition of Wood*. Chichester: John Wiley.
- ROBBINS, W. J., HERVEY, A., DAVIDSON, R. W., MA, R. & ROBBINS, W. C. (1945). A survey of some wood-destroying and other fungi for antibacterial activity. *Bulletin of the Torrey Botanical Club* **72**, 165–190.
- ROSSALL, S. & MANSFIELD, J. W. (1978). The activity of wyerone acid against *Botrytis*. *Annals of Applied Biology* **89**, 359–362.
- SIEPMANN, R. (1987). Wachstumshemmung von Stammfäulepilzen und von *Gremmeniella abietina* durch *Bacillus subtilis*. *European Journal of Forest Pathology* **17**, 59–64.
- SONNENBICHLER, J., BLIESTE, I. M., PEIPP, H. & HOLDENRIEDER, O. (1989). Secondary fungal metabolites and their biological activities. I. Isolation of antibiotic compounds from cultures of *Heterobasidion annosum* synthesized in the presence of antagonistic fungi or host plant cells. *Biological Chemistry Hoppe-Seyler* **370**, 1295–1303.
- STALPERS, J. A. (1978). Identification of wood-inhabiting fungi in pure culture. *Studies in Mycology* no. 16. Baarn: Centraalbureau voor Schimmelcultures.
- WEDEKIND, E. & FLEISCHER, K. (1923). Über die Konstitution des Sparassols. *Berichte der deutschen chemischen Gesellschaft* **56**, 2556–2563.
- WEDEKIND, E. & FLEISCHER, K. (1924). Zur Kenntniss des Sparassols. *Berichte der deutschen chemischen Gesellschaft* **57**, (Abt. B), 1121–1123.
- WILKINS, W. H. (1945). Investigation into the production of bacteriostatic substances by fungi. Cultural work on basidiomycetes. *Transactions of the British Mycological Society* **28**, 110–114.
- WILKINS, W. H. (1946). Investigation into the production of bacteriostatic substances by fungi: preliminary examination of the fifth 100 species, all basidiomycetes, mostly of the wood-destroying type. *British Journal of Experimental Pathology* **27**, 140–142.
- WILKINS, W. H. & HARRIS, G. C. M. (1944). Investigation into the production of bacteriostatic substances by fungi. VI. Examination of the larger basidiomycetes. *Annals of Applied Biology* **31**, 261–270.
- WOODWARD, S. & PEARCE, R. B. (1988). The role of stilbenes in resistance of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) to entry of fungal pathogens. *Physiological and Molecular Plant Pathology* **33**, 127–149.