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Short Communication

Incrustoporin, a New Antibiotic from *Incrustoporia carneola* (Bres.) Ryv. (Basidiomycetes)

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During a screening of fungal extracts for new antibiotics, extracts of cultures of the Basidiomycete *Incrustoporia carneola* (strain 9170) were found to possess antifungal activity. We wish to report the isolation and structure determination of the active constituent from the extracts, as well as its antimicrobial and cytotoxic activities.

During a typical fermentation of *Incrustoporia carneola* in a 20 l scale (see the Experimental section for details), glucose and maltose are converted primarily into ethanol, which apparently is the carbon source for most of the biomass of the fungus. The cultures were harvested after 480–500 h, when the biomass and antifungal activity had reached their maxima. The metabolite responsible for the antifungal activity was isolated by bioassay guided fractionation, and it was found to be a new compound for which we suggest the name incrustoporin (see Fig. 1). Incrustoporin does not exhibit antibacterial activity in the serial dilution assay (maximum concentration 100 μg ml^{-1}) against *Acinetobacter calcoaceticus*, *Arthrobacter citreus*, *Bacillus brevis*, *Bacillus subtilis*, *Escherichia coli* K12, *Micrococcus luteus*, *Salmonella typhimurium* TA 98, and *Streptomyces spec.* ATCC 23836. The antifungal and cytotoxic activities are given in Tables 1 and 2.

High resolution mass spectroscopy data determined the composition of incrustoporin to be $C_{13}H_{14}O_2$, and NMR data revealed that the compound is a *para*-substituted methylbenzene. The presence of a γ-lactone functionality was suggested[1] by a proton signal at 4.98 ppm (correlated with a doublet at 81.4 ppm in the ^{13}C spectrum), the corresponding proton couples with the methylene protons of an ethyl group and an olefinic proton. The latter proton gives a long-range correlation to a carbonyl carbon (171.8 ppm). The position of the 4-methylphenyl group on the α carbon of the lactone moiety is suggested[1] by the chemical shift of the γ-lactone olefinic proton (7.48 ppm), and this could be confirmed by long-range $^1H–^{13}C$ and NOESY correlation experiments (summarised in Fig. 1).

Experimental

The producing organism *Incrustoporia carneola* strain 9170 was collected in Ethiopia and isolated from wood,[2] and the strain has been deposited in the culture collection of the Lehrbereich Biotechnologie, University of Kaiserslautern. Mycelial cultures were obtained from spore prints of the fruiting body, and for maintenance on agar slants the fungus was grown on YMG medium (g dm^{-3}: yeast extract 4, malt extract 10, glucose 4; pH 5.5). Fer-

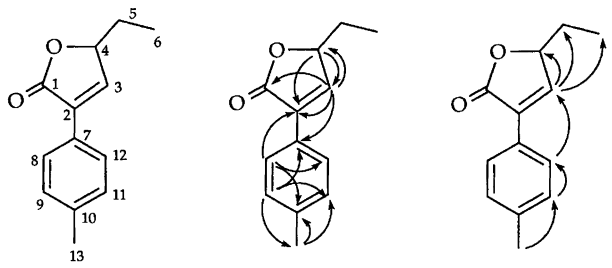

Fig. 1. The structure of incrustoporin, with significant long-range $^1H–^{13}C$ (middle) and NOESY (right) correlations.

Table 1. Antifungal activities of incrustoporin in the agar diffusion assay (YMG medium). The activity is measured as the diameter of inhibition zone (in mm) when the given amount of incrustoporin (adsorbed on a paper disc with diameter 6 mm) was put on the medium with the respective fungus. — Signifies that no inhibition zone was observed.

Organism	10	50	100 µg/disc
Absidia glauca (+)	9	16	20
Absidia glauca (−)	—	8	14
Aspergillus ochraceus	—	9	11
Cladosporium cladosporioides	—	18	23
Curvularia lunata	—	12	18
Fusarium oxysporum	—	10	11
Fusarium fujikuroi	—	12	13
Mucor miehei	10	18	22
Nematospora coryli	—	9	11
Paecilomyces variotii	—	8	11
Penicillium islandicum	—	10	12
Penicillium notatum	—	11	13
Ustilago nuda	—	14	15

mentations were carried out in a Biostat U fermentor equipped with an MFCS system (B. Braun Biotech) containing 20 l of Y2MG medium: g dm^{-3}: yeast extract 4, malt extract 20, glucose 4. The pH was adjusted to 5.5 prior to sterilization. After inoculation with a well grown seed culture in YMG medium (500 ml) the fermentor was incubated at 24 °C with aeration (3 l min^{-1}) and agitation (100 rpm). The oxygen content in the medium was determined on-line. The contents of glucose, maltose, ethanol (determined enzymatically) and incrustoporin (determined by HPLC), as well as the mycelial dry weight and the pH of the culture fluid were measured daily. The antifungal activity of crude extracts of the cultures and the chromatography fractions was monitored with the agar plate diffusion assay with *Mucor miehei* as the test organism. The antibacterial, antifungal (see Table 1) and cytotoxic (see Table 2) activities were measured as described previously.[3]

After fermentation, the mycelia were separated by filtration and discarded. The culture fluid (14 l) was passed through Mitsubishi Diaion HP 21 resin, the resin was washed with water–acetone 1:1 (1 l), whereafter a fraction containing incrustoporin was eluted with 1 l of acetone. The solvent was evaporated off, and the crude product (795 mg) was fractionated by chromatography (Merck 60 SiO_2 column, 60–200 µm, eluted with cyclohexane–EtOAc 7:3). The enriched product (45 mg) was purified by preparative HPLC (Merck LiChrosorb Diol

Table 2. The cytotoxic activities of incrustoporin. IC_{50} and IC_{100} signifies the concentrations causing 50% and 100% lysis of cells after 48 h.

Cell line	IC_{50} (µg ml^{-1})	IC_{100} (µg ml^{-1})
BHK 21 (ATCC CCL 10)	50	> 100
L 1210 (ATCC CCL 219)	50	100
HL 60 (ATCC CCL 240)	25	50
HeLa S3 (ATCC CCL 2.2)	25	> 100

7 µm, column size 2.5 × 25 cm, eluted with cyclohexane–*tert*-butyl methyl ether 95:5). The final yield of pure incrustoporin was 24 mg. The spectral data were recorded with Bruker IFS 48 (IR), Perkin-Elmer λ16 (UV), Jeol SX102 (MS) and Bruker ARX500 (NMR) spectrometers.

Incrustoporin was obtained as a colourless oil, $[\alpha]_D$ −4° (*c* 0.3 in $CDCl_3$). UV (methanol) λ_{max} (ε): 264 nm (8900). IR (KBr): 2970, 1750, 1515, 1120, 965 and 825 cm^{-1}. ^1H NMR ($CDCl_3$, δ in ppm relative to TMS, *J* in Hz): 7.75 (d, *J* = 8.1, 9-H and 11-H), 7.48 (d, *J* = 1.7, 3-H), 7.22 (d, *J* = 8.1, 8-H and 12-H), 4.98 (ddd, *J* = 1.7, 5.4, 6.8, 4-H), 2.37 (s, 13-H), 1.87 (ddq, *J* = 5, 7, 14, 5-Ha), 1.79 (ddq, *J* = 7, 7, 14, 5-Hb), 1.06 (t, *J* = 7.4, 6-H_3). ^{13}C NMR ($CDCl_3$, δ in ppm relative TMS, multiplicity): 171.8 (s, C-1), 146.5 (d, C-3), 139.4 (s, C-10), 131.7 (s, C-2), 129.2 (d, C-9 and C-11), 126.9 (d, C-8 and C-12), 126.8 (s, C-7), 81.4 (d, C-4), 26.8 (t, C-5), 21.3 (q, C-13), 9.2 (q, C-6). MS (direct inlet, EI, 70 eV): *m/z* 202.1000 (M^+, 55%, calc. for $C_{13}H_{14}O_2$ 202.0994), 145 (29%), 117 (100%), 84 (37%).

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